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The growth of non-stoichiometric apatites using the constant composition method

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The kinetics of the crystal growth of calcium-deficient hydroxyapatites with different stoichiometry ($Ca_{5-\mu}(HPO_4)_\mu(PO_4)_{3-\mu}(OH)_{1-\mu}$) have been investigated at 37 °C using the constant composition method. The growth was performed in solutions supersaturated only with respect to $Ca_5(PO_4)_3(OH)$ (HAp) by inoculating with well-characterized seed crystals. The stoichiometry of the grown apatites was consistent with values of $0 \leqslant \mu \leqslant 0.185$. The deviation μ from HAp stoichiometry of the growing apatite increased with increasing supersaturation degree (S). The constant composition method also provides relevant information about the solubility behaviour of the growing phase with a definite composition. From the decrease of the normalized growth rate j with decreasing S, an estimate could be made of the composition of the solution for which the growth ceases. The determined solubility product of the grown apatite (4.28×10^{-54} M^9) was higher than the value obtained by the equilibration of the seed material. The results were interpreted on the basis of differences in crystal lattice perfection.

1. Introduction

The main inorganic phase of bone and teeth of the higher vertebrates, as well as most pathological calcified tissues, is commonly described as calcium-hydroxyapatite, $Ca_5(PO_4)_3(OH)$ (HAp) [1–7]. The calcification processes in these biological systems can only be understood by studying the growth and solubility behaviour of these calcium phosphates. The application of HAp-like materials as orthopedic implants has increased this need.

In order to elucidate the mineralization and demineralization processes of biominerals, numerous attempts have been made to determine the solubility product of biological apatites using equilibration methods. However, in these experiments, a surface layer with *a priori* unknown characteristics determines the solubility behaviour of the solid [8].

Recently, several studies have been performed on the crystal growth of apatites using the constant composition method (CC-method) [9–17]. This method maintains the lattice ionic concentrations and ionic strength constant during growth by adding reagent solutions with suitable composition. Most of these literature reports have been devoted to the crystal growth of HAp in media of high supersaturation and low solid : solution ratio [9–15]. However, little work has been done to study the seeded growth kinetics of apatite in solutions which are slightly supersaturated with respect to HAp only and at high solid : solution ratio [16, 17]. These conditions more closely approach those in real biological systems.

The CC-method is ideal for studying the crystal growth kinetics of apatites in slightly supersaturated solutions since reliable growth rates can be obtained. Moreover, from the progress of the normalized growth rate (j) as a function of the supersaturation degree (S), an estimate can be made of the composition of the solution for which the growth ceases. Consequently, apart from a study of the kinetics of the growth process, a quantitative relation between the stoichiometry of the grown apatite and its solubility can be made as well.

In this study, the CC-method was used to investigate the crystal growth of apatites at 37 °C by seeding solutions, supersaturated only with respect to HAp, with well-characterized seed crystals. These conditions are representative of biological calcifications. With an extrapolation method, an estimate was made of the solubility product of the grown apatite, which was compared with the solubility product obtained by equilibration of the seed material [18]. Hitherto, relating the kinetics of the apatite crystal growth experiments to the solubility product of the grown phase has never been reported.

2. Materials and methods

Analytical reagent grade chemicals and freshly double-distilled, CO_2-free water were used throughout for the

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preparation of solutions. The solutions were filtered before use (0.22 μm Millipore filters).

Solutions, supersaturated with respect to HAp, were prepared from stock solutions of $CaCl_2$ and KH_2PO_4. KCl was used to adjust the ionic strength to 0.15 M. The calcium content of the stock solutions was determined by complexometric titration with an EDTA/Zn-EDTA solution [19]. The phosphorus content was determined spectrophotometrically as phosphovanadomolybdate using a slight modification of the method of Brabson *et al.* [20]. The relative uncertainties on the analysed amounts were estimated to be 0.2% for both methods.

The seed crystals, commercially available apatite "Merck" used for bioceramic applications, were analysed for Ca and P using the same methods (Ca/P molar ratio = 1.666 ± 0.003). The specific surface area was determined by the BET method to be $63.66 \pm 0.77\ \mathrm{m^2\,g^{-1}}$. The solubility product K_{sp} of the seed material, determined by equilibration, was $5.83 \times 10^{-58}\ \mathrm{M}^9$ [18].

Crystal growth experiments were carried out using the CC-method with a Radiometer pH-stat unit. The unit consisted of a PHM84 Research pH meter, a TTT80 Titrator, two coupled ABU 80 Autoburettes and a REC 80 Servograph recorder equipped with REA 270 pH-stat and REA 160 Titrigraph modules. The pH was measured with a combined glass electrode (Ingold) which was calibrated at 37 °C before and after each experiment with a phosphate and borax buffer prepared according to the National Bureau of Standards [21]. The uncertainty in the pH measurements is estimated as 0.02 pH units.

Solutions (250 ml), supersaturated with respect to HAp and 0.15 M in ionic strength, were pipetted in a specially constructed water-jacketed round-bottom reaction cell thermostated at 37.0 ± 0.1 °C. The suspension was stirred by an electronically driven and controlled paddle stirrer at 300 ± 2 rpm so that no solid sedimented on the bottom of the cell. Nitrogen, presaturated with water vapour was bubbled through the solution to exclude carbon dioxide contamination from the atmosphere. The final solution pH was adjusted to 8.00 before the start of the growth experiment by the addition of 0.05 M potassium hydroxide.

After thermal equilibration and following verification of the stability of the supersaturated solutions for at least 3 h, crystal growth was initiated by the addition of 100 mg of dried seed "Merck". This relatively high solid:solution ratio is relevant with respect to physiological systems, such as the bone–serum interface, where the mineral surface areas involved are very large. Moreover, at lower ratios, a non-constancy of the solution composition would be analytically undetectable within reasonable growth times.

The onset of growth resulted in a decrease of the solution pH, which triggers the addition of titrants from a pH-stat unit in order to keep the pH, the ionic strength as well as the calcium and phosphate concentrations in the solution constant during the crystal growth of the HAp phase. The concentrations of the titrant solutions were derived from the stoichiometry of the growing phase as described by Heughebaert *et al.* [16].

The concentrations of the titrant solutions for the growth of non-stoichiometric apatites were calculated using the following relationships

$$\text{Titrant 1}\begin{cases}\mathrm{CaCl_2} & T_1 = (5-\mu)C + 2W_1 \\ \mathrm{KCl} & T_2 = -(10-\mu)C + 2W_2\end{cases}$$

$$\text{Titrant 2}\begin{cases}\mathrm{KH_2PO_4} & T_3 = 3C + 2W_3 \\ \mathrm{KOH} & T_4 = (7-2\mu)C + 2W_4\end{cases}$$

where T_i represents the typical titrant concentration of the particular salt i, W_i is the total concentration of that salt in the supersaturated solution and μ is the non-stoichiometric coefficient of the titrants reflecting the general composition of calcium-deficient apatites represented by the formula [8]

$$\mathrm{Ca_{5-\mu}(PO_4)_{3-\mu}(HPO_4)_{\mu}(OH)_{1-\mu}} \qquad (1)$$

with $0 \leqslant \mu \leqslant 1$. C represents the effective molar titrant concentration with respect to the growing apatite [16].

At the end of the crystal growth experiments (for a period of 5 h unless otherwise specified), the suspensions were quickly filtered (0.22 μm Millipore filters). Calcium and phosphate analyses were performed on the filtered solutions in order to verify the constancy of the solution composition. The calcium was analysed by atomic absorption using a Varian 30 AA-spectrophotometer. The phosphorus content was determined spectrophotometrically by using a slight modification of the heteropoly blue method of Chen *et al.* [22]. The uncertainties were estimated as 2% and 0.5%, respectively, of the amounts of Ca and P determined.

The solid samples were dried under vacuum at 25 °C and analysed for the specific surface area using a triple point BET method (Quantasorb, Quantachrome Corporation).

3. Results

In this study, the supersaturation degree, S, with respect to dicalcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$; DCPD), tricalcium phosphate ($Ca_3(PO_4)_2$; TCP), octacalcium phosphate ($Ca_4(HPO_4)(PO_4)_2 \cdot 2.5H_2O$; OCP) and HAp is defined as

$$S = (I_p/K_{sp})^{1/\nu} \qquad (2)$$

where ν represents the number of ions in a formula unit of the calcium phosphate phase, I_p is the ion activity product in the solution and K_{sp} the thermodynamic solubility product.

The relative supersaturation, σ, is then given by

$$\sigma = S - 1 \qquad (3)$$

The ion activity products were calculated from the molar activities a_x of the species X in solution by an iterative procedure described in detail elsewhere [23]. The thermodynamic dissociation constants at 37 °C for phosphoric acid and for water were taken from Robinson and Stokes [24]. For the association constants of the ion pairs $CaH_2PO_4^+$ and $CaHPO_4$,

TABLE I Composition of the solutions and their relative supersaturation, σ, with respect to DCPD, TCP, OCP and HAp

	Composition				σ			
Solution	$c_{\rm Ca} \times 10^4$ (mol l^{-1})	$c_{\rm P} \times 10^4$ (mol l^{-1})	$c_{\rm KCl}$ (mol l^{-1})	Ca/P	DCPD	TCP	OCP	HAp
OA$_{\rm II}$	3.600	1.800	0.1490	2.000	– 0.8265	0.4868	– 0.03241	4.431
OB$_{\rm I}$	3.000	1.800	0.1491	1.667	– 0.8413	0.3345	– 0.1156	3.914
OB$_{\rm II}$	3.300	1.650	0.1491	2.000	– 0.8407	0.3644	– 0.1024	4.032
OC$_{\rm I}$	2.750	1.650	0.1492	1.667	– 0.8544	0.2246	– 0.1797	3.552
OE$_{\rm I}$	2.250	1.350	0.1493	1.667	– 0.8806	– 0.02085	– 0.3104	2.816
OE$_{\rm II}$	2.400	1.200	0.1493	2.000	– 0.8837	– 0.004061	– 0.3185	2.803
OH$_{\rm I}$	1.500	0.9000	0.1496	1.667	– 0.9201	– 0.3285	– 0.5862	1.668

TABLE II Ca/P molar ratio, percentage growth with respect to the initial amount of seed material and normalized growth rate $\bar{j}$ of apatites grown at 37°C as a function of $I_{\rm P}^{\rm HAp}$. Standard deviations are given in brackets

Solution	Ca/P	Percentage growth	$I_{\rm P}^{\rm HAp}$ (mol^9 l^{-9})	$\bar{j}$ (mmol min^{-1} m^{-2})
OA$_{\rm II}$	1.605	40	2.40×10^{-51} (0.52×10^{-51})	1.818×10^{-5} (0.079×10^{-5})
OB$_{\rm I}$	1.605	32	9.7×10^{-52} $(2.1 \times 10^{-52}$	1.496×10^{-5} (0.054×10^{-5})
OB$_{\rm II}$	1.610	38	1.21×10^{-51} (0.26×10^{-51})	1.714×10^{-5} (0.034×10^{-5})
OC$_{\rm I}$	1.610	34	4.9×10^{-52} (1.1×10^{-52})	1.478×10^{-5} (0.044×10^{-5})
OE$_{\rm I}$	1.635	15	1.00×10^{-52} (0.22×10^{-52})	5.28×10^{-6} (0.14×10^{-6})
OE$_{\rm II}$	1.630	17	9.7×10^{-53} (2.1×10^{-53})	6.91×10^{-6} (0.29×10^{-6})
OH$_{\rm I}$	1.667	8	4.00×10^{-54} (0.87×10^{-54})	8.22×10^{-7} (0.90×10^{-7})

respectively, the values of 8.4 and 542 M^{-1} were used [25]. The activity coefficients of the ionic species were approximated with the extended form of the Debye–Hückel equation proposed by Davies [26]. The values used for the thermodynamic solubility products of the various calcium phosphate phases at 37 °C were, $K_{\rm sp}$ (DCPD) = 2.06×10^{-7} M^2 [27], $K_{\rm sp}$ (TCP) = 2.83×10^{-30} M^5 [28], $K_{\rm sp}$ (OCP) = 1.13×10^{-49} M^8 [27] and $K_{\rm sp}$ (HAp) = 5.83×10^{-58} M^9 [18].

The composition of the solutions used for the seeded growth experiments and their relative supersaturation, σ, with respect to DCPD, TCP, OCP and HAp are summarized in Table I. All initial solutions given in Table I were undersaturated with respect to DCPD and OCP and slightly supersaturated with respect to TCP and HAp. The formation of TCP has never been observed under these experimental conditions. At a constant S with respect to HAp, the Ca/P molar ratio of the solution was chosen to be 1.67 or 2.00 (solutions with subscript I or II, respectively).

The results of the seeded growth experiments in the slightly supersaturated solutions with respect to HAp (Table I) are summarized in Table II. The table shows that the apatites grown under sustained supersaturation were non-stoichiometric apatites, with a Ca/P molar ratio varying from 1.605 to 1.667. This ratio tends to increase with increasing S but is not affected by the Ca/P ratio of the solution. Typical plots of volume titrant as a function of time are shown in Fig. 1. It can be seen from these plots that the crystal growth showed an initial growth surge during the first minutes of the growth.

The amounts of grown apatite ($n_{\rm HAp}$) could be calculated at each moment from the volume of the added titrants. The rate of crystal growth was then obtained by a numerical differentiation of $n_{\rm HAp}$ as a function of time. Fig. 2 shows that the specific surface area (SSA) decreased during growth so that the rates of growth have to be normalized for changes in total surface area of the crystals in solution.

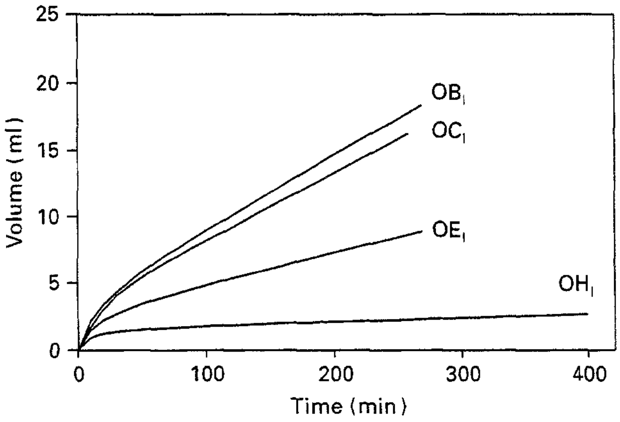

Figure 1 Volume titrant added versus time for the seeded growth experiments in supersaturated solutions with a Ca/P molar ratio of 1.667 (Table I).

In Fig. 3, the normalized crystal growth rates, j, are given as a function of time for two crystal growth experiments in supersaturated solutions OB$_{\rm I}$ and OE$_{\rm I}$. The general shape of these curves again illustrates the initial surge. Following the surge, j slowly decreases with extent of growth and reaches a plateau of constant growth rate within experimental error. This constant normalized growth rate was calculated as the mean value in the plateau. The resulting weighted mean of the normalized growth j ($\bar{j}$) was determined on the basis of at least five parallel experiments and the values are given in Table II. The standard deviation on $I_{\rm P}^{\rm HAp}$ was calculated according to error

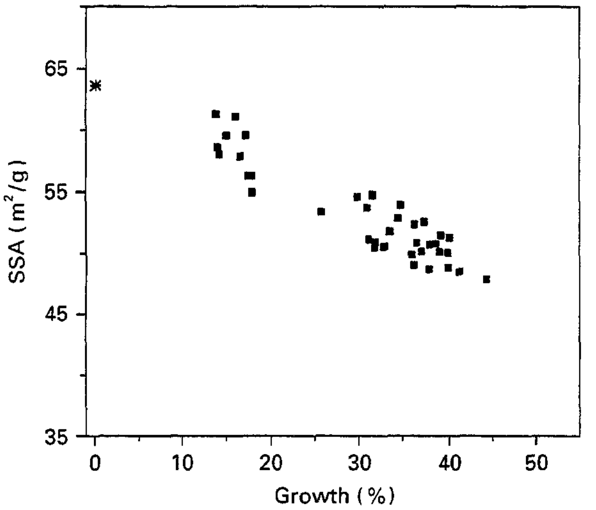

Figure 2 Specific surface area SSA as a function of extent of growth with respect to the amount of original seed. The specific surface area of the original seed is also indicated (*).

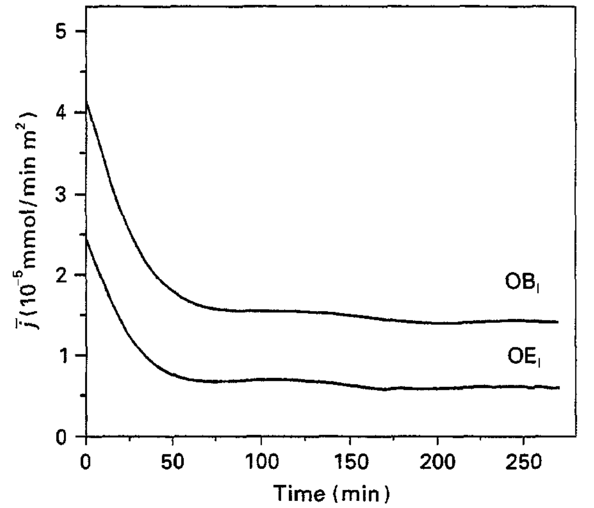

Figure 3 Normalized growth rate j as a function of time for a typical seeded growth experiment in the supersaturated solution OB$_I$ and OE$_I$.

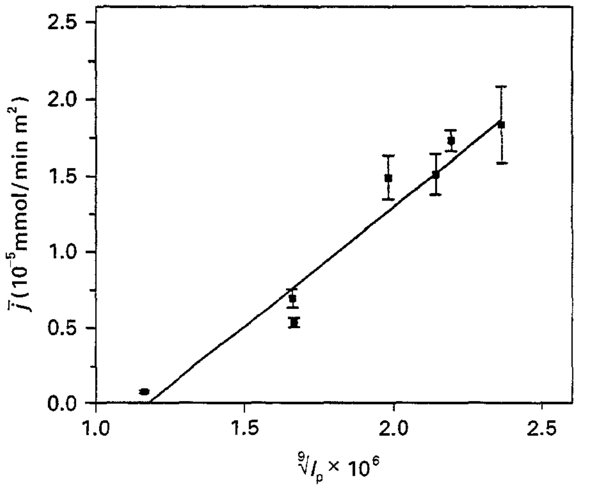

Figure 4 Normalized growth rate $\bar{j}$ as a function of $\sqrt[9]{I_p^{HAp}}$ for the results of Table II.

propagation. The seeded growth experiment in supersaturated solution OH$_I$ was performed for more than 24 h. The growth rate was very small indicating that the saturation degree, with respect to HAp, was approximated.

Table II shows that the normalized crystal growth increases with increasing I_p^{HAp} and hence with increasing S as well as with an increasing Ca/P molar ratio of the solution. This is more clearly illustrated in Fig. 4 where $\bar{j}$ is plotted against $\sqrt[9]{I_p^{HAp}}$ for the seeded growth experiments.

4. Discussion

The crystal growth experiments of the present investigation showed that non-stoichiometric apatites grow in slightly supersaturated solutions. These results were interpreted according to Equation 1 with $0 \leqslant \mu \leqslant 0.185$. The growing non-stoichiometric apatites had apparent deficiencies in both calcium and hydroxide lattice ions that were associated with the inclusion of acid phosphate into the crystal lattice [16].

The initial growth surge, as illustrated in Fig. 3, might be attributed to the adjustment of the seed surfaces to the conditions of the supersaturated solutions as discussed by Zhang and Nancollas [13, 29]. This clearly indicates that the use of initial rates of crystal growth for interpretive purposes will lead to significant errors. Since the decrease in rate with extent of growth is not due to a change in available surface area for growth, it may be attributed to a surface maturation phenomenon in which the number of available growth sites decreased to a steady value under the conditions of sustained supersaturation [13].

With an extrapolation method, an estimate could be made of the composition of the solution for which the growth ceases. According to Equations 2 and 3 the corresponding $I_{p.eq}^{HAp}$ then equals the solubility product of the growing phase, K_{sp}. A linear regression analysis of $\bar{j}$ as a function of $\sqrt[9]{I_p^{HAp}}$ for the growth experiments mentioned in Table II yields $I_{p.eq}^{HAp} = (4.28 \pm 1.96) \times 10^{-54}\ \text{M}^9$. As μ decreases with decreasing S, it can be expected that this value of $I_{p.eq}^{HAp}$ will approach the solubility product of stoichiometric HAp (see Table II).

Hohl *et al.* [14] studied the crystal growth of HAp with relatively small amounts of seed crystals and over a relative wide range of supersaturation and pH values. Their results are shown in Fig. 5. A regression analysis of the combined data in Fig. 5 shows that j varies linearly with $\sqrt[9]{I_p^{HAp}}$. Extrapolation to growth rate zero as a function of $\sqrt[9]{I_p^{HAp}}$ for the experiments of Hohl *et al.*, over the pH range of 7.0–8.5, yields a value of $I_{p.eq}^{HAp} = (1.46 \pm 1.03) \times 10^{-54}\ \text{M}^9$. This is in agreement with the value obtained in this study.

We can conclude that for both studies $I_{p.eq}^{HAp} > 5.83 \times 10^{-58}\ \text{M}^9$, which corresponds to the solubility product obtained by equilibration of the seed material "Merck" [18].

Crystal growth experiments at constant pH performed by Nancollas *et al.* [30] confirmed that the solubility product of the grown apatite is substantially higher than that of the original seed crystals. A recalculation of the data of Nancollas *et al.* [30] shows that

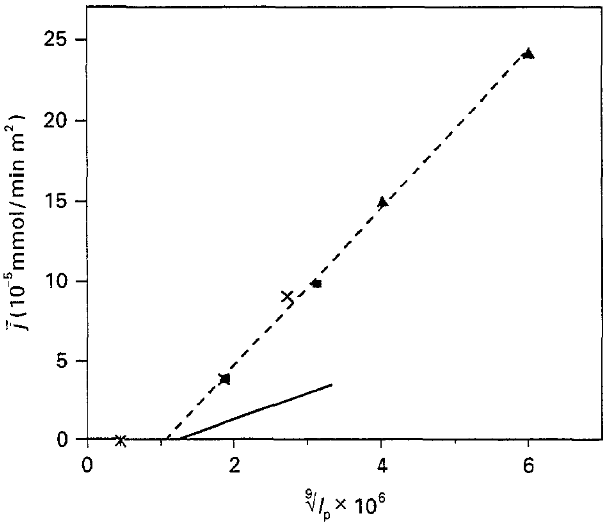

Figure 5 Normalized growth rate $\bar{J}$ as a function of $\sqrt[9]{I_p^{HAp}}$ for the results of Table II (—) and for the results of Hohl *et al.* [14] (×) pH = 7.0; (◇) pH = 7.4; (△) pH = 8.5. The thermodynamic solubility product K_{sp} of the seed material of the present study is also indicated (∗)

after 4 days growth the I_p^{HAp} of the solution reached a constant value of 5×10^{-54} M^9 [31].

Generally, the seeded growth of HAp in slightly supersaturated solutions and low solid : solution ratio is a surface-controlled growth corresponding to a dislocation process [15, 32]. For the crystal growth experiments at high solid : solution ratio Nancollas *et al.* [33] suggested that in the initial surge, the effective order of reaction had a value of $n > 3$, indicating the participation of a polynuclear surface nucleation process. At larger extents of growth, the value of n decreases and approaches 2, suggesting a spiral growth mechanism controlled by screw dislocations. This is corroborated by the experiments of Hohl *et al.* [14] who also found a screw dislocation controlled growth for HAp. Consequently, at the low supersaturation degrees used in the present study, the grown apatite will contain numerous dislocations. Hence, the high value of $I_{p,eq}^{HAp}$ corresponding to the solubility product of a stoichiometric HAp ($\mu \cong 0$), merely reflects the increase of the free energy of the apatite by these lattice effects.

Contrary to crystal growth, equilibration of seed crystals with dislocations will lead to recrystallization into crystals with less imperfections [18]. Hence the corresponding equilibrium solubility product will be determined by an almost perfect HAp surface layer with unknown properties.

It can be concluded that, in opposition to the equilibration method, the results of crystal growth by the CC-method can be directly related to the characteristics of the grown phase. These findings are important in understanding mineralization in biological systems for which the supersaturation is low and solid : solution ratio high.

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