Precipitation of Calcium Phosphates
from Electrolyte Solutions

II. The Formation and Transformation of the Precipitates *

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The kinetics of the formation and transformation of calcium phosphate precipitates obtained by mixing equal volumes of solutions, 6×10^{-3} M in total calcium and/or total phosphate was investigated at 25°. The phosphate solutions were preadjusted to pH 7.4. Changes of the pH and turbidity of the solutions were followed simultaneously as a function of time. Precipitates were isolated at various time intervals and characterized by different physico-chemical methods. Initially a precipitate with a molar Ca/P ratio of 1.5, amorphous to X-ray and electron diffraction was formed. IR spectra indicated the presence of PO_4^{3-} and HPO_4^{2-} ions. After a period of metastability, precipitation of a crystalline material within or upon the amorphous matter occurred. Twenty four hours after sample preparation the precipitates showed mainly the characteristics of octacalcium phosphate.

Key words: Calcium — Phosphate — Precipitation — Kinetics — Chemistry.

La cinétique de la formation et de la transformation des précipités de phosphate de calcium, obtenus en mélangeant de volumes égaux de solutions à 6×10^{-3} M de calcium total et/ou phosphate total est étudiée à 25° C. Les solutions de phosphate sont préajustées à un pH de 7.4. Les changements de pH et de turbidité des solutions sont suivis simultanément en fonction du temps. Les précipités sont isolés à des intervalles de temps variables et caractérisés par diverses méthodes physico-chimiques. Initialement un précipité avec un rapport molaire Ca/P de 1.5, amorphe aux rayons X et en diffraction électronique, est formé. Le spectre IR indique la présence de PO_4^{3-} et de HPO_4^{2-}. Après une période métastable, on observe la précipitation d'un matériel cristallin dans ou sur la phase amorphe. Vingt quatre heures après préparation de l'échantillon les précipités présentent surtout les caractères du phosphate octocalcique.

Die Kinetik der Bildung und Transformation von Calciumphosphat-Niederschlägen wurde bei 25° C untersucht. Es wurden dazu gleiche Volumen von Lösungen gemischt, bei einer Konzentration von 6×10^{-3} M totales Calcium und/oder totales Phosphat. Die Phosphat-lösungen wurden zuerst auf pH 7,4 eingestellt. Veränderungen des pH und Trübung der Lösungen wurden gleichzeitig als eine Funktion der Zeit aufgezeichnet. Niederschläge wurden in verschiedenen Zeitintervallen isoliert und mit verschiedenen physiko-chemischen Methoden charakterisiert. Am Anfang wurde ein Niederschlag mit einem molaren Ca/P-Verhältnis von 1,5, im Röntgenbild und in der Elektronendiffraktion amorph, gebildet. Infrarotspektren deuteten die Anwesenheit von PO_4^{3-}- und HPO_4^{2-}-Ionen an. Nach einer metastabilen Periode erfolgte ein Niederschlag aus kristallinem Material innerhalb oder auf der amorphen Substanz. 24 Std nach der Herstellung der Proben zeigten die Niederschläge in der Hauptsache die Charakteristiken von Octacalciumphosphat.

Introduction

There is considerable evidence [10–12, 17, 18, 35], that under conditions of rapid precipitation from neutral or basic solutions calcium phosphate first separates as a cryptocrystalline material ("amorphous" calcium phosphate, ACP) which is transformed into crystalline calcium phosphate in contact with the mother liquor. The chemical nature of the first precipitate and the mechanism of the conversion process is still the subject of some controversy [10, 14, 17]. It is indicated [1, 18, 19] that different results may be obtained under different experimental conditions. In a previous communication [18] the range of solution compositions resulting in the formation of octacalcium phosphate (OCP) via an amorphous precursor was defined by a precipitation diagram at 25°. The purpose of this paper is to present, in some detail, results pertaining to the mechanism of this transformation.

Methods

All experiments were carried out at 25°. Equal volumes of solutions of sodium phosphate ([NaOH]/[H_3PO_4] molar ratio approx. 1.75, pH 7.4) and calcium chloride were mixed [18, 33] to obtain a final concentration of 3×10^{-3} M in total phosphate and calcium at zero time. Changes of the pH and relative turbidity were then continuously recorded as a function of time. A Radiometer Model 26 pH meter in connection with a Hewlett-Packard Moseley 7100 BM recorder and a Zeiss recording turbidimeter (Spekol) were used. Turbidities were measured at 540 mμ against a 6.28×10^{-5} g/ml suspension of latex LS-54-2 ($R_{90°} = 0.040$ cm^{-1} at 546 mμ). Particle charge was determined by microelectrophoresis. For electron microscopy and electron diffraction the suspension was dropped on a copper grid covered with a Movital membrane, the supernatant was immediately removed and the sample placed in a vacuum desiccator. Samples for analysis were collected by thorough vacuum packing on Millipore filters and placed over silicagel in a vacuum desiccator. Separation was completed within 1–2 min. X-ray amorphous precipitates could only occasionally be obtained by this technique; in most cases precipitates crystallized during the drying process. In order to avoid this difficulty, in another set of experiments samples were frozen immediately after separation and the moisture was evaporated under high vacuum. X-ray amorphous precipitates thus prepared yielded the same infrared (IR) spectra as did X-ray amorphous precipitates obtained by the first technique. IR spectra were taken from KBr pellets in the range of 4000—400 cm^{-1} using a Perkin Elmer Model 221 spectrophotometer. Electron microscopes KM4-Trüb, Täuber & Co. and Elmiskop I, Siemens were used. X-ray diffraction powder patterns, refractive indices and chemical analyses were obtained as previously [18] described, except that calcium was determined by atomic absorption.

Results

In Fig. 1 a typical pH and turbidity curve is represented. Electron micrographs of the precipitates obtained at different time intervals and the corresponding X-ray diffraction powder patterns are shown in Fig. 2, IR spectra are given in Fig. 3 while the results of chemical and X-ray diffraction analyses and refractive index determinations are listed in Tables 1 and 2. It is evident that precipitation proceeds in two distinctly separate stages, the onset of the second stage being indicated by a discontinuous drop in pH and a turbidity maximum (Fig. 1). The amount of time which elapsed before the onset of the second stage varied from experiment to experiment, but reproducibility was within $\pm 12\%$.

The initial precipitate was rapidly formed and consisted of nearly isoelectric colloid particles amorphous to X-ray and electron diffraction. Electron micro-
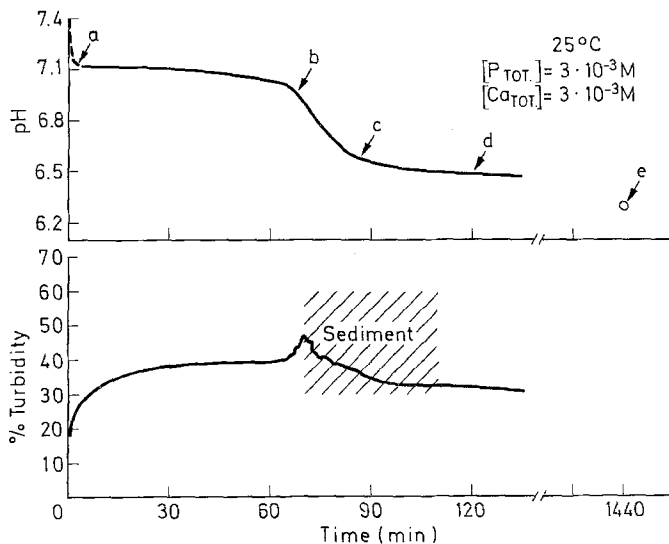
Fig. 1. Changes of pH and turbidity as a function of time at 25° when sodium phosphate (preadjusted to pH 7.4) and calcium chloride are mixed to give a concentration of 3×10^{-3} M in both at zero time

Table 1. Calcium/phosphorus molar ratios and refractive indices $\bar{n}_D^{25°}$ of precipitates isolated at various time intervals compared with literature data

Time t of separation	pH at time t	Molar Ca/P	$\bar{n}_D^{25°}$	
<2 min	7.13	1.50 ± 0.03[a]	—[b]	
120 min	6.48	1.35	1.577	
150 min	6.44	1.35	1.577	
24 h	6.27	1.35[c]	1.578	
14 days	6.02	1.49	1.591	
4 weeks	5.83	1.50	1.593	
Literature data				
OCP [5]		1.33	n_α	1.576
			n_β	1.583
			n_γ	1.585
HA [5]		1.67	n_ε	1.640
			n_ω	1.646
DA [26]		1.33–1.67	1.582–1.645	

[a] Average of 15 determinations, 95% confidence level of the t distribution test.
[b] Refractive indices of ACP are not listed since these values are highly dependent on the degree of drying.
[c] Average of 3 determinations.

graphs (Fig. 2a) show chain-like agglomerates of round particles approx. 0.1 μ in diameter, similar to those described by Weber *et al.* (1967). The material was soft and decomposed easily under the electron beam. IR spectra of this material (Curve 1 in Fig. 3) are similar to those of amorphous calcium phosphate, obtained
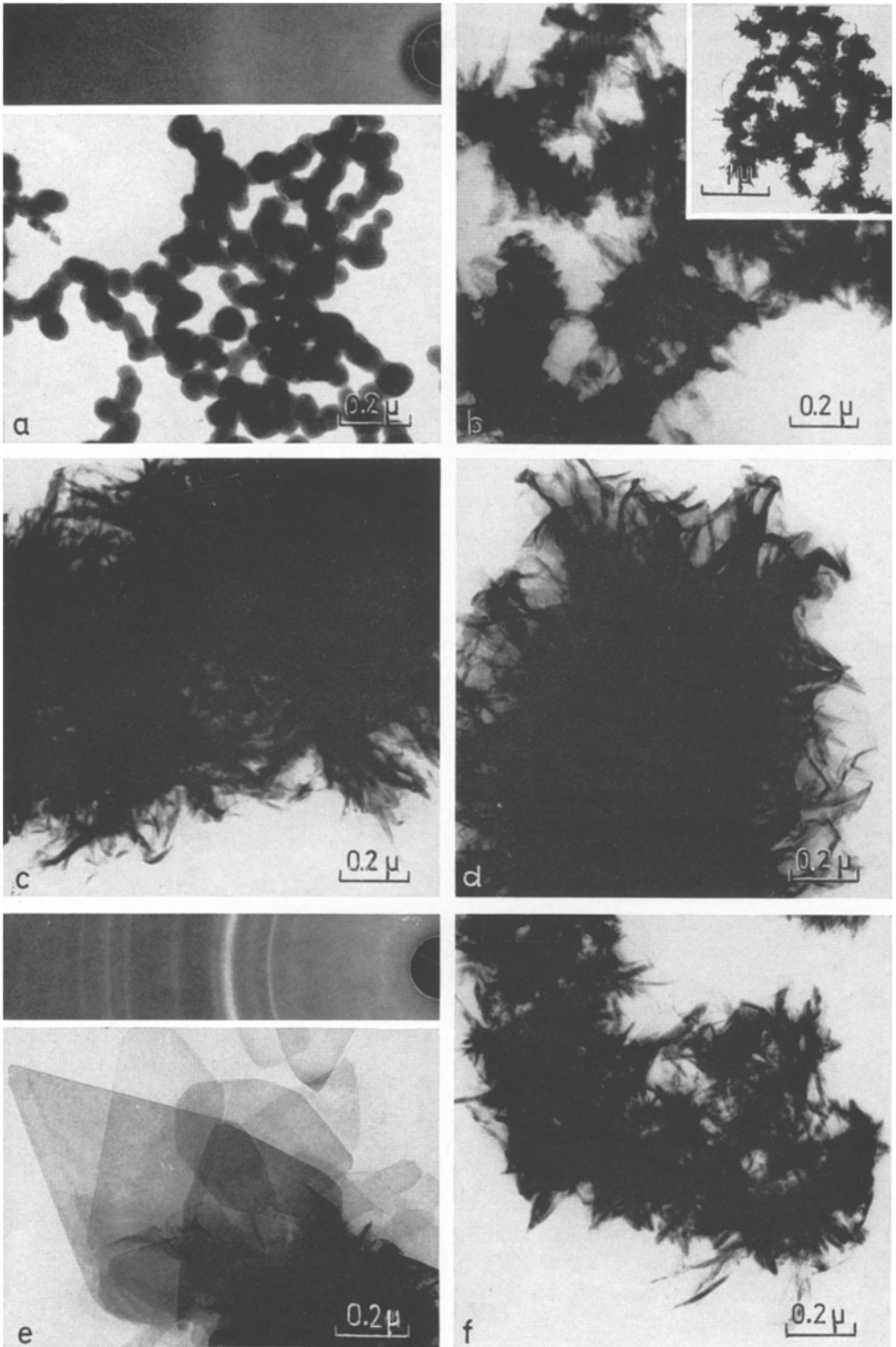
Fig. 2 a—f. Electron micrographs and X-ray diffraction powder patterns (a, e) of precipitates isolated at time intervals a—e, denoted on the pH curve in Fig. 1. f Electron micrograph of a sample isolated 4 weeks after preparation
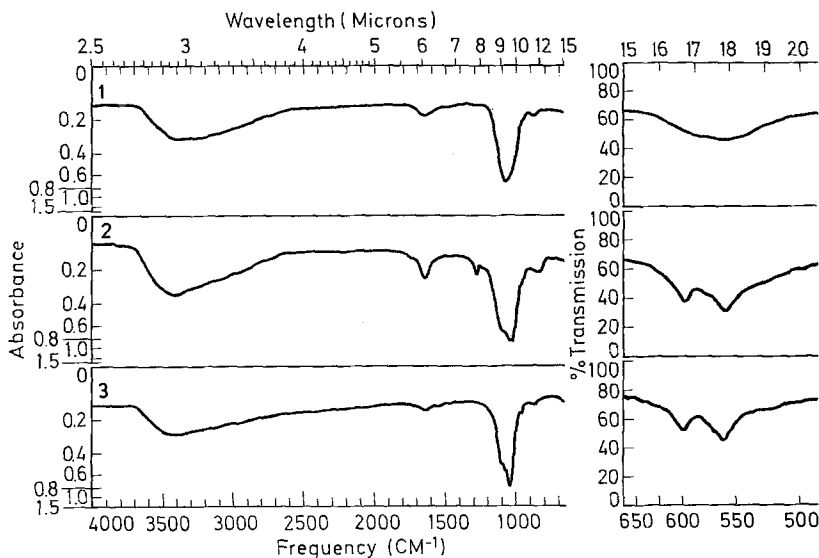
Fig. 3. IR spectra of an amorphous (1) and a crystalline (2) precipitate both isolated immediately after sample preparation; the latter crystallized during drying. The IR spectrum of a sample which was aged for 24 h is shown at (3)

by Stutman et al. (1965). However, in addition to the absorption bands listed by these authors, the spectra represented by Curve 1 in Fig. 3 exhibit a band at approx. 870 cm^{-1}, which was assigned to the P—(OH) stretching frequency (after ref. [2, 8, 9]). The H—O—H bending mode is shifted towards higher frequencies (1 640 cm^{-1}) indicating bound water. Precipitates separated less than 2 min after mixing and crystallized during drying exhibited both the P—(OH) stretching (~855 cm^{-1}) and O...H—O bending (1 280 cm^{-1}) mode (Curve 2 in Fig. 3). Both bands were better defined than in samples which had been in contact with the mother liquor for 24 h (Curve 3 in Fig. 3). X-ray spectra of the immediately isolated and crystallized material (Column 1 in Table 2) show primarily d-values characteristic for OCP.

Considering the electron micrographs (Fig. 2b–d, note the insert in Fig. 2b) and X-ray diffraction powder patterns it seems that during the second stage a crystalline precipitate was formed within and/or upon the amorphous material. This was more acidic than the precursor (Table 1). Thus, hydrolysis being excluded, the drop in pH accompanying this change (Fig. 1) indicates rapid additional precipitation [18]. Apart from this discontinuity, continuous separation of material throughout the whole reaction period is indicated (compare also the first part of the turbidity curve). Corresponding changes of solution calcium and phosphorus have also been observed [15].

Precipitates isolated 24 h after preparation exhibited mainly the characteristics of OCP; molar Ca/P ratios, refractive indices (Table 1) and X-ray diffraction powder patterns were in good agreement with the corresponding literature data (compare refs. 3 and 27 for d-spacings of OCP). In some of the samples

Table 2. d-spacings [Å] and relative intensities [I] of reflections obtained from X-ray diffraction powder patterns of precipitates which were isolated at different time intervals

Aging time <2 min[a]		Aging time 24 h		Aging time 4 weeks	
d	I	d	I	d	I
		18.26	3		
9.27	3	9.29	3		
		(8.34)[b]	1	8.24	2
5.53	1	5.49	2	5.38	1
		5.10	1		
		4.44	1		
				4.09	1
				3.96	1
3.90	1	3.89	1		
3.66	1	3.65	1		
3.42	4	3.43	4	3.43	4
3.15	1	3.16	2	3.17	1
				3.07	1
2.841	5	2.811	5	2.802	5
2.773	2	(2.717)[b]	1	2.724	3
		2.658	2	2.635	2
		2.280	1	2.281	1
		2.166	1	2.155	1
		2.074	1	2.071	1
1.952	2	1.951	2	1.952	2
		1.893	1	1.892	1
1.842	2	1.834	3	1.846	3
				1.760	1
1.712	1	1.718	2	1.720	2

[a] Precipitates isolated immediately after sample preparation. Crystallization occurred during drying.
[b] Lines in brackets were obtained only occasionally.

weak reflections characteristic of HA were also obtained (Table 2) indicating slight hydrolysis of the precipitates. After longer periods of time further hydrolysis into calcium-deficient apatites (DA) was observed (Fig. 2f, Tables 1, 2).

Discussion

The above results are in general agreement with other authors [11, 16, 17] who showed that the conversion of amorphous into crystalline calcium phosphate is a discontinuous process. It seems that if prepared under the above experimental conditions, ACP contains both PO_4^{3-} and HPO_4^{2-} ions. This follows from its IR spectrum (Curve 1 in Fig. 3) and from the fact that crystallization of this material isolated from the mother liquor yielded OCP and/or DA (Table 2, Curve 2 in Fig. 3). Other workers have reported amorphous calcium phosphates with molar Ca/P ratios below 1.33 [17, 30] as well as amorphous tricalcium phosphate [14]; all were precipitated under different experimental conditions. The apparent disagreement between all these results

can be easily explained in terms of the hypothesis of Holmes and Beebe (1971) proposing that the spherical particles of ACP (Fig. 2a) are agglomerates consisting of hydrated calcium and phosphate ions with little or no three-dimensional order. In accord with this hypothesis, the IR spectrum of ACP (Curve 1 in Fig. 3, ref. [31]), shows no splitting of the $P=0$ stretching and bending modes and exhibits the bands of hydration water. It is quite conceivable, then, that the composition of the precursor phase depends on the composition of the environment (concentrations of calcium and phosphate species, the latter depending on the concentrations of total phosphate, calcium and pH) at the time of precipitate formation.

It has been proposed that the conversion of amorphous into crystalline calcium phosphate proceeds by ACP surface catalysis [7] and/or as an independent autocatalytic process, following dissolution of the precursor [10]. The latter concept does not fit our experimental results, since dissolution should cause the pH to rise. Moreover, our electron micrographs (Fig. 2b–d) indicate a definite connection between the precursor and the secondary precipitate, which makes ACP appear as a template to OCP nucleation. Crystals are most probably formed initially by deposition of new material from solution, while in the later stages both crystal growth and perfection (probably by aging processes such as recrystallization, Ostwald ripening etc.) are encountered. The length of the induction period, preceeding secondary precipitation depends on the experimental conditions [32]. It is possible that during this period rearrangements (loss of excess hydration water, crystal ordering) are taking place which make ACP a suitable template for nucleation.

The formation of OCP via an amorphous precursor, although not described earlier, is thermodynamically and kinetically reasonable at the given temperature and pH range, provided the reactant concentrations are within the range previously [18] described. However, until more is known about the composition of the microenvironment at the calcification site it will be difficult to asses the physiological significance of the above findings. It is questionable, whether the ionic concentrations in this environment are comparable to those of the blood serum. For instance, it was suggested [22, 24] that the pH at the surface of bone mineral might be significantly lower, i.e. about 6.8. Human intracellular pH has been found to vary between 6.8 and 7 [34] and the intracellular pH of avian shell glands has been reported to drop from 7 to 6.4 during egg shell formation [29]. Thus, it is not unreasonable to assume, that experiments in which the pH is allowed to change freely throughout the reaction might be suitable models for physiological mineralization.

Although hydrolysis at 37° is much faster than at 25° [18] precipitates prepared at this temperature under the above experimental conditions shortly after mixing exhibited some characteristics of the OCP structure (Füredi-Milhofer, H., Brečević, Lj., Oljica, E., Purgarić, B., Gass, Z., and Perović, G., in preparation). It is possible that the kinetics of transformation are significantly altered in an "in vivo" situation.

Finally, it is of interest to note that both ACP (13, 25, 28) and octacalcium phosphate (and/or carbonate octacalcium phosphate) [4, 6, 20, 23] have been reported to be constituents of bone mineral.

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