

Heterogeneous Nucleation of Calcium Phosphates. II. Inhibition by Cupric Ions

H. E. LUNDAGER MADSEN

Department of Inorganic Chemistry, Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Copenhagen V, Denmark

Inhibition of nucleation of $\text{Ca}_4\text{H}(\text{PO}_4)_3$ on $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ by Cu^{2+} has been studied. At low concentrations of copper a slight increase in incubation time and a slight decrease in precipitation rate was observed, whereas at higher concentrations the rate of precipitation was initially very low; after a lapse of time which increased with increasing $[\text{Cu}^{2+}]$ the rate increased strongly. The observations are explained in terms of sorption of Cu^{2+} in the adsorbed and surface layers of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ and by the subsequent transfer of Cu^{2+} to the interior of the crystals. There was no indication of a direct interference of Cu^{2+} with the crystal growth of $\text{Ca}_4\text{H}(\text{PO}_4)_3$.

In a previous paper¹ investigations on the hydrolysis of brushite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) in pure water or dilute phosphoric acid were reported. The process which takes place under the existing conditions is



The crystallization of tetracalcium monohydrogen phosphate is accompanied by a pH drop from about 7 to below 6. The rate of crystallization was shown to depend strongly on pH of the initial solution saturated with respect to brushite.

It has long been known that this process is strongly sensitive to trace amounts of other substances, particularly copper.² For this reason, water of high purity, preferably glass-distilled, has to be used for the experiments.

In the present paper is reported a detailed examination of the influence of cupric ions on the heterogeneous nucleation of basic calcium phosphates on secondary calcium phosphate as substrate.

EXPERIMENTAL

In the following, only new procedures and changes from those applied in the previously reported investigation are described.

Apparatus. For pH measurements a Radiometer digital pH meter type 52 was used. Calibration was carried out using two standard buffers, 0.05 m potassium hydrogen phthalate and 0.025 m $\text{KH}_2\text{PO}_4 + 0.025$ m $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$. For pCu measurements at concentrations above 10^{-5} M a Radiometer ion-selective electrode type F 1112 Cu (sensing element: copper selenide single crystal) and a mercurous sulfate reference electrode were used. In this case, calibration was performed using various standard cupric nitrate solutions, which were 0.1 M with respect to potassium nitrate, as were the samples; in this way a constant liquid-junction potential was ensured.³

Precipitates were examined by X-ray powder diffraction using a General Electric diffractometer equipped with a Cu-anode X-ray tube and a scintillation counter, and by infrared spectrometry using a Perkin-Elmer spectrophotometer model 457. Samples for IR spectrometry were prepared as nujol mulls, and KBr windows were used.

Radioactive samples were counted on a Frieske and Hoepfner counting equipment type 49 B using a well-type scintillation counter.

Materials. Stock solutions of $\text{Cu}(\text{NO}_3)_2$ were standardized by adding an excess of solid potassium iodide to an acidified sample and titrating the iodine liberated with sodium thio-sulfate, which had previously been standardized against potassium iodate.

Radioactive copper (^{64}Cu) of high specific activity was obtained by a Szilard-Chalmers process.⁴ A sample of copper-phthalocyanine (α form) was irradiated with thermal neutrons in the DR 2 reactor at Risø. The activity of the sample after irradiation was 20 mCi per g Cu-phthalocyanine. 50 mg was dissolved in 1 ml hot (80–100 °C) concentrated H_2SO_4 .

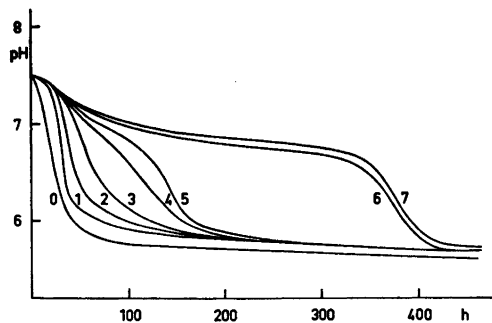


Fig. 1. pH variation as a function of time. Numbers on curves indicate cupric ion concentration in μM .

Subsequent dilution with 10 ml water caused complete precipitation of the Cu-phthalocyanine, which was filtered off. The filtrate, which contained most of the radioactive copper, was electrolyzed between platinum electrodes, until the solution was no longer radioactive. The cathode was rinsed in water and the copper dissolved in nitric acid, which was then evaporated under a heat lamp; the residue was finally dissolved in water, and this solution was used in the experiments. $[\text{Cu}^{2+}]$ was determined by solvent extraction of the diethyl-dithiocarbamate complex followed by spectrophotometry.⁵ A specific activity of 36 Ci per g Cu was found, corresponding to an enrichment factor of 230. The retention was 11 %.

RESULTS

Unless otherwise stated, the experiments were carried out as described in the previous paper.¹ The initial solid/solution ratio was always 2 g to 1 litre.

Experiments with addition of calcium nitrate up to a concentration of 1 mM showed that the nitrate ion had no effect. When the initial phosphoric acid concentration was too low, no appreciable inhibition by cupric ions in the concentration range of interest was observed. For this reason an initial phosphoric acid concentration of 10^{-4} M was chosen in most of the experiments.

Fig. 1 shows the results of a series of experiments with $[\text{Cu}^{2+}]$ ranging from 0 to 7×10^{-6} M. The limiting value of pH as $t \rightarrow \infty$ was about 5.6 which corresponds to saturation with respect to $\text{Ca}_4\text{H}(\text{PO}_4)_3$. The lapse of time before the large pH drop showed variations up to ~ 20 %, but the same regular pattern was observed in all

series of experiments with the pH drop always occurring in the order of increasing $[\text{Cu}^{2+}]$. A cupric ion concentration of about $4 \mu\text{M}$ appeared to be critical; below this, the incubation time was increased somewhat by the copper, and the rate of the process was altogether a little lower, whereas at higher concentrations there was a considerable delay. In the latter case, several stages of the process could be observed; first the initial incubation period, where pH was about 7.5; then a drop to about pH 7.0, followed by a very slow, but steady decrease of pH; finally, pH dropped to < 6 within a day or two. The time at which this occurred depended strongly on $[\text{Cu}^{2+}]$. The initial incubation time was the same at all cupric ion concentrations $\geq 2 \mu\text{M}$ within the same series of experiments, and above the critical concentration the rates of both the slow pH decrease and the final drop were virtually independent of $[\text{Cu}^{2+}]$.

Table 1 shows the results of a series of measurements of the second incubation time. The first incubation time was in this case about 20 h.

By X-ray diffractometry it was shown that the product of hydrolysis was a mixture of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ and $\text{Ca}_4\text{H}(\text{PO}_4)_3$, as was expected. In the microscope the crystals of $\text{Ca}_4\text{H}(\text{PO}_4)_3$ appeared as the characteristic spherulites, and they were particularly well developed when precipitated at high Cu concentration. Before the final pH drop only brushite could be detected, even in an experiment with a cupric ion concentration as high as 1 mM. In the latter case the brushite had a distinct blue-green colour, and the lines in the diffractogram were slightly displaced, but changes of unit-cell dimensions did not exceed 0.01 \AA in any direction; there was no change in the IR spectrum. When brushite was boiled with reflux for 4 h in a 2 mM cupric nitrate solution it was transformed mainly to monetite (anhydrous CaHPO_4); a small amount of $\text{Ca}_4\text{H}(\text{PO}_4)_3$ was also present. The monetite was

Table 1.

$[\text{Cu}^{2+}]$, μM	4.5	5.0	5.5
t_{i2} , h	87	151	213
	6.0	6.5	7.0
	248	257	297
			407

blue-green, whereas the other compound was colourless. With pure water instead of cupric nitrate solution brushite is transformed completely to hydroxylapatite, $\text{Ca}_5(\text{PO}_4)_3\text{OH}$, presumably *via* $\text{Ca}_4\text{H}(\text{PO}_4)_3$, after a short time of boiling.

The reproducibility of results from experiments with radioactive copper was rather poor. Apparently, single-layer absorption corresponded to 0.6 ± 0.1 mmol Cu per kg $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$; that is 1.2 ± 0.2 μmol from 1 litre of solution with 2 g solid. At a Cu^{2+} concentration of about 5 μM the amount in the solid phase increased strongly. This amount apparently depended on pH, although no regularity could be found, except that it increased with increasing pH.

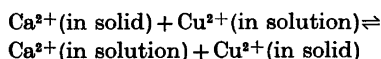
Finally, an experiment was carried out in which brushite was added to a 1 mM solution of $\text{Cu}(\text{NO}_3)_2$. The suspension was stirred constantly, and $a(\text{Cu}^{2+})$ was monitored potentiometrically and was found to decrease by a factor of 100 within half an hour. When a similar experiment was carried out with tetracalcium monohydrogen phosphate instead of brushite no decrease in cupric ion activity could be measured, even after nearly 20 h.

DISCUSSION

It is obvious that precipitation of tetracalcium monohydrogen phosphate under the circumstances studied requires the presence of brushite as a substrate for nucleation; there is no evidence for homogeneous nucleation. The inhibiting action of cupric ions is due to their adsorption on the surface of the brushite crystals or to exchange of calcium and copper in the outermost atomic layers. The effect is a delay of crystallization of $\text{Ca}_4\text{H}(\text{PO}_4)_3$. No foreign solid phase could ever be detected, so it is reasonable to assume that only brushite and tetracalcium monohydrogen phosphate occur in the system. At 100 °C brushite is transformed to Cu-containing monetite; there is still a strong inhibition of precipitation of $\text{Ca}_4\text{H}(\text{PO}_4)_3$.

Brushite is seen to have a high affinity for Cu^{2+} , whereas there is no sign of cupric ion uptake by $\text{Ca}_4\text{H}(\text{PO}_4)_3$. It was hoped that the experiments with radioactive copper would reveal the details of copper uptake, but, as stated above, the results were subject to high

uncertainty, presumably due to the difficulty of avoiding contamination by decomposition products of phthalocyanine; these may well be highly active surfactants. The pH dependence may be explained by an exchange equilibrium of the kind



because the calcium ion concentration in solution decreases as pH increases. However, it was not possible to fit the experimental data to any particular hypothesis.

As a fast precipitation of $\text{Ca}_4\text{H}(\text{PO}_4)_3$ eventually sets in even at the highest Cu^{2+} concentration the cupric ions must somehow disappear from the surface of the brushite crystals. The only place where they can go is the interior of the crystals, and it may happen either by recrystallization or by solid-state diffusion. That the latter process is just as plausible as the former may be realized by the following argument. We assume that the decrease of surface concentration of copper due to inward diffusion follows an exponential rate law:

$$\Gamma = \Gamma_0 \exp(-t/\tau)$$

At a definite value of Γ , Γ^* , the copper in the surface, can no longer prevent nucleation of $\text{Ca}_4\text{H}(\text{PO}_4)_3$; the value Γ^* is reached after the incubation time t_i :

$$\Gamma^* = \Gamma_0 \exp(-t_i/\tau)$$

In this equation Γ_0 is the independent and t_i the dependent variable, whereas Γ^* and τ are constants. Taking the logarithm on both sides, we get

$$t_i = \tau(\ln \Gamma_0 - \ln \Gamma^*)$$

Assuming that no copper is left in solution we may substitute the total amount of copper added for Γ_0 , to which the former is proportional. Instead of Γ^* we then find the critical copper concentration c^* mentioned in the preceding section. A least-squares fit of the data in Table 1 yields

$$c^* = 3.8 \mu\text{M} \\ \tau = 543 \text{ h} = 1.95 \times 10^6 \text{ s}$$

The value of c^* agrees well with experiments. In order to see whether the value of τ is con-

sistent with the diffusion hypothesis, we make use of the equation ⁶

$$1/\tau = \nu_0 \exp(-E_a/RT)$$

where ν_0 is the vibration frequency of positive ions in the crystal, and E_a is the activation energy for diffusion. With $\nu_0 = 3 \times 10^{12} \text{ s}^{-1}$ (corresponding to a wavenumber of 100 cm^{-1}) we get $E_a = 111 \text{ kJ/mol}$

No other determinations of this quantity have been carried out, but the order of magnitude is the same as for alkali halides and related ionic solids.⁶

In the rest of our discussion it will be advantageous to consider three different ranges of Cu^{2+} concentrations: (1) $0 < [\text{Cu}^{2+}] < 2 \mu\text{M}$, (2) $2 \mu\text{M} \leq [\text{Cu}^{2+}] < 4 \mu\text{M}$, and (3) $[\text{Cu}^{2+}] \geq 4 \mu\text{M}$. In range 3 the precipitation of $\text{Ca}_4\text{H}(\text{PO}_4)_3$ takes place in two stages, the slow and the fast stage. The rate of precipitation in range 2 is almost equal to the rate at the fast stage in range 3. If we call this rate v_1 , the rate at the slow stage in range 3, v_2 , and the rate when $[\text{Cu}^{2+}] = 0$, v_0 , the following approximate ratio of the three rates is found

$$v_0:v_1:v_2 = 1:0.45:0.0105$$

The rate of precipitation is presumably nearly proportional to the rate of nucleation. Hence the activation energy of nucleation is increased by the presence of copper (assuming the same frequency factor) by an amount, which in range 2 is equal to $-RT \ln 0.45 \approx 2.1 \text{ kJ/mol}$ and in range 3, slow stage, equal to $-RT \ln 0.0105 \approx 11.7 \text{ kJ/mol}$.

In the previous paper¹ the nucleation mechanism was postulated to be composed of the four elementary reactions occurring at the

- I. $\text{Ca}^{2+} + \text{PO}_4^{3-} \rightarrow \text{CaPO}_4^-$
- II. $\text{CaPO}_4^- + \text{Ca}^{2+} \rightarrow \text{Ca}_2\text{PO}_4^+$
- III. $\text{Ca}_2\text{PO}_4^+ + \text{PO}_4^{3-} \rightarrow \text{Ca}_2(\text{PO}_4)_2^{2-}$
- IV. $\text{Ca}_2(\text{PO}_4)_2^{2-} + \text{Ca}^{2+} \rightarrow \text{Ca}_3(\text{PO}_4)_2$

surface of the brushite crystals, and with III as the rate-determining step. The encounter of calcium and phosphate ions presumably takes place in the mobile adsorbed layer, but the stationary surface layer of the crystal plays an important role in stabilization of the nucleus. In range 2 calcium ions in the adsorbed layer are displaced by cupric ions; before nucleation

can take place, this exchange must be reversed, and the activation energy for exchange of ions between solution and the adsorbed layer—presumably the desorption energy of Cu^{2+} —must be added to the activation energy of nucleation to obtain the activation energy of the overall process. Hence we ascribe the value 2.1 kJ to the molar desorption energy of Cu^{2+} . In range 3 the calcium ions are also displaced from the surface layer, so that the stabilizing effect of this layer is missing. Hence an ion exchange in this layer must take place prior to nucleation, and the activation energy for exchange in this layer is considerably higher than in the adsorbed layer; we ascribe the value of 11.7 kJ/mol to this second activation energy or, maybe more correct, this value is equal to the sum of the two activation energies. These assignments are consistent with the theory of Giddings and Eyring for multibarrier kinetics.⁷

CONCLUSION

When a copper salt is added to a suspension of brushite in dilute phosphoric acid, the cupric ions are taken up by the brushite crystals, at low concentration only in the adsorbed layer, at higher concentrations also in the surface layer. As copper cannot replace calcium in $\text{Ca}_4\text{H}(\text{PO}_4)_3$, the nucleation of this compound is thereby hindered. Ion exchange in the adsorbed layer is relatively fast, and at low concentrations of copper only a slight inhibition is observed. Copper in the surface layer disappears by exchange with calcium either in solution or in the interior of the crystal; the latter is achieved by recrystallization or by solid-state diffusion. Exchange in the surface layer is much slower than in the adsorbed layer, so nucleation is slowed down considerably in this case. Eventually the inward transport of Cu^{2+} ions brings their surface concentration down to a level where nucleation of $\text{Ca}_4\text{H}(\text{PO}_4)_3$ is possible without prior exchange in the surface layer; then an increase in nucleation rate sets in.

Acknowledgements. The author wishes to thank Professor A. Tovborg Jensen for his interest and valuable advice. The equipment for radioactivity measurements has been purchased on a grant from the Carlsberg Foundation.

REFERENCES

1. Madsen, H. E. L. *Acta Chem. Scand.* 24 (1970) 1677.
2. Rowles, S. R., quoted in: Bjerrum, N. *Kgl. Dan. Vidensk. Selsk. Mat.-Fys. Medd.* 31 (1958) No. 7.
3. Madsen, H. E. L., Hansen, B. S. and Olafsson, S. A. *Kgl. Vet.- og Landbohøjsk. Årsskr.* (1974) 29.
4. Sharp, R. A., Schmitt, R. A., Suffredini, C. S. and Randolph, D. F. *USAEC report GA-910* (1959).
5. Henriksen, A. *Tidsskr. Planteavl* 61 (1957) 685; *Arbejdsmetoder III, Fælles metoder til jordbundsanalyser*, Copenhagen 1966.
6. Frenkel, J. *Kinetic Theory of Liquids*, Oxford 1946, Chapter 1.
7. Giddings, J. C. and Eyring, H. *J. Phys. Chem.* 62 (1958) 305.

Received September 12, 1974.