Heterogeneous Nucleation of Calcium Phosphates

I. Kinetics of Nucleation of Tetracalcium Monohydrogen Phosphate on Crystals of Calcium Monohydrogen Phosphate Dihydrate

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The nucleation kinetics of tetracalcium monohydrogen phosphate (Ca$_4$H(PO$_4$)$_3$) on brushite (CaHPO$_4$·2H$_2$O) has been studied. The incubation time is shown to be strongly dependent on initial pH in an interval about pH 7. The experimental data are consistent with a second-order dependence of nucleation rate on phosphate ion concentration. A surface reaction mechanism is proposed in which the process Ca$_4$PO$_4$$^+$ + PO$_4$$^-$ → Ca$_4$(PO$_4$)$_4$$^+$ is considered the rate-determining step.

The behaviour of supersaturated calcium phosphate solutions in regard to what they may precipitate — if anything — how much of it, and how fast, is a matter of considerable importance, not only to physical inorganic chemistry, but to many related sciences, including physiology of hard tissue. A high degree of supersaturation is very often encountered in solutions of calcium phosphate and other calcium or phosphate salts, whereas for instance silver halides precipitate readily from solutions just above saturation. A connection seems to exist between the hardness of a compound and its behaviour upon precipitation. Only salts with a Moh’s hardness below ~3 readily form well-defined crystalline precipitates at room temperature. A consistent explanation of this phenomenon has not yet been given.

Ignoring different hydrates, five pure orthophosphates of calcium may crystallize from aqueous solution:

I. Primary calcium phosphate

II. Secondary calcium phosphate

III. Tetracalcium monohydrogen phosphate

IV. Whitlockite

V. Hydroxylapatite

I crystallizes only from strong phosphoric acid, anhydrous or with one mole of water. II exists in two forms, the anhydrous form, monetite, which is stable above $\sim 36^\circ$C, and the dihydrate, brushite. Either form is precipitated from slightly acid solution, pH $\leq 6$. III is the normal product of precipitation in neutral or slightly basic medium. It is metastable and will slowly recrystallize to hydroxylapatite. III is by some authors claimed to be a dihydrate, by others a trihydrate. In the present treatment, the formula will always be written without water of crystallization, as this is immaterial. IV, which is anhydrous, is formed only under special circumstances. V is the stable final product of precipitation from neutral or basic solution. As its rate of crystallization is very low at ordinary temperature, it often appears as submicroscopic crystals.

In addition to the five salts listed above there is a number of more or less crystalline compounds with a Ca/P ratio ranging continuously from 1.25 to 1.67. Their X-ray powder diagrams resemble that of hydroxylapatite, and they are normally described as calcium-deficient apatites.

The multitude of possible solid phases which may occur in the calcium phosphate system makes the quantitative description of precipitation and dissolution extremely complex. Most of the salts dissolve incongruently, and unless the ionic strengths of the solutions are adjusted to a constant high value by addition of a neutral salt, a lengthy iteration procedure is needed to find the actual values of stoichiometric solubility products and dissociation constants. Calculations of solubility in the conventional sense, i.e. the amount of a given salt that may dissolve in a given solution, are infrequent. With reliable solubility products electronic data processing will rationally solve most of these problems.

The time needed for a calcium phosphate system to attain equilibrium may vary from minutes to practically infinity. An important consequence is seen in the reported values of the various solubility products. For brushite and monetite the agreement between different investigators is good, for the other salts poor. * Bjerrum reported two values for tetracalcium monohydrogen phosphate and for hydroxylapatite, one for precipitation and another for dissolution, the former being from 10 to 30 times the value of the latter. In addition to this, problems of metastability arise.

A few investigations of the kinetics of calcium phosphate crystallization have been reported recently. Walton et al. determined the Ca/P ratio at critical supersaturation in solutions of different pH and demonstrated that in the range of pH from 6.3 to 9.1 the stoichiometry of the nucleus corresponds to the formula Ca₃(PO₄)₂. This was explained as being due to the low entropy change for formation of Ca₃(PO₄)₂ in contrast to Ca₄H(PO₄)₂. However, the crystal may well change its stoichiometry on growing. Mukherjee has shown that the effect of an increase in calcium or phosphate concentration or pH is an increase in the rate of precipitation of calcium phosphate. The effect of pH was most significant near 8.0.

* Primary calcium phosphate is too soluble to have a measurable solubility product.
NUCLEATION OF CALCIUM PHOSPHATES

PREVIOUS STUDIES ON HYDROLYSIS OF BRUSHITE

In a series of experiments with the object of determining the solubility of brushite Bjerrum and Damsgaard-Sørensen observed the precipitation of tetracalcium monohydrogen phosphate in a suspension of brushite in neutral or slightly acid medium at 37°C. The process was studied in this laboratory in further details by Tovborg Jensen, Krogh Andersen, and Rowles. Brushite was rotated with 5 x 10^-4 M primary calcium phosphate solution at 37°C for 2 or 3 days. Then the solid was filtered off and rotated with fresh solution. In the course of a week a complete transformation into tetracalcium monohydrogen phosphate was achieved. From pH measurements and analysis of the solution an approximate solubility product of tetracalcium monohydrogen phosphate could be determined. Impurities in the solution seriously affect the nucleation and growth of tetracalcium monohydrogen phosphate. The method has been widely used for synthesis. The overall reaction is

5 CaHPO_4(solid) → Ca_4(HPO_4)_2(solid) + Ca(H_2PO_4)_2(solution)

During precipitation of Ca_4(HPO_4)_2 the pH of the solution will decrease until equilibrium between the solid phases and the solution is established. At 37°C this will occur at pH = 5.56, corresponding to pK_sp for CaHPO_4 = 6.646 and pK_sp for Ca_4(HPO_4)_2 = 11.59.

Lerch et al. studied the hydrolysis of brushite partly in pure water, partly at constant pH, obtained by automatic addition of NaOH or HNO_3. In the latter case they observed an immediate reaction at pH up to 8. Above pH 8 the onset of crystallization was delayed 2-4 h. This was interpreted in terms of the dependence of nucleation and growth rate on pH, the latter increasing and the former decreasing - as a result of slower dissolution of brushite - with increasing pH. The end product of hydrolysis in pure water as well as the first product at pH ~ 8 was described as crystalline hydrated normal calcium phosphate, Ca_4(PO_4)_2, aq, with tetracalcium monohydrogen phosphate as an intermediate in pure water.

In the present paper is reported an examination of the kinetics of precipitation of tetracalcium monohydrogen phosphate in suspensions of brushite in neutral or weakly acid solution at 37°C. The experimental methods are to a large part identical with those applied formerly by Tovborg Jensen.

EXPERIMENTAL

Apparatus. pH was measured with a calomel and a glass electrode using a Radiometer pH Meter type 4d. The precipitates were examined with a Leitz phase contrast microscope and by X-ray powder diffraction using a Bradley type camera with a diameter of 190 mm. Co-radiation was used.

Materials. Brushite was prepared by the method of Tovborg Jensen and Rathlev. The other chemicals were analytical or C. P. grade. Glass-distilled water was used for the hydrolysis experiments.

Analytical methods. Calcium was determined by complexometric titration. To the sample was added an excess of 0.01 M EDTA disodium salt. 1-2 drops of methyl red indicator was added, and the solution was neutralized with 0.1 M sodium hydroxide. pH was adjusted to 10 with ammonia buffer, and the excess of EDTA was titrated with 0.01 M magnesium sulphate using erio-T as indicator. With this method there is no
possibility of calcium phosphate precipitation, and a sharp end point is obtained. The reproducibility was ±0.2%.

Phosphate was determined by Bondorf's modification of the usual molybdenum blue method. 5 ml sample was pipetted into a 25 ml volumetric flask. 2 ml ammonium molybdate (25 g/litre) in 2 M sulphuric acid was added dropwise under shaking. After 5 min was added 1 ml of an aqueous solution containing per litre 125 g anhydrous sodium sulphite, 25 g sodium hydrogen sulphite, and 5 g metol. 10 min later the solution was diluted with water to 25 ml. After further 10 min the absorbance was measured at 780 nm using a Zeiss PM QII spectrophotometer. Deviation from Beer's law was significant at phosphate concentrations above ~10^{-2} M. For the phosphate molarity c, the following expression was found

\[ c = (1.277 + 0.0086d) \times 10^{-3} \]

where d is the absorbance of the sample. The blue colour is more stable when developed with metol than with stannous chloride.

Method of hydrolysis. 0.5 g brushite was weighed in a polyethylene bottle with screw-cap, 250 ml water or phosphoric acid solution was added, and the bottle was placed in a water thermostat at 37°C, where it was rotated constantly. pH was measured at selected time intervals without separation of the precipitate. For analysis, the suspension was filtered by suction, the precipitate was washed three times with acetone, and dried in the air. In most cases the Ca/P ratio of the solid phase was determined from the composition of the solution. Control analyses showed no significant error.

RESULTS

In a preliminary series of experiments the hydrolysis in pure water was studied in detail. The onset of nucleation within 30 min was evident from a drop in pH of the solution. In the course of a few hours small crystals of a new phase became visible on the surface of the brushite crystals when viewed under the microscope. After 144 h of hydrolysis the brushite lines in the X-ray diffraction pattern of the precipitate had disappeared, and the pattern was that of Ca₃H(PO₄)₂, but with very diffuse lines. The Ca/P ratio of the precipitate was 1.42. Evidently the product of hydrolysis in pure water is not well-crystallized tetracalcium monohydrogen phosphate. A plausible cause is the high initial supersaturation, which results in the formation of a large number of nuclei.

In the experiments with solutions of phosphoric acid instead of pure water, only pH was measured, and all other quantities regarding the composition of the system were calculated. This was possible because the preliminary experiments had shown that the solution was saturated with respect to brushite.

![Fig. 1. Variation of pH during hydrolysis. Numbers on curves denote initial phosphoric acid concentration in mM.](image)

as long as any solid brushite was left at all, which was always the case except at the end of the experiments with the lowest initial phosphoric acid concentrations. The calculations have been described elsewhere.10

The concentrations of phosphoric acid ranged from 0.025 to 0.375 mM. A selection of the results is plotted in Fig. 1. The numbers on the curves are the initial phosphoric acid concentrations in mM.

From the pH values the amount of tetracalcium monohydrogen phosphate precipitated at any time may be calculated. Fig. 2 is a plot of the course of precipitation for an initial phosphoric acid concentration of 0.100 mM. The ordinate is the amount precipitated from 1 litre of solution. The incubation time, \( t_i \), is taken as the intersection of the tangent at the steepest part of the curve with the abscissa axis. The incubation time could not be determined for systems with an initial concentration of phosphoric acid below 0.050 mM.

Fig. 3 is a logarithmic plot showing the relation between the incubation time and the concentration of the ion \( \text{PO}_4^{3-} \) when the solution is saturated.

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*Fig. 2. Course of precipitation of tetracalcium monohydrogen phosphate for initial phosphoric acid concentration 0.1 mM.*

*Fig. 3. Dependence of incubation time on phosphate ion concentration. Slope of line drawn is $-2.00$.*
with respect to brushite, but before any tetracalcium monohydrogen phosphate is precipitated. The slope of the straight line, as determined by the method of least squares, is $-2.02$. The line drawn in Fig. 3 has a slope of $-2.00$.

This series of experiments showed that the larger the initial phosphoric acid concentration, the better developed tetracalcium monohydrogen phosphate crystals were obtained. They mostly appeared as spherulites or were arranged in bundles. The largest single crystals had a length of about 0.1 mm. The X-ray powder pattern of the crystals grown at an initial phosphoric acid concentration of 0.375 mM showed, in addition to the brushite lines, 11 of the strongest lines of tetracalcium monohydrogen phosphate. The sharpness of the lines was characteristic of a well-crystallized compound.

**DISCUSSION**

Heterogeneous nucleation is normally described by an equation of the form $^{18,19}$

$$J = Cn^2\exp\left(\frac{2\Delta G_{\text{des}} - \Delta G_{\text{dil}} - \Delta G^+}{kT}\right)$$

where $J$ is the steady-state rate of formation of nuclei, $n$ is the concentration in solution of the nucleating species, $\Delta G_{\text{des}}$ is the activation free energy per molecule for desorption, $\Delta G_{\text{dil}}$ is the activation free energy for surface diffusion, and $\Delta G^+$ is the standard free energy for formation of a critical nucleus, i.e. an ion cluster in unstable equilibrium with the adsorbed layer. This, in turn, is assumed to be in quasi-equilibrium with the solution. $C$ accounts for the rate constant of diffusion of ions to the substrate surface, the Zeldovich correction factor, the distance between equivalent sites on the surface, and the vibration frequencies of the adsorbed ions. It is usually treated as a constant.

$\Delta G_{\text{des}}$ and $\Delta G_{\text{dil}}$ are treated as constants, whereas $\Delta G^+$ depends on the size and shape of the critical cluster. For a square cluster, we have

$$\Delta G^+ = \frac{4yd^2\varphi^2}{mkT \ln s}$$

where $d$ is the average ionic diameter in the cluster, $\varphi$ is a correction factor for lattice mismatch, $\varphi$ is the edge energy per unit length, $m$ is the number of ions needed to form a neutral “molecule”, and $s$ is the supersaturation ratio. The condition for validity of this expression is that the critical nucleus is large enough to allow the application of macroscopic thermodynamics for the description of its properties, i.e. it must contain more than $\sim 40$ ions.$^{20}$

At low supersaturation, $\Delta G^+$ is numerically much greater than $\Delta G_{\text{des}}$ and $\Delta G_{\text{dil}}$ and is therefore the rate-determining term. Consequently, the range of supersaturation ratios, for which the nucleation rate is measurable, is very narrow. The critical supersaturation, $s^+$, is defined as the value for which $J = 1$ nucleus per cm$^2$ per sec. Below this value of $s$, nucleation is practically unobservable, and above, it appears instantaneous. This follows from the dependence of $J$ or $s$, viz. $J = c_1 \exp(-c_2/\ln s)$, where $c_1$ and $c_2$ are constants.

and $c_0 \gg \ln s$. The theory has been tested for the nucleation of alkali halides on mica \(^{19}\) by measuring the critical supersaturation at different temperatures. Qualitative agreement was found. The same problems are encountered in this theory as in the surface nucleation theory for crystal growth,\(^{21,22,23,p.191}\) viz. the rate of nucleation predicted at the observed critical supersaturation (a few per cent) is so extremely low that the process must be controlled by lattice defects on the surface where adsorption is strong.

In the case of tetracalcium monohydrogen phosphate nucleating on brushite a different approach is needed. The values of $s$, for which the incubation time was measured, ranged from 150 to 6500 (5.0$\leq \ln s \leq 8.8$). There is no evidence of a definite critical supersaturation. As the critical nucleus is probably small, $\Delta G^*$ is low, and nucleation on a perfect surface has a reasonable probability. When the nucleus contains only a few ions, $\Delta G^*$ is not a continuously decreasing function of supersaturation, but a step function, eventually a constant in the range considered. This implies a critical nucleus of constant size, and hence the expression for $J$ (eqn. 1) reduces to $J = k_2n^2$, i.e. nucleation is a second-order process.

In spite of the simplicity of the relation $J = k_2n^2$, and its obvious agreement with the observed dependence of incubation time on phosphate ion concentration, viz. $t_i = k_2\left[\text{PO}_4^{3-}\right]^2$, this relation should be taken with care. The incubation time must depend on both nucleation and growth rate. If we consider nucleation and growth as two separate processes, both contributing to the total amount of deposited material, we may regard the incubation time as the time elapsed before the growth rate (expressed as the amount of solid deposited per unit time) becomes greater than the nucleation rate. This is consistent with the way of determining $t_i$. At the time the formation of a new phase is detected by a drop in pH, the composition of the solution has not altered significantly. The rate of nucleation is therefore constant during the incubation period, whereas the total growth rate depends on the number of nuclei already formed. We do not know the mathematical form of this relation, but we shall assume the simple equation

$$\frac{dn}{dt} = A + Bn$$  \hspace{1cm} (3)

where $n$ is the total amount of material in the new phase at time $t$. $A$ and $B$ depend on the ion concentrations in solution and are taken as time-independent. This holds to a good approximation during the incubation period. The solution of (3) with $n = 0$ for $t = 0$ is

$$n = \frac{A}{B} \left( e^{Bt} - 1 \right)$$  \hspace{1cm} (4)

We assume that the new phase is detected when an amount $n_0$ has been formed. Then we have

$$t_i = \frac{1}{B} \ln \left( \frac{Bn_0}{A} + 1 \right)$$  \hspace{1cm} (5)

With the same value of $n_0$ in all cases, eqn. 5 expresses the dependence of $t_i$ on $A$ and $B$. For small values of $n_0$, $t_i$ depends mainly on $A$ ($t_i \approx n_0/A$), otherwise.

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B is decisive. From the simple relationship between \( t_i \) and \([\text{PO}_4^{3-}]\) it follows that both \( A \) and \( B \) are proportional to the square of phosphate ion concentration. Hence

\[
t_i = \frac{1}{b[\text{PO}_4^{3-}]^2} \ln \left( \frac{bn_0}{a} + 1 \right) = k'_2[\text{PO}_4^{3-}]^2
\]

(6)

It should be emphasized that even if the right side of (3) were of the form \( A + Bn^m \) with \( m < 1 \), we should arrive at an expression analogous to (6). A remarkable feature is, that no explicit dependence on calcium or monohydrogen phosphate ion concentration is reflected in the data plotted in Fig. 3.

A plausible model for the course of processes during the incubation period is, with the results of Ref. 12 in mind, an epitaxial overgrowth of a \( \text{Ca}_3(\text{PO}_4)_2 \) phase on the brushite crystals. The nucleation itself may be a reaction of the type

\[
3 \text{Ca}^{2+} + 2 \text{PO}_4^{3-} \longrightarrow \text{Ca}_3(\text{PO}_4)_2
\]

occurring either anywhere on the surface or — more probably — at definite active sites, which may be the same as the growth sites of the mother substance. The most probable partial processes are

I. \( \text{Ca}^{2+} + \text{PO}_4^{3-} \longrightarrow \text{CaPO}_4^- \)

II. \( \text{CaPO}_4^- + \text{Ca}^{2+} \longrightarrow \text{Ca}_3\text{PO}_4^+ \)

III. \( \text{Ca}_3\text{PO}_4^+ + \text{PO}_4^{3-} \longrightarrow \text{Ca}_3(\text{PO}_4)_2\text{PO}_4^- \)

IV. \( \text{Ca}_3(\text{PO}_4)_2\text{PO}_4^- + \text{Ca}^{2+} \longrightarrow \text{Ca}_3(\text{PO}_4)_2 \)

I is energetically favoured and therefore fast. There is a steady-state surface concentration of \( \text{CaPO}_4^- \)-complexes given by

\[
\Gamma(\text{CaPO}_4^-) = K_1 \Gamma(\text{Ca}^{2+}) \Gamma(\text{PO}_4^{3-})
\]

where \( \Gamma \) denotes surface concentration in the adsorbed layer. As the solution is saturated with respect to brushite, \( \Gamma(\text{Ca}^{2+}) \) is equal to the constant saturation value. Reaction II may be expected to have a lower rate constant than I, but the reaction is favoured by the high calcium concentration. Again, there is a steady-state surface concentration

\[
\Gamma(\text{Ca}_3\text{PO}_4^+) = K_{II} \Gamma(\text{CaPO}_4^-) \Gamma(\text{Ca}^{2+}) = K_{II} K_1 \Gamma(\text{Ca}^{2+}) \Gamma(\text{PO}_4^{3-})
\]

III is the rate-determining process. The rate expression is

\[
J = k_{III} \Gamma(\text{Ca}_3\text{PO}_4^+) \Gamma(\text{PO}_4^{3-}) = k_{III} K_{II} K_1 \Gamma(\text{Ca}^{2+}) \Gamma(\text{PO}_4^{3-})^2
\]

(7)

Finally, reaction IV is fast, because it is favoured by energetics and reactant concentration. The phosphate concentration is so small that we may write

\[
\Gamma(\text{PO}_4^{3-}) = K_{ads}[\text{PO}_4^{3-}]
\]

The resultant rate is

\[
J = k'''[\text{PO}_4^{3-}]^2
\]

(8)

with $k'' = K_{ads}k_{III}K_{II}K_{I}(Ca^{2+})^2$. An analogous expression applies, according to Walton,\textsuperscript{25} for further growth of the primary germs, because an energy minimum is reached any time an electrically neutral unit is completed. $k''$ is, of course, greater for growth than for nucleation. The two values of $J$ are equal to $A$ and $B$, respectively, in eqns. 3–5. The agreement of this model with the observed kinetics is obvious.

On further growth, HPO$_4^{2-}$ ions are deposited on the germs, and the primary phase is gradually transformed into crystalline tetracalcium monohydrogen phosphate. The phosphate ion concentration is still rate-determining, and when growth is slow, there is high probability for redissolution of germs. This is in accordance with the observed dependence of crystal size on initial phosphoric acid concentration.

In the present treatment, complex formation between calcium and phosphate in solution is neglected. As far as the nucleation mechanism proposed above is concerned, it is immaterial whether the CaPO$_4^-$-complexes are adsorbed as such from the solution or formed by a reaction in the adsorbed layer, if their concentration is low. The solubility products used for the calculations of phosphate ion concentrations have been determined on the assumption that no complexes are formed. The values of the rate and equilibrium constants are altered, if complexing is accounted for, but not the general features.

CONCLUSION

The pattern of heterogeneous nucleation of tetracalcium monohydrogen phosphate on brushite reflects an aspect of nucleation kinetics different from the classical picture as encountered among the alkali halides and most other simple salts of strong acids. Recent books on crystallization kinetics\textsuperscript{22,24,25} treat the mechanism of nucleation in terms of phase boundary properties. Nielsen\textsuperscript{26} considers the surface energy as the important factor not only in nucleation, but even in crystal growth at moderate supersaturation. The different behaviour of a calcium phosphate system is due to the fact that the solution is highly incongruent with respect to the precipitating ions. The critical nucleus is small and has a constant free energy of formation as a consequence of very high supersaturation. It is therefore not the degree of supersaturation, which is of major importance, but the concentration of the ion present in least amount.

The nucleation reaction of Ca$_4$H(PO$_4$)$_3$ is shown to be of second order with respect to phosphate ion concentration, and this is consistent with a mechanism of four consecutive bimolecular reactions between oppositely charged ion species ending up with an 'electrically neutral "molecule"'. The accordance of this mechanism with the kinetics of crystal growth of ionic salts from solution suggests that the nucleation reaction might be considered as epitaxial growth of the new phase from the growth sites of the substrate. As precipitation has the nature of an autocatalytic process, an incubation time will be observed if both nucleation and growth are sufficiently slow. The dependence of the incubation time on ion concentration is closely connected with the mechanism of the primary precipitation reaction.

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