The Nucleation and Growth of Calcium Phosphate Crystals*

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The growth of seed crystals in supersaturated solutions of calcium phosphate at 25° C is discussed. In the pH range 5—6, the crystallization of dicalcium phosphate follows a second order rate equation in a process which is surface reaction rather than diffusion controlled. Under physiological pH conditions, the growth of hydroxyapatite seed crystals proceeds through the formation of a precursor phase which has a calcium : phosphate ratio of 1.45 ± 0.05 and which attains the HAP composition after slow interaction with the medium; the process again appears to be interface controlled.

The control of supersaturation and the nucleation and growth of crystals in calcium phosphate systems are important in relation to the physiological deposition of bone and tooth mineral. The pathological deposition of calcium salts in abnormal or unusual locations from supersaturated tissue fluids will also be significantly dependent upon such processes. Numerous studies of spontaneous precipitation of calcium phosphates have been made in an attempt to determine the mechanism of the process. In almost all experiments made at approximately the physiological pH, the stoichiometry of the initially formed phase does not correspond to that of hydroxyapatite [HAP, $Ca_{10}(PO_4)_6$ $(OH)_2$]; the calcium : phosphate ratio being invariably less than the required 1.67. After prolonged periods of ageing, the ratio rises and eventually reaches that for the pure HAP phase. In an attempt to explain this phenomenon, several theories have been put forward. Francis[1] proposed a surface complex of calcium hydrogen phosphate (DCP, $CaHPO_4$) to account for the solubility behaviour of HAP under moderately acidic conditions ranging from pH 3.5 to 6. At higher pH's (6.3 to 7.5) the assumption of a surface complex of the general formula $Ca_2(HPO_4)(OH)_2$ by Rootare, Dietz and Carpenter[2] yielded ionic products which were reasonably consistent. Neuman and Neuman[3], on the other hand, proposed an exchange of surface ions to account for the observed variation of composition of precipitated HAP.

More recently, a number of physical methods have been used in an attempt to determine the nature of the amorphous precursor in HAP precipitation. The results of Posner and his co-workers[4] indicate an approximate composition of this phase corresponding to tricalcium phosphate

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* Based on a lecture presented at the II International Summer Conference on the *Chemistry of Solid/Liquid Interfaces*, »Rudjer Bošković« Institute, Rovinj — Yugoslavia, September 1970.
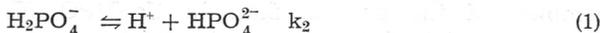
(TCP, $Ca_3(PO_4)_2$). Subsequent changes to the more crystalline HAP phase, followed by an x-ray diffraction method, were found to be autocatalytic in nature. Brown[5] has made extensive x-ray studies of calcium phosphate phases and has determined the crystal structure of another phase, octacalcium phosphate (OCP, $Ca_8H_2(PO_4)_6 \cdot 5\,H_2O$) which is precipitated at physiological pH's. The close epitaxial similarity of OCP to HAP led to the suggestion that the former phase was a precursor in the process of calcification[5].

Previous studies of calcium phosphate precipitation have usually been concerned with the spontaneous formation of solid phase in supersaturated solutions. The supersaturated solutions are made by the slow mixing of solutions containing the lattice ions and it is assumed that the initial solid phases are formed by homogeneous nucleation. However, it is doubtful whether heterogeneous nucleation can be avoided in any practical medium and in any event, biological calcification almost certainly results from a heterogeneous rather than a homogeneous nucleation. In addition, in spontaneous precipitation experiments, both nucleation and the subsequent growth of the nuclei occur simultaneously and the results are markedly dependent upon the fluid dynamics of the systems and upon the rates of mixing of the reagents. The difficulty of reproducing the results of such experiments renders them unattractive as a means of studying the mechanism of formation of crystals or for assessing the effectiveness of additives in inhibiting the rate of crystal growth.

The existence of well-defined metastable limits for the spontaneous crystallization of most sparingly soluble electrolytes makes it possible, by careful control of experimental conditions, to prepare supersaturated solutions which are stable for considerable periods[6,7]. The kinetics of growth of well — characterized seed crystals may then be studied, under reproducible conditions, by inoculation of these supersaturated solutions and by following the concentrations of lattice ions as a function of time[7-9]. In this paper, we discuss two calcium phosphate crystal growth systems, DCP and HAP, for which this method has been applied.

Dicalcium phosphate dihydrate

There is considerable interest in the mechanism of growth of DCP crystals from supersaturated solutions of the salt since this phase may play an important part in the production of dental caries[10]. Studies have been made in calcium phosphate solutions within the pH range 5 to 6 in which the equilibria

$$H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-} \qquad k_2 \qquad (1)$$

$$Ca^{2+} + H_2PO_4^- \rightleftharpoons CaH_2PO_4^+ \qquad K^+ \qquad (2)$$

and $\qquad Ca^{2+} + HPO_4^{2-} \rightleftharpoons CaHPO_4 \qquad K° \qquad (3)$

must be taken into account. The concentrations of ionic species in the supersaturated calcium phosphate solutions at any instant were calculated from mass balance and electroneutrality conditions and the known values for the equilibrium constants[11] by successive approximation of the ionic strength[12].

As stated previously, the seeded growth experiments yield highly reproducible results and in Fig. 1 are shown typical plots of total calcium and
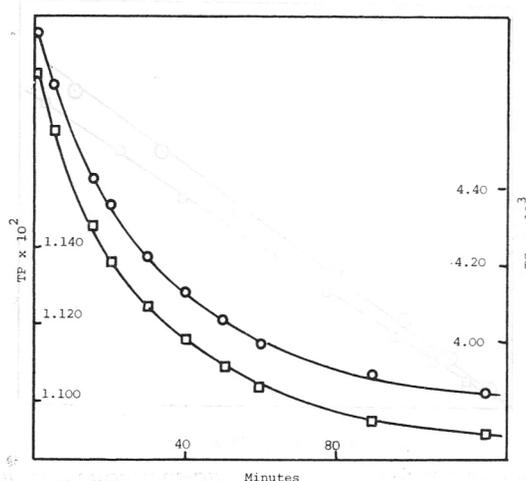
Fig. 1. Plots of total calcium and total phosphate concentrations as a function of time. Initial conditions: pH = 5.939, T_{Ca} = 4.808 $\times$ 10^{-3} M, T_{PO4} = 1.185 $\times$ 10^{-2} M

total phosphate concentrations as a function of time. It is seen that growth commences immediately upon the addition of DCP seed crystals to the supersaturated solutions prepared by the careful mixing of calcium chloride, disodium hydrogen phosphate and potassium dihydrogen phosphate solutions. After a brief initial fast period the growth process, represented by the smooth curves in Fig. 1, follows equation (4)[10]

$$- \left(\frac{wi}{w} \right)^{2/3} \frac{dT}{dt} = ks \left([Ca^{2+}][HPO_4^{2-}] - K_{sp}/f_2^2 \right) \qquad (4)$$

in which the rate of growth, dT/dt, is expressed as a change in either total calcium or total phosphate concentrations, K_{sp} is the thermodynamic solubility product, f_2 the activity coefficient of divalent ions, k the rate constant and s, the surface area. The correction factor $(w_i/w)^{2/3}$ is introduced to correct the rate of growth at time t to that corresponding to the initial surface area of the seed crystals. w_i is the weight of seed crystals at time zero and w, the weight present at time t. Adherence to the second order kinetic equation (4) indicates that the growth of DCP crystals is surface rather than diffusion controlled[10]. It is interesting to note that similar conclusions have been reached for the growth of crystals of several other sparingly soluble electrolytes. The rate of growth is independent of the rate of stirring within the crystallization cells. For DCP, the kinetic plots in Fig. 2 confirm the second order dependence of equation 4.

The striking effect of additives in reducing the rate of crystallization has been described for a number of systems[7]. If it is assumed that the additive is adsorbed at growth sites on the crystal surface and effectively prevents further deposition, the retarding action can be described in terms of a Langmuir adsorption isotherm. The excellent reproducibility of the experimental results for DCP crystal growth has enabled such a study of the effect of sodium pyrophosphate upon the growth rate. Pyrophosphate has been
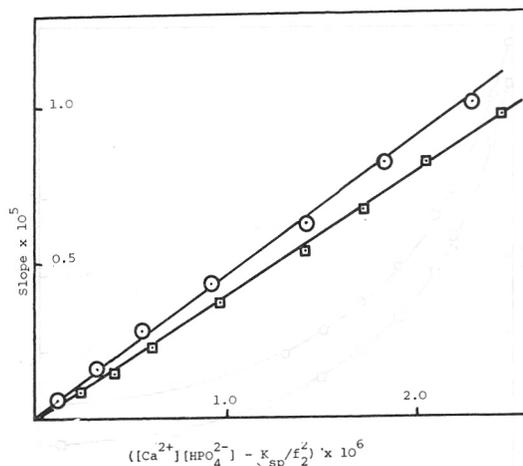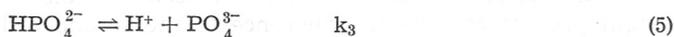
Fig. 2. Plots of $-(\text{Wi}/\text{W})^{2/3}\,dT/dt$ (= "slope") against $([Ca^{2+}][HPO_4^{2-}] - K_{sp}/f_2^2)$. Initial conditions: (○) $pH = 5.595$, $T_{Ca} = 1.211 \times 10^{-2}$ M, $T_{PO4} = 1.131 \times 10^{-2}$ M; (□) $pH = 5.59$, $T_{Ca} = 1.171 \times 10^{-2}$ M, $T_{PO4} = 1.083 \times 10^{-2}$ M.

invoked as a substance which is effective in preventing pathological calcification in the body[13]. Its action in inhibiting the growth of DCP crystals is striking, a concentration as low as 5×10^{-6} M being sufficient to prevent almost entirely the crystal growth process. The reduction in rate constant k (equation 4) is satisfactorily described in terms of a Langmuir adsorption isotherm[10].

Hydroxyapatite

The existence of a precursor in the precipitation of HAP from supersaturated solutions of calcium phosphate has already been discussed. Our own studies have concerned the growth of well characterized HAP preparations in supersaturated solutions in which the pH was maintained constant at a value of 7.4 by means of pH-stat-controlled additions of alkali. Under such conditions, in addition to equilibria 1—3, it is necessary to take into account the following reactions[14]

$$HPO_4^{2-} \rightleftharpoons H^+ + PO_4^{3-} \qquad k_3 \qquad\qquad (5)$$

$$Ca^{2+} + PO_4^{3-} \rightleftharpoons CaPO_4^- \qquad K^- \qquad\qquad (6)$$

The additional species and known values for the equilibrium constants are introduced into the computational procedure described previously. After the addition of HAP seed crystals to the supersaturated solutions the concentrations of calcium and phosphate and the alkali uptake were monitored as a function of time[14]. The typical rate plots in Fig. 3 indicate that crystal growth takes place immediately upon inoculation with crystals and the overall growth curves consist of two main regions. In the first, of 60—90 min duration, growth proceeds rapidly with the precipitation of a calcium phosphate phase having a calcium : phosphate ratio of 1.45 ± 0.05. Thereafter, this precursor phase undergoes a slow interaction with the medium and the ratio of calcium : phosphate taken up from the supersaturated solution increases to a value
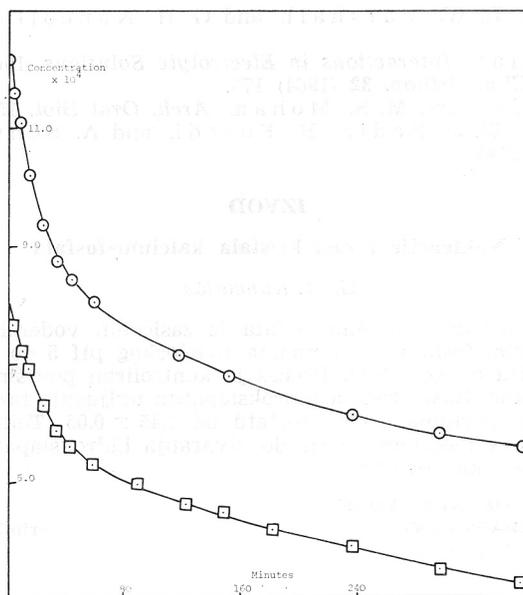
Fig. 3. Plots of total calcium (O) and total phosphate (□) concentrations as a function of time at a constant $pH = 7.4$. Initial conditions: $T_{Ca} = 1.244 \times 10^{-3}$ M, $T_{PO4} = 0.782 \times 10^{-3}$ M.

approaching 2.0. Equilibrium is attained after 6—7 days at the end of which time, the overall calcium: phosphate ratio of the material deposited is close to the 1.67 required for HAP.

The stoichiometry of the precursor phase is close to that, 1.5, for TCP. It is interesting to note, however,[15] that the calcium : phosphate ratio is consistently 3—5% lower than that required for pure TCP and thus it is not possible to rule out other phases, such as DCP or OCP which may participate in the early stages of the crystal growth process. As was found with DCP, the rates of change of concentrations with time and hence the rate of crystal growth are independent of the rates of stirring. This evidence strongly suggests that the seeded growth of HAP is also controlled by a reaction at the surface of the crystals rather than by a diffusion process.

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IZVOD

Nukleacija i rast kristala kalcium-fosfata

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Opisan je rast kristala kalcium-fosfata iz zasićenih vodenih otopina kod 25°. Proces rasta dikalcium-fosfata u otopinama fiziološkog pH 5 do 6 slijedi kinetičku jednadžbu za reakciju drugog reda. Proces je kontroliran površinskim pojavama, a ne difuzijom iz otopine. Rast kristala hidroksiapatita uključuje fazu predstanja (prekurzora) s odnosom kalciuma prema fosfatu od 1.45 ± 0.05. Tek iz tog predstanja sporom interakcijom s medijem dolazi do stvaranja hidroksiapatita. I ovaj proces kontroliran je stanjem na površini.

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