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Nucleation and crystal growth of dicalcium phosphate dihydrate on titanium powder

C. COMBES, M. FRECHE, C. REY
INPT-ENSCT, Laboratoire des Matériaux, Equipe Physico-Chimie des Solides, URA 445, 38 rue des 36 Ponts, 31400 Toulouse, France

The nucleation and crystal growth of dicalcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$ DCPD or brushite) on titanium powder surface has been studied in metastable supersaturated solutions at 37 °C and pH=5.50. These experiments were carried out using the constant composition method. In order to determine the effect of substrate size on the kinetics of brushite formation, we used four ranges of titanium particle size (< 20 μm, 20–45 μm, 63–90 μm and 90–125 μm). The induction period τ, before the initial crystal growth of DCPD, varied markedly with the relative supersaturation σ. The initial growth rate R_c of brushite is strongly size-dependent, whereas τ is relatively constant for the different particle sizes. Plots of Ln R_c versus Ln σ, for each particle size, gave an apparent order of 3.4 ± 0.2 for the crystallization of brushite on titanium surface. The interfacial energy γ estimate of 22.9 ± 1.2 mJ m^{-2}, from the kinetic results, was in the same order of magnitude than that for sparingly soluble salts. Observations by scanning electron microscopy showed many platelets of DCPD covering the titanium powder surface.

1. Introduction

Titanium metal and its alloys are widely used as biomaterials especially for hip joints and dental implants. Titanium powder-sprayed on TA6V substrate increases the porosity and roughness of the biomaterial and improves the adhesion and osteo-integration between the bone and the implant [1]. It has been shown that the titanium surface can nucleate apatite crystals analogous to bone mineral in supersaturated solution at physiological pH [2]. However the surgical trauma associated with biomaterial implantation induces locally acidic pHs. These conditions may favour the initial formation of dicalcium phosphate dihydrate (DCPD or brushite) which is the most stable calcium phosphate salt in acidic solutions. Indeed, DCPD has been proposed as one of the precursors of calcium phosphate in the formation of hydroxyapatite during the mineralization of bone [3] and previous studies have already shown that titanium metal is a good nucleator of DCPD [4].

In this work, experiments were carried out using the constant composition method at different relative supersaturations. This method has been described by Nancollas and co-workers [5] and has been widely used for understanding crystal growth processes. In order to determine the role of the metal surface in the process of mineralization, we studied the kinetics of nucleation and crystal growth of DCPD on titanium powder of different granulometries.

2. Materials and methods

2.1. Titanium powder

We passed titanium powder (Aldrich 99.9%) through several sieves in order to obtain four ranges of titanium particle size: < 20 μm, 20–45 μm, 63–90 μm and 90–125 μm. The BET (Brunauer Emmet Teller) specific surface areas, determined with 30/70 nitrogen/helium (Micromeritics Flow sorb II 2300), were 0.40, 0.19, 0.12, 0.10 m^2 g^{-1} respectively.

2.2. Crystallization experiments

The constant composition crystal growth method was used to study the nucleation and growth of dicalcium phosphate dihydrate crystals from metastable supersaturated solutions of calcium phosphate. When titanium particles are added to the supersaturated solution, they may induce brushite crystallization depending on their ability to initiate the formation of first nuclei. The nucleation and growth of crystals consume ions from the solution lowering the pH which triggers the addition of two titrant solutions that serve to maintain the pH, the concentration of calcium and phosphate and the ionic strength of the solution constant.

All experiments reported herein were done at pH 5.50 in a 250 ml, double-walled glass reactor thermostated to 37 ± 0.1° C by circulating water. The stock solution contained KH_2PO_4, $CaCl_2$ and KCl at the desired concentrations for calcium, phosphate ([Ca] = [P] = 8 to 9.5 mM) and ionic strength (IS = 0.1 mol l^{-1}) respectively. The pH of the metastable solution (total volume 100 ml) was carefully adjusted by the addition of standard potassium hydroxide solution (0.1 M) to the magnetically agitated thermostated reactor. After thirty minutes of equilibration, 10 mg of titanium powder were introduced into the reactor. The pH changes were detected by

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a combined glass electrode with a Ag/AgCl internal reference element. When the calcium phosphate crystals began to grow, there was a small decrease of pH (ΔpH = 0.01) which controlled the simultaneous addition of the two titrant solutions ($CaCl_2$ + KCl and KH_2PO_4 + KOH) from electrically coupled automatic burettes (Metrohm). The concentrations of the titrant solutions were such that their addition exactly compensated for the phosphate and calcium ions precipitated and maintained the concentrations, pH and ionic strength constant.

The burettes were connected to a recorder, and from the plot of the volume of titrant added, V (ml), against time, t(min), the initial growth rate per square metre, R_c (mol min^{-1} m^{-2}), were accurately calculated as follows:

$$R_c = (\Delta V\, C)/(\Delta t\, m_0\, SSA)$$

where SSA (m^2 g^{-1}) is the specific surface area of the different titanium powder size ranges, C (mM) is the effective titrant concentration and m_0 is the mass of titanium powder. The induction time τ was measured on the time axis from the start of the recording until the intersection of the straight line with the time axis.

2.3. Characterizations

After each crystal growth experiment, the formation of brushite was confirmed by IR spectroscopy (Perkin Elmer 1600 FTIR) and X-ray diffraction (Inel CPS 120). The morphology of the growing phase was observed by scanning electron microscopy SEM (JEOL JSM 6400).

3. Experimental results and discussion

3.1. Kinetic results

The aim of these experiments was to show the effect of the titanium particle size on the crystallization of DCPD at various relative supersaturations σ($0.26 < \sigma < 0.47$). σ is defined by:

$$\sigma = (IP/K_{so})^{1/v} - 1$$

where IP is the ionic product, K_{so} the solubility product of DCPD at 37 °C and v the total number of ions in the formula ($v = 2$ for DCPD). These values of σ correspond to calcium and phosphorus concentrations of the supersaturated solutions in the range 8 to 9.5 mM.

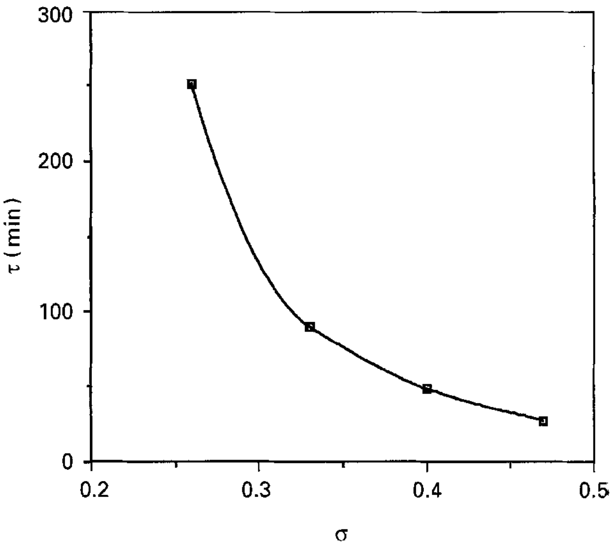

Figure 1 Induction time τ versus relative supersaturation σ for titanium particle sizes of 20–45 μm.

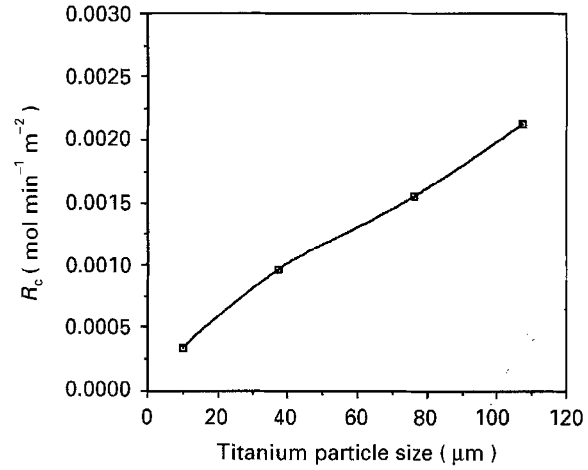

Figure 2 Dependence of R_c on titanium particle size for $\sigma = 0.33$.

The kinetic results (induction time τ and initial crystal growth rate R_c) are reported in Table 1. τ represents the time needed to form stable nuclei on the titanium powder surface or more precisely on the oxide layer at the surface of the metal. It can be seen, in Table 1, that whatever the relative supersaturation was, there was always an induction time τ before initial crystal growth of DCPD. Fig. 1 illustrates the dependence of τ on the relative supersaturation and Fig. 2, the dependence of R_c on substrate size. For

TABLE I Induction time τ (min) and initial growth rate R_c (mol min^{-1} m^{-2})

		Titanium particle sizes (μm)							
		< 20		20–45		63–90		90–125	
[Ca] mM	σ	τ	$R_c(10^4)$	τ	$R_c(10^4)$	τ	$R_c(10^4)$	τ	$R_c(10^4)$
8	0.26	269	1.4	252	3.5	335	4.7	277	7.5
8.5	0.33	124	3.35	90	9.6	110	15.5	80	21.3
9	0.40	47	6.8	49	14.6	44	27.9	54	41.5
9.5	0.47	42	10	28	22.3	30	41.5	28	64.4

$V_{total} = 100$ ml, $T = 37$ °C, pH = 5.51, $m_{Ti} = 10$ mg, [Ca] = [P]

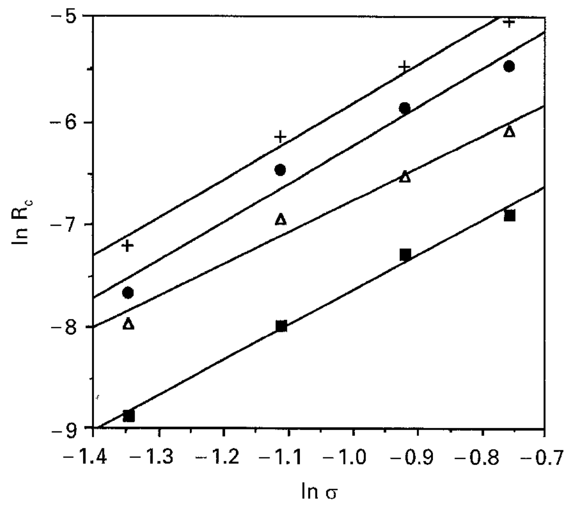

Figure 3 Plots of ln R_c versus ln σ for each titanium particle size: ■; < 20 μm; △; 20–45 μm; ●; 63–90 μm; + ; 90–125 μm.

a given particle size, when the relative supersaturation increased, the induction period decreased and the initial crystal growth rate increased. For the same relative supersaturation, τ was relatively constant but R_c increased strongly with titanium particle size ($R_{c(90-125\,\mu m)} = 6 \times R_{c(<20\,\mu m)}$). This showed that the growth rate was size-dependent, increasing with titanium particles size. Nucleation and growth of DCPD seemed to be favoured by large titanium particles and not by a large total surface area in the solution. This size contribution to the growth process has been encountered for the crystallization of several salts, and is attributed to a diffusion dependence that increases with the size of the substrate [6, 7]. Indeed, the probability that surface nucleation will occur is proportional to the surface area. Consequently, increasing supersaturation and size enhances the nucleation frequency and the growth rate.

The plots of ln R_c versus ln σ, for each particle size, presented in Fig. 3, show a series of approximately parallel straight lines, with slopes that give the order of the reaction n: the average value for n was 3.40 ± 0.4. The crystallization of DCPD on DCPD leads to an order $n = 2$ related to a spiral growth mechanism [8, 9, 10]. The order we found, different from 2, can be interpreted as being indicative of a polynucleation mechanism [11].

3.2. Estimation of the interfacial energy γ

An important parameter for the description of both nucleation and crystal growth rates is the interfacial energy, γ, between the brushite and the supersaturated solution.

The induction time can be considered as inversely proportional to the nucleation rate, that is proportional to the change in free energy [12]. Crystallization at surfaces may be induced at supersaturations lower than those required for spontaneous precipitation. The overall free energy for the formation of the critical nuclei under heterogeneous conditions, ΔG_{het}, is lower than that corresponding to homogeneous nucleation, ΔG_{hom}, by a factor φ which is < 1.

$$\Delta G_{\text{het}} = \phi . \Delta G_{\text{hom}}$$

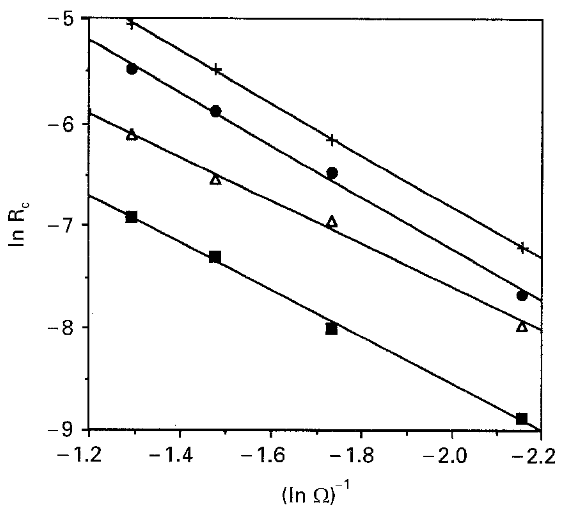

Figure 4 Initial growth rate of crystallization of DCPD R_c as a function of the supersaturation ratio Ω, for all particle sizes, according to the polynuclear model. Particle size: ■; < 20 μm; △; 20–45 μm; ●; 63–90 μm; + ; 90–125 μm.

φ is related to the contact angle θ between the crystalline deposit and the substrate [13].

$$\phi = (2 + \cos\theta)(1 - \cos\theta)^2/4$$

φ depends on the lattice match between the substrate and precipitating phase; the better the fit, the lower the value of φ and the greater the rate of nucleation.

According to the polynuclear model [14, 15, 16], the rate of growth is given by:

$$R_c = A \exp\left[-\frac{4\lambda^2 a^2}{3k^2 T^2 \ln \Omega}\right]$$

where A is a constant, a the mean molecular parameter of the crystallizing phase ($a_{\text{DCPD}} = 4.98 \times 10^{-10}$m), λ the edge free energy ($\lambda a = \gamma a^2$), Ω the supersaturation ratio ($\Omega = IP/K_{\text{so}}$), k the Boltzmann constant and T the absolute temperature. The representation ln R_c versus $(\ln \Omega)^{-1}$, for each particle size, yielded a straight line as shown in Fig. 4. From the slopes of these lines an average value of 22.9 ± 1.2 mJ m^{-2} was obtained for the interfacial energy γ_{R_c} of the growing brushite.

The dependence of the induction time on the supersaturation ratio may also be used to estimate the interfacial energy, in the case of homogeneous nucleation, by plotting ln τ as a function of $(\ln \Omega)^{-2}$ for all particle sizes, see Fig. 5. From the slope α_τ of this straight line, we can calculate γ_τ following the empirical equation given by Nielsen [17]:

$$\gamma_\tau = kT\left[\frac{5\alpha_\tau}{2\beta v^2}\right]^{\frac{1}{3}}$$

where v is the mean ionic volume of the crystallizing phase ($v = 6.175 \times 10^{-29}$ m^3), β the shape factor (assuming cubic shape nuclei β = 32), k the Boltzmann constant and T the absolute temperature. The average value calculated was 10.6 ± 0.4 mJ m^{-2}.

As foreseen, the estimation of the interfacial energy with the induction time is lower because the theory for homogeneous nucleation does not take into account

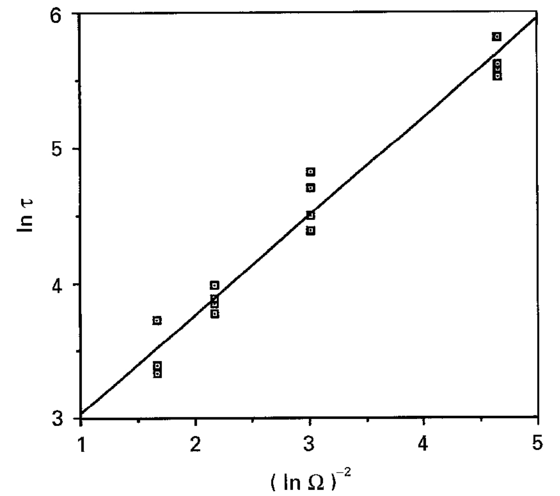

Figure 5 Induction time τ versus supersaturation ratio Ω, for all particle sizes, according to Nielsen.

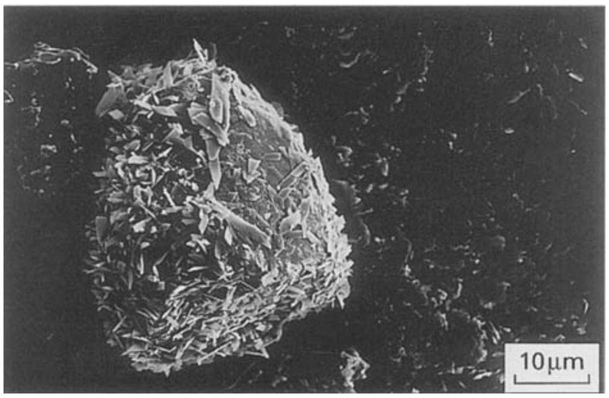

Figure 6 Scanning electron micrograph of brushite crystals on titanium powder surface (x 1500).

the role of the substrate. If the ϕ parameter, defined earlier, is introduced then [18]:

$$\gamma_{R_c}\,\phi^{1/3} = \gamma_\tau$$
$$\text{and } \phi = 0.09$$

Such a low value for ϕ has been calculated for heterogeneous nucleation of other calcium phosphate salts [18]. We could think that there is a relatively close lattice matching between DCPD and titanium surface.

The relatively low value for the interfacial energy between brushite and the solution (22.9 mJ m^{-2}) proved that this calcium salt crystallizes easily on a foreign substrate like titanium.

3.3. Characterizations

The products obtained after crystal growth were analyzed by X-ray diffraction and IR spectroscopy: they were, in all cases, only composed of titanium and DCPD.

The presence of platelets of brushite at the surface of the titanium powder was confirmed by scanning electron microscopy, see Fig. 6. The photograph shows many crystals of DCPD entirely covering the metal surface. This confirms that titanium is a good nucleator of brushite.

4. Conclusion

In the present work, it has been shown that titanium may serve as a substrate for the nucleation of dicalcium phosphate dihydrate. We used the constant composition crystal growth method to study the effect of substrate size and relative supersaturation on the heterogeneous nucleation and crystal growth of DCPD on titanium. This method leads to a precise determination of the two main parameters, the induction time τ and the initial crystal growth rate R_c. Brushite was exclusively formed at the surface of the titanium powder following an induction time. The particle size of the titanium powder has an important effect on the initial crystal growth rate R_c (R_c for the particles of 90–125 μm was about six times higher than R_c for particles of < 20 μm), while the induction time τ is determined only by changes in relative supersaturation. The estimation of the interfacial energy for the overgrowing brushite (22.9 mJ m^{-2}), from kinetic data, gives a typical value for DCPD nucleation and growth. The direct nucleation and growth of DCPD on titanium powder confirms that titanium surfaces are good nucleators for calcium phosphate compounds; this could be related to their good integration in bone tissues.

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