Kinetics of dissolution of calcium phosphate (Ca-P) bioceramics

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Abstract

Hydroxyapatite (HAp) and β-tricalcium phosphate (β-TCP) are widely used bioceramics for surgical or dental applications. This paper is dealing with dissolution kinetics of synthetically prepared β-TCP and four types of HAp granules. Two groups of HAp, treated at different temperatures, each of them with two different granule sizes, were tested. Three corrosive solutions with different pH and simulated body fluid (SBF) were used for immersing of the samples. Changes in concentrations of calcium and phosphate ions, pH level and weight changes of the samples were observed. It was found that presence of TRIS buffer enhanced dissolution rate of the β-TCP approximately two times. When exposed to SBF solution, calcium phosphate (most probably hydroxyapatite) precipitation predominates over β-TCP dissolution. Results from HAp samples dissolution showed some unexpected findings. Neither heat treatment nor HAp particle size made any major differences in dissolution rate of the same mass of each HAp sample.

Keywords: calcium phosphate, kinetics, dissolution

I. Introduction

Hydroxyapatite (HAp) $Ca_{10}(PO_4)_6(OH)_2$ as well as β-tricalcium phosphate (β-TCP) $Ca_3(PO_4)_2$ belong to the group of the most important bioceramics used for surgery or dental applications. These bioactive materials have an important role in hard tissue repairs, because of their good resorbability (β-TCP) or ability to bond with surrounding bone tissue (HAp) in body fluid environment [1]. Osteoconductive properties of hydroxyapatite are also used for scaffolds for bone tissue engineering [2]. HAp is widely used in the form of powder or granules (sintered powder), but also very often as a layer (coating) on metallic materials, for example titanium and its alloys or NiTi alloys. This composite material combines good mechanical properties of metals and bioactive properties of hydroxyapatite. Its applications are in surgery and dentistry, when some load-bearing bone has to be replaced by implant [3–5].

This paper deals with dissolution kinetics of HAp and β-TCP, both prepared synthetically. The aim was to observe relationship between the porosity, way of preparation and granule size of the Ca-P material and the rate of the material dissolution. For the *in vitro* experiments several solutions were used: i) demineralized water, ii) water buffered to various pH levels or iii) simulated body fluid. *In vitro* tests are useful in finding of a dissolution mechanism of the Ca-P material after interaction with body fluids. Knowledge of calcium phosphate dissolution and precipitation rates is important for the estimation and optimizing of healing period. Too slow dissolution and precipitation rate could cause unacceptably long healing period. On the other hand too fast HAp precipitation could negatively influence surrounding living tissue because of strong local concentration decrease of precipitated ions.

II. Experimental

Materials and methods

β-TCP granules and four types of HAp granules were tested in this work. All materials were prepared synthetically by company Lasak, Ltd. In brief hydroxyapatite powder was prepared by coagulating of $Ca(OH)_2$ solution and H_3PO_4. From the arisen powder the granules were rolled and consequently heat treated. Samples

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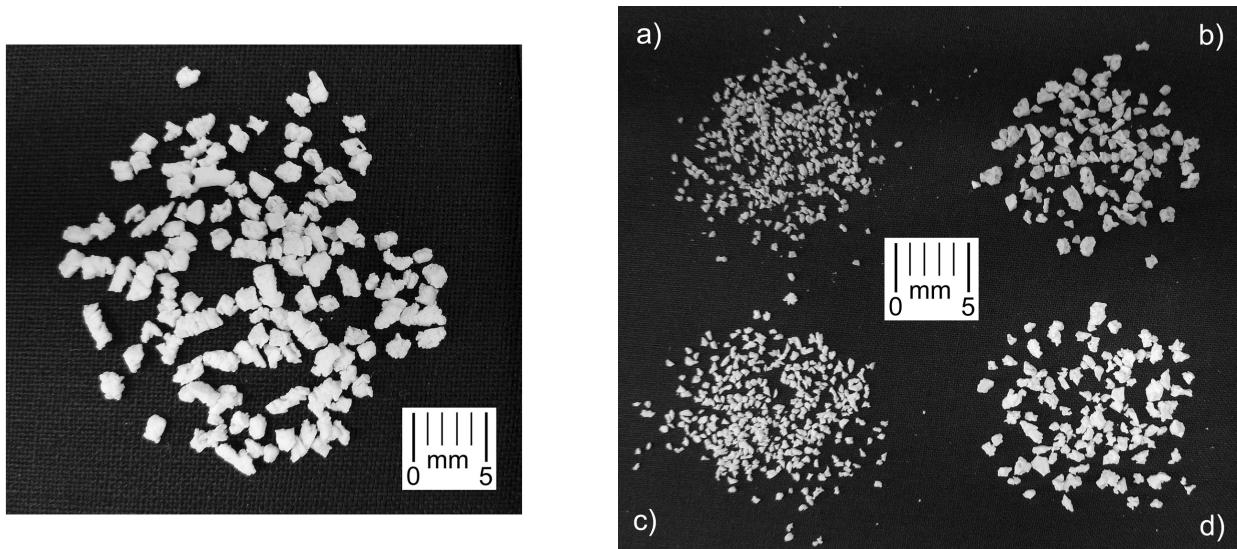e-mail: *lukas.brazda@vscht.cz*

Figure 1. Photographs of materials used for experiments – β-tricalcium phosphate (TCP), HAp-N-1 (a), HAp-N-2 (b), HAp-700-1 (c) and HAp-700-2 (d)

of HAp differ from one another in size of granules and in temperature of heat treatment during preparation of the material (Fig. 1). Sample notation, sintering condition and some characteristics of the used materials are summarized in Table 1. The surface area was measured with BET method using krypton (TCP samples) or nitrogen (HAp samples). Size of granules was measured by optical microscope.

The β-TCP and HAp samples were exposed to several corrosive solutions. In this work four diffrent solutions were used (pH values correspond to 20°C):

a) demineralized water, pH = 6.0
b) demineralized water with initial pH value set up with HCl, pH ~ 3.0
c) demineralized water buffered by TRIS (tris(hydroxymethyl)aminomethane) and HCl, pH = 7.5
d) SBF (Simulated Body Fluid) buffered with TRIS and HCl, pH = 7.6

The simulated body fluid is the commonly used solution for *in vitro* tests of bioactivity. The *in vivo* formation of an apatite layer on the surface of implanted material can be correlated to this *in vitro* test [6]. Composition of the simulated body fluid is similar to inorganic part of human blood plasma as shown in Table 2.

Weight of each sample was measured before and after exposition in corrosive solution. During the test, the concentrations of calcium and phosphate ions as well as pH value were followed in leached solutions. Calcium ions concentrations were measured by Atomic Absorption Spectrometry (VARIAN Spectr AA 300, 422.7 nm), phosphate ions concentrations were measured by UV-VIS Spectrometry (SHIMADZU UV-1601, 830 nm) and pH values were measured using glass electrode (inoLab). From these data process of calcium phosphate dissolution was evaluated.

Table 1. Characterization of used materials

Sample signification	Material	Sintering temperature [°C]	Average size of granules [mm]	Specific surface area [m²/g]
HAp-N-1	hydroxyapatite	150	0.8–1.2	64.4
HAp-N-2	hydroxyapatite	150	1.5–2.5	66.0
HAp-700-1	hydroxyapatite	700	0.8–1.2	25.6
HAp-700-2	hydroxyapatite	700	1.5–2.5	25.8
TCP	β-tricalcium phosphate	1200	1.5–2.5	0.17

Table 2. Composition of the simulated body fluid (SBF) [mmol/l] [9]

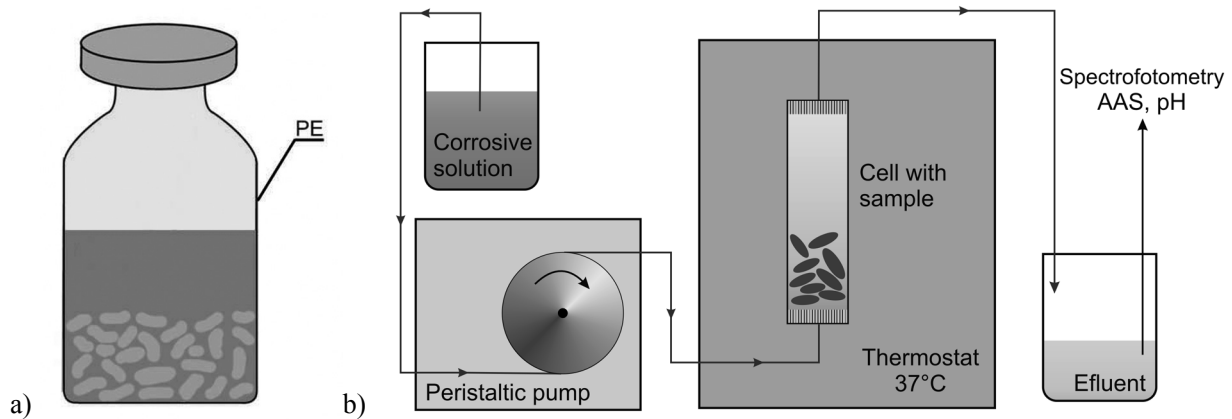|	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	$H_2PO_4^-$	SO_4^{2-}
Blood plasma	137.0–147.0	3.6–5.5	2.1–2.6	1.5	95.0–107.0	27.0	1.0	0.7–1.5
SBF	142.0	5.0	2.5	1.0	126.0	10.0	1.0	1.0

Figure 2. Experimental settlement of static (a) and dynamic (b) *in vitro* test

In vitro static test

There are two basic settlements of testing material behaviour in various solutions. For the first estimation, the static test of materials is used. The static test means that fine measured samples (approx. 0.5 g) were put into the plastic flask with 100 or 200 ml of corrosive solution (Fig. 2a). Flasks with samples were placed in thermostat or in shaking apparatus with temperature adjusted to 37°C. The shaking apparatus (150 rpm) was used in this 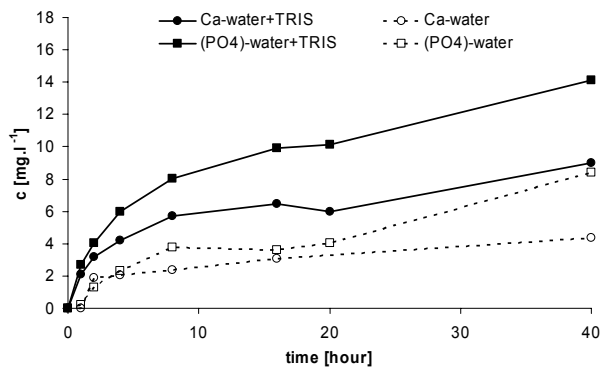work to accelerate transport of dissolved ions from the solution adjacent to the surface of samples. Testing at static conditions has simple adjustment, but there are also some disadvantages. The *S/V* ration (i.e. ratio between sample surface and volume of corrosive solution) is predominant factor. If the *S/V* ratio is too high, the leaching solution could turn saturated with products of sample corrosion and this would slow down or stop further dissolution. Back precipitation of corrosion products from the solution could also appear. On the other hand if the *S/V* ration is too low, there is no danger with solution saturation, but changes in ions concentrations caused by material dissolution are very small and difficult to analyze.

In vitro dynamic test

Due to the elimination of static test disadvantages described in previous paragraph, samples are tested under dynamic conditions. Dynamic test could be helpful in evaluation of material dissolution at steady state conditions. In Fig. 2b the scheme of dynamic test used in this work is shown. Samples were enclosed in flow cells placed in thermostat set to 37°C. Fresh corrosive solution from storage flask flowed round the material in the cell. Flow rate of the solution was set up with the peristaltic pump to 48 ml/day. Solutions after interaction with material were taken away in certain time periods to analyze calcium and phosphate ions concentration and to measure pH value. Data shown in graphs are average values from two independent experiments.

III. Results and Discussion

β-tricalcium phosphate

First results of material solubility from static test showed a difference between the rates of β-TCP dissolution in pure water an water buffered by TRIS solution. The β-TCP sample dissolved approximately 2 times faster in water buffered by TRIS (pH = 7.5) than in non-buffered demineralized water (starting pH value of demineralized water was 6.0). The ion concentration changes during the test are showed in Fig. 3. The authors [7] described reaction of TRIS buffer solution with Ca^{2+} and Mg^{2+} ions to form a soluble complex

Figure 3. The ion concentration changes in water and water buffered by TRIS, static conditions of β-TCP exposition, 37°C

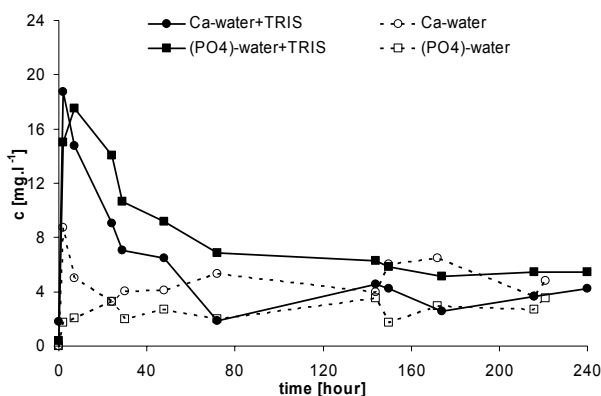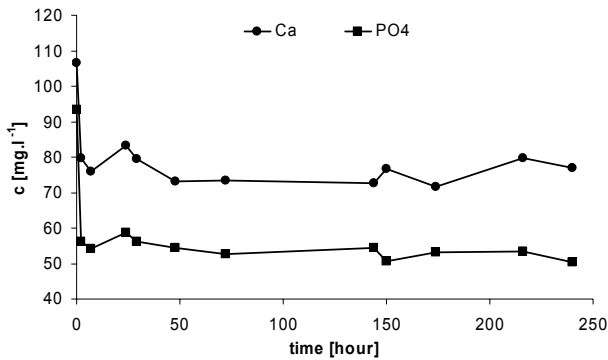

Figure 4. The ion concentration changes in water and water buffered by TRIS, dynamic conditions of β-TCP exposition, 37°C

Figure 5. The ion concentration changes in SBF during β-TCP exposition, dynamic conditions, 37°C
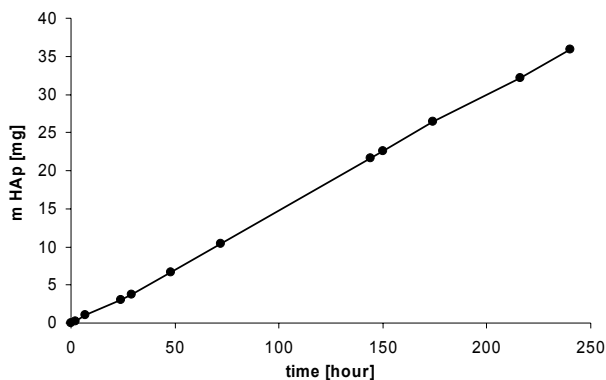

Figure 6. Total mass of the hydroxy- apatite precipitated from SBF, dynamic conditions, 37°C

compound. This process could cause increase in the β-TCP dissolution rate. The Ca/P weight ratio (calculated from solution analysis) was close to the theoretical Ca/P ratio for β-TCP. This could indicate congruent dissolution of the β-TCP material in water buffered by TRIS.

The faster dissolution of the β-TCP in TRIS solution was later confirmed by dynamic tests. The dissolution process started immediately after exposition of the β-TCP sample in corrosive solutions. As can be seen in Fig. 4, there was a rapid increase of both calcium and phosphate ions in effluent from the sample cell in first several hours. This could be caused by preferential dissolution of smaller β-TCP particles [8]. After approx. 50 hours of the β-TCP exposition the steady state of dissolution was achieved. The concentrations of Ca^{2+} and $(PO_4)^{3-}$ ions released into water buffer with TRIS were higher than concentrations analyzed in demineralized water in agreement with the static tests results.

Bioactivity of the β-TCP sample (indicated by calcium phosphate precipitation) has been confirmed by *in vitro* test in the simulated body fluid. It is supposed that when the β-TCP is exposed in SBF solution, dissolution of β-TCP and back precipitation of HAp is running at the same time. That is why we subtracted amount of Ca^{2+} and $(PO_4)^{3-}$ ions coming from β-TCP dissolution in TRIS solution from concentrations measured during β-TCP exposition in SBF. The graph in Fig. 5 shows

that calcium phosphate precipitation started immediately after exposition of the β-TCP sample to SBF. After few hours the rate of calcium phosphate precipitation became constant. The Ca/P weight ratio calculated from solution analysis was in good correlation with Ca/P ratio of HAp. The hydroxyapatite precipitation on β-TCP material was confirmed by small increase of sample weight. Total weight of precipitated HAp during the dynamic test is shown in Fig. 6. The data were calculated from calcium and phosphate ions decrease from the simulated body fluid. XRD measurements did not confirm presence of HAp on the β-TCP granules probably because the experiment time was too short and only small particles or thin film of HAp precipitated. This assumption should be confirmed by SEM in next study. Large change in the sample surface area is also worth mentioning. The surface area of the β-TCP after exposition in the simulated body fluid increased 10 times to 1.60 m^2/g. This phenomenon could be caused partially by disintegration of β-TCP surface and partially by precipitation process of hydroxyapatite from SBF solution.

Hydroxyapatite

Four types of hydroxyapatite material were exposed to water with a small amount of HCl at first. Starting pH value of this solution was approximately 3.0. During exposition of HAp the pH level of corrosive solution was rising until it got settled to value about 6.0.

Static in vitro test

Results from static *in vitro* have already showed some kind of unexpected behaviour of HAp samples. Although samples with heat treatment at 150°C (non-sintered) and at 700°C (sintered) have quite different specific surface area (Table 1), no significant differences in behaviour of materials during the exposition in corrosive solution were observed, when similar sample weight of material was exposed. This phenomenon was observed both for the static and dynamic settlement of corrosion test. Differences in dissolution rates of the non-sintered and sintered hydroxyapatite samples appeared when dissolved masses of ions were counted per sample surface area (see Table 3). The static test dissolution rates were calculated from initial hours of exposition. Fig. 7 shows concentration changes during the experiment for all kinds of hydroxyapatite. Within 48 hours calcium and phosphate ions concentration in corrosive solution settled down. In the case of phosphate ions a small difference in final concentrations appeared. Concentrations for the sintered HAp were about 4 mg/l higher than for the non-sintered samples. 14-day-test confirmed, that these concentrations were no more changing and so that the corrosive solution could be considered as saturated. The concentrations in steady state were 31–33 mg/l for calcium ion and 32–33 or 36–37 mg/l for phosphate ions.

Table 3. Rates of HAp dissolution at static and dynamic conditions (calculated from concentration changes in effluents)

R_c	Static test		Dynamic test	
	[μg g^{-1}h^{-1}]	[μg m^{-2}h^{-1}]	[μg g^{-1}h^{-1}]	[μg m^{-2}h^{-1}]
HAp-N-1	3372	52	285	4.4
HAp-N-2	3496	53	280	4.3
HAp-700-1	5003	195	304	11.9
HAp-700-2	3344	130	289	11.2
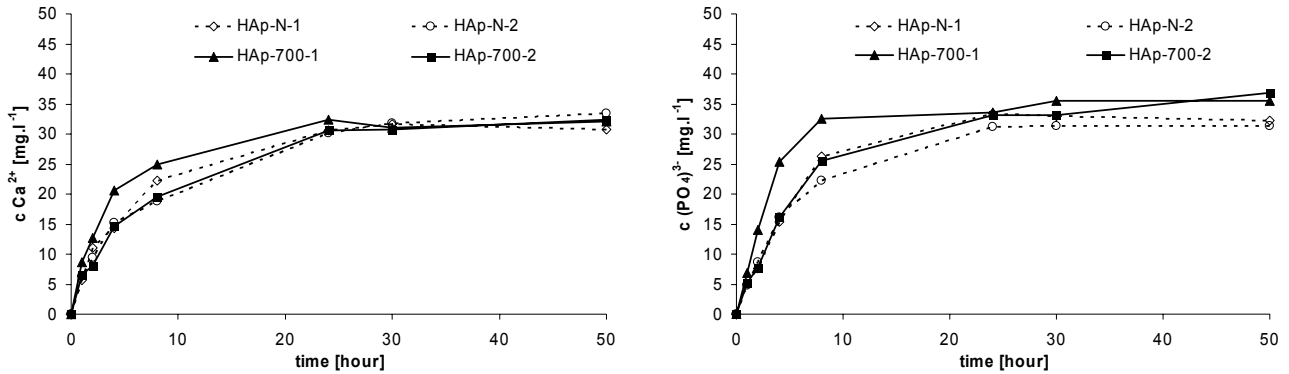
Figure 7. The calcium and phosphate ions concentration changes in solution during HAp dissolution, static conditions, 37°C
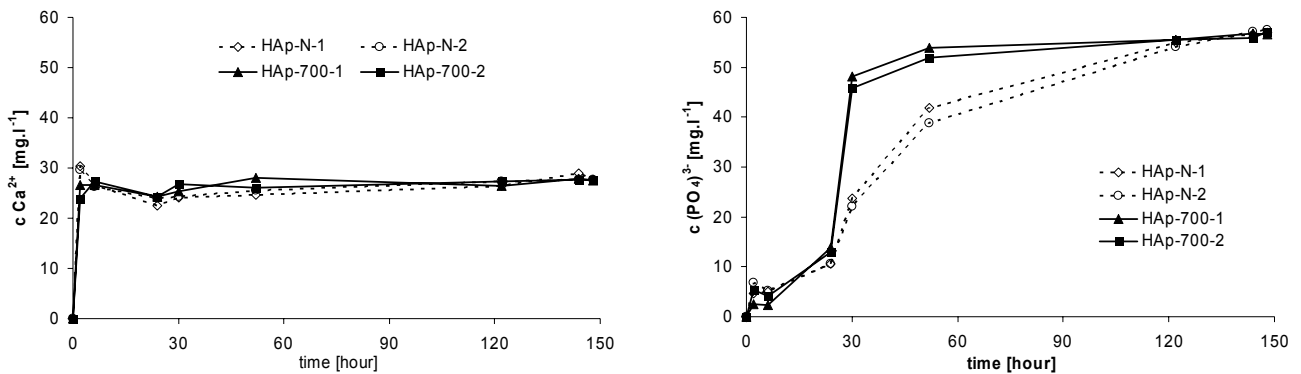
Figure 8. The calcium and phosphate ions concentration changes in solution during HAp dissolution, dynamic conditions, 37°C

Dynamic in vitro test

Results from solution analysis during the dynamic test are presented in Fig. 8. Within 40–50 hours the dissolution process achieved the steady state. That means the concentrations as well as pH in corrosive solution flowing out of the cells with samples did not change significantly with time and the HAp dissolved by approximately constant rate. Only for dissolution of phosphate ions from the non-sintered samples longer time was necessary for steady state adjustment. The steady state concentrations were similar for each kind of material again - 27–28 mg/l of Ca^{2+}, 57–58 mg/l of (PO$_4$)$^{3-}$ and pH level around 6.0. These concentrations were similar (Ca) or higher (PO$_4$) than the results from previous static test. Since the effluent solutions are close to saturation the dissolution rate of hydroxyapatite (Table 3) is supposed to be influenced. In on going experiments the faster flow rate (120 ml/day) of corrosive solution is used to eliminate the influence of effluent saturation with dissolved material.

IV. Conclusions

β-TCP

The presented results allow us to say that the β-TCP material dissolves congruently in water and water buffered with TRIS under both static and dynamic conditions of *in vitro* test. Presence of the buffer solution (TRIS) enhances the dissolution rate of the β-TCP approx. 2 times. Probably it could be caused by masking Ca^{2+} ions in soluble complex. When exposed to the simulated body fluid (SBF), the β-TCP material consequently induces on its surface calcium phosphate formation. Although it was not confirmed by IR and XRD analysis because of too low amount of precipitated material, it is supposed that the precipitating phase is hydroxyapatite. After subtracting of calcium and phosphate ions released into the solution buffered with TRIS, rates of HAp precipitation were calculated (854 μg m^{-2}h^{-1} or 145 μg g^{-1}h^{-1}).

These results were in good correlation with the HAp precipitation rates calculated from samples weight changes after exposition in SBF (868 μg m^{-2}h^{-1} or 148 μg g^{-1}h^{-1}).

HAp

There are some surprising results of hydroxyapatite dissolution. As it was demonstrated by both static and dynamic *in vitro* test, if the same amount of various hydroxyapatite materials was exposed to corrosive solution, approximately the same mass of each material was dissolved. Neither specific surface area, nor size of the investigated HAp granules made any difference. The average dissolution rate of the non-sintered hydroxyapatite at steady state was 4.4 μg m^{-2}h^{-1} and 11.6 μg m^{-2}h^{-1} for the sintered hydroxyapatite. These dissolution rates are probably influenced by solution saturation with corrosion products. Another dynamic test with higher flow rate of corrosive solution should confirm this expectation in future study.

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