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Fibrous hydroxyapatite grown in the gel system: effects of pH of the solution on the growth rate and morphology

MASAMI TANAHASHI, KANICHI KAMIYA, TOHRU SUZUKI, HIROYUKI NASU
Department of Chemistry for Materials, Faculty of Engineering, Mie University, Kamihama-cho, Tsu-shi, Mie-ken, 514, Japan

In the preparation of fibrous hydroxyapatite (HAp), using the gel system consisting of agar gel containing calcium nitrate and over-layered $(NH_4)_2HPO_4$ solution, the pH of the solution was found to greatly influence the growth rate and morphology of the resultant products. In particular, a pH value of about 9–10 produced straight fibrous HAp in the shortest time. Also, the Ca/P molar ratio of the product increased with the pH value of the starting solution. The growth rate and morphology of the product were correlated to the ion species present in the solution at different pHs.

1. Introduction

Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$(HAp), is a major component of vertebrate animals' bones and teeth, and thus artificially synthesized HAp is one of the important biocompatible materials [1]. Therefore, HAp ceramics have been considered as a substitute for human bone or bone implant [2–4]. The bonding of sintered hydroxyapatite ceramics to the bone is known to be achieved by a direct chemical bond between the apatite in the ceramic and in the bone [5]. Fibrous HAp is considered to be useful as teeth and bone fillers.

In the technological field, it has been an attractive material for chromatographic separation [6, 7], as a catalyst, and for ion-exchange [8]. So far, many preparation methods of HAp powders have been published [9–11], but only two consider fibrous HAp [12, 13]. In one of those [13], the preparation of hollow fibrous HAp by using an agar gel system has been reported by the present authors. In that study fibrous HAp was grown when the PO_4^{3-}-bearing solution was layered over the agar gel containing Ca^{2+} ions. The fibrous HAp obtained was Ca-deficient [14], poor in crystallinity and transformed into fragile β-tri-calcium phosphate (β-TCP) at about 800 °C in air. However, these problems have been solved by adding F^- ions into the PO_4^{3-}-bearing solution in the preparation stage and forming F^--containing fibrous HAp [15].

The low growth rate and zigzag feature of the fibrous products is another problem which should be overcome for its practical use.

In the present work, straight fibrous HAp was obtained and its growth rate was enhanced. Moreover, effect of the pH value of the solution on the morphology of the product is discussed on the basis of existing ion species in the $(NH_4)_2HPO_4$ solution.

2. Experimental

2.1. Preparation of fibrous hydroxyapatite in the agar gel system by using $Ca(NO_3)_2\cdot 4H_2O$ and $(NH_4)_2HPO_4$ solutions at various pH values

The analytical grade $Ca(NO_3)_2\cdot 4H_2O$ and $(NH_4)_2HPO_4$ (Nacalai Tesque Co.) were used as starting reagents. The 0.4 M aqueous solutions of those materials were prepared. The pH of $Ca(NO_3)_2\cdot 4H_2O$ and/or $(NH_4)_2HPO_4$ solution was varied in the range from 6 to 11 by adding NH_4OH or HNO_3. The 1.5 g of agar of reagent grade was dissolved in 30 ml of the $Ca(NO_3)\cdot 4H_2O$ solution at boiling temperature. The hot $Ca(NO_3)_2\cdot 4H_2O$ solution containing agar was then poured into the test tube of diameter 30 mm, and cooled to room temperature to be gelled. The 30 ml of $(NH_4)_2HPO_4$ solution was warmed up below boiling temperature to remove the dissolved CO_3^{2-} ions which might have been incorporated into the products, and then carefully layered over the gel phase in the test tube. The gel system thus set up was kept standing in the ambient atmosphere at room temperature. In a few minutes, fibrous products emerged from the gel phase as shown in Fig. 1 and were grown upwards in the $(NH_4)_2HPO_4$ solution. The products were carefully taken out of the system, washed thoroughly with distilled water and dried in the room for several days. Some of the dried fibrous products were immersed in the saturated $Ca(OH)_2$ solution to compensate the Ca deficiency, then heated to 1000 °C at a heating rate of 5 °C min^{-1} in air and kept there for 10 h.

2.2. Characterization of the products

The crystalline species in the fibrous products were examined by a conventional powder X-ray diffraction

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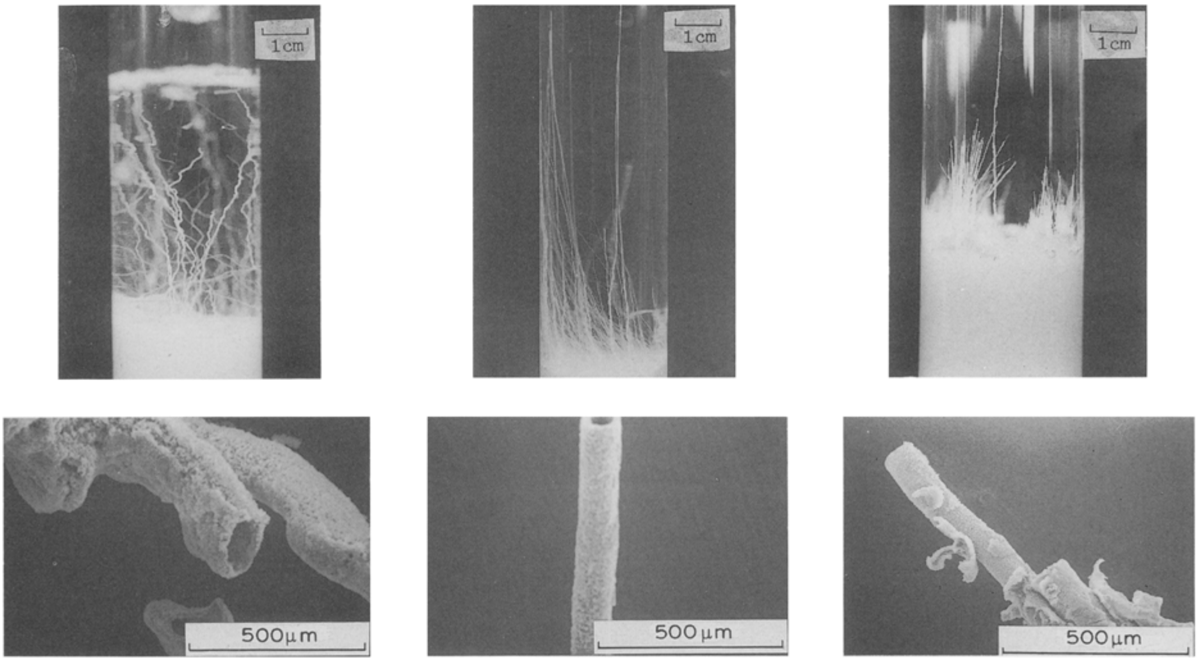


pH = 8.417 pH = 9.35 pH = 10.3

Figure 1 Photographs and SEM photographs of products obtained at various pH values.

(XRD) technique, using Ni-filtered CuK_α radiation. The fibrous products were subjected to the scanning electron microscopic (SEM) observation with Hitachi S-2300S. The Ca content of the products was determined by atomic absorption spectroscopy and that of P by phosphomolybdate techniques, respectively. The lengths of fibrous products grown in the gel system were measured by a rule.

3. Results

3.1. The pH effect on the growth rate of the product

Fig. 1 shows photographs and SEM photographs of fibrous products obtained in the gel system at various pH values. As can be seen, zigzag HAp, relatively straight and needle-like HAp are obtained at pH = 8.47, 9.35 and 10.3, respectively. The outer surface of samples obtained at pH = 10.3 is seen to be peeled off, which is not observed in the products obtained at pH = 8.47 and 9.35.

Table I shows that crystalline species in the products aged for 1 day in the mother solutions and morphology of products at various pHs, where pH of the solution was adjusted by adding NH_4OH. Needle-like dicalcium phosphate dihydrate, $CaHPO_4 \cdot 2H_2O$ (DCPD) was obtained in the range $6 < pH < 7$, and hydroxyapatite in the range $7 < pH < 11$. The morphology of HAp changed from zigzag to straight and needle-like as the pH was increased, as already mentioned. At $pH > 11$, no product was obtained.

The change of the product with ageing time in $(NH_4)_2HPO_4$ solution is shown in Table II. It is seen that DCPD and amorphous calcium phosphate (ACP) is converted to HAp as the ageing time is increased at pH = 8.47 and 9.35. At pH = 10.3, only ACP is formed and is converted to HAp. Therefore, it can be said that the fibrous product grown over the gel phase in the early stage of reaction is ACP, which is then converted to HAp in $(NH_4)_2HPO_4$ solution during standing.

TABLE I Crystalline species in the products aged for 1 day in the mother solution and morphology of products at various pH values

pH	$(NH_4)_2HPO_4$-NH_4OH	
	Product	Type
6–7	DCPD	Needle
7–8	HAp	Zigzag
8–9	HAp	Zigzag
9–10	HAp	Straight
10–11	HAp	Needle
11–12	No product	—
12–13	No product	—
13–14	No product	—

TABLE II The change of the product with ageing time in $(NH_4)_2HPO_4$ solution

pH Time min	8.47	9.35	10.30
2	DCPD + ACP	DCPD + ACP	—
6	DCPD + ACP	ACP	ACP
7	DCPD + ACP	ACP	ACP
8	ACP	ACP	ACP
13	ACP	ACP	ACP
15	ACP	ACP	ACP
120	HAp	HAp	HAp

Fig. 2 shows the relation between length of the fibrous product and ageing time. The growth rate of fibrous product was determined from the slope of linear part in this relation and is shown in Fig. 3 as a

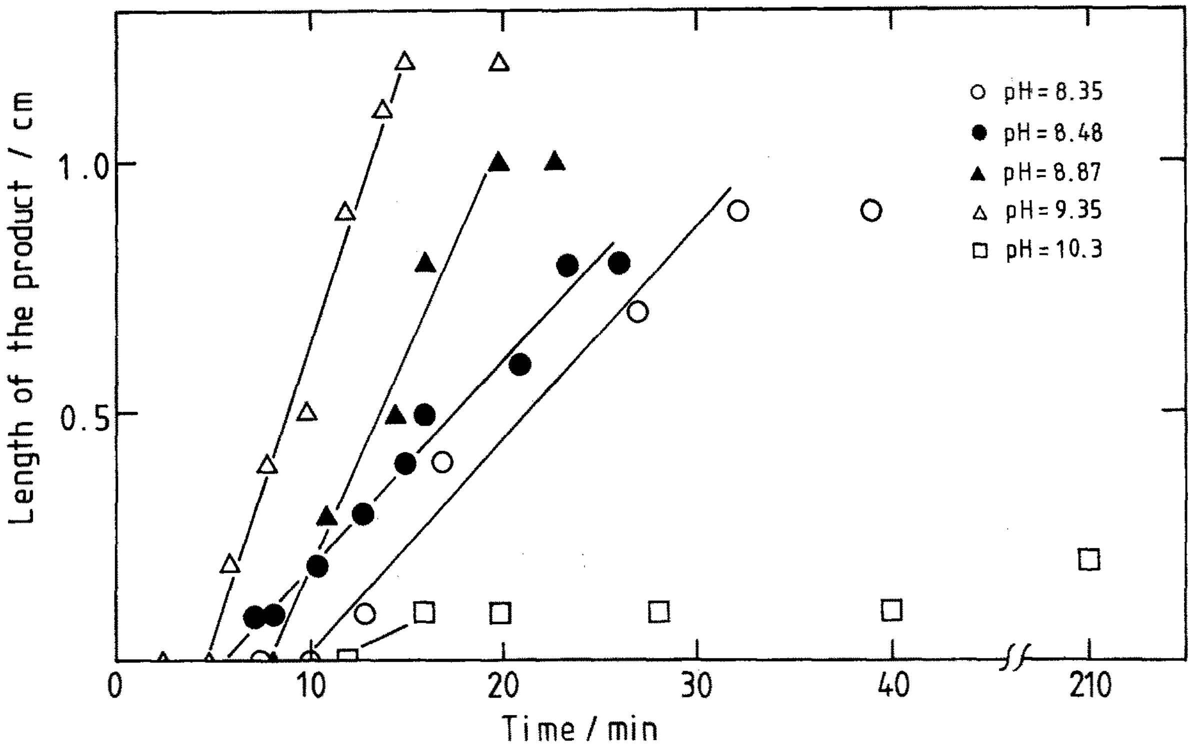


Figure 2 Length of the fibrous product as a function of ageing time.

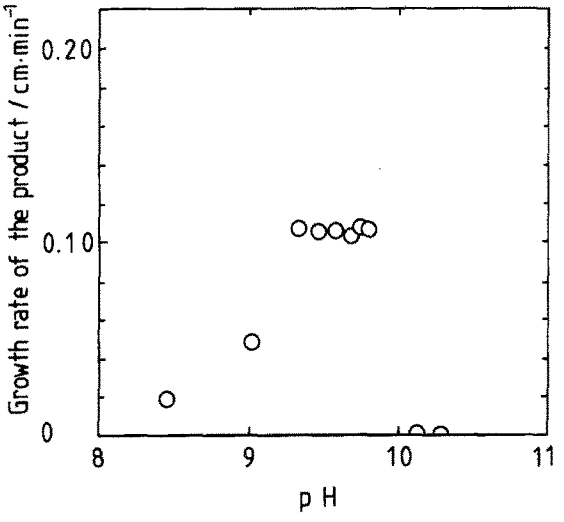


Figure 3 Growth rate of fibrous product at different pH values.

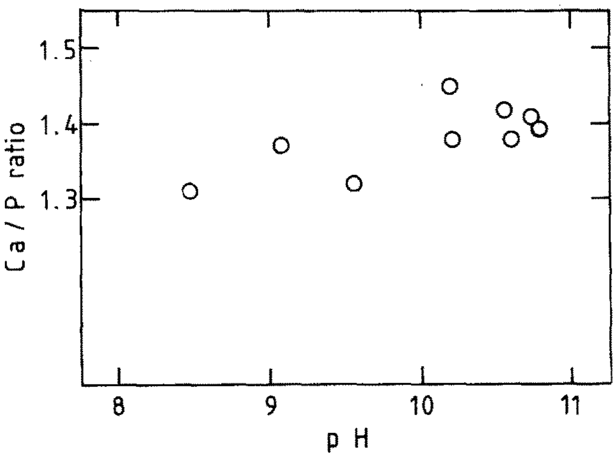


Figure 4 Relation between Ca/P molar ratio of fibrous HAp and pH of the starting solution.

function of pH value of the solution. It is seen that the growth rate is increased with pH value and decreased drastically above pH = 10.

In Fig. 4, the Ca/P molar ratio of fibrous products is plotted against pH value. The Ca/P molar ratio is increased as the pH value of the starting solution is increased. This agrees with the result of Kanazawa *et al.* [16], who prepared samples by mixing the Ca^{2+} and PO_4^{3-}-containing solutions.

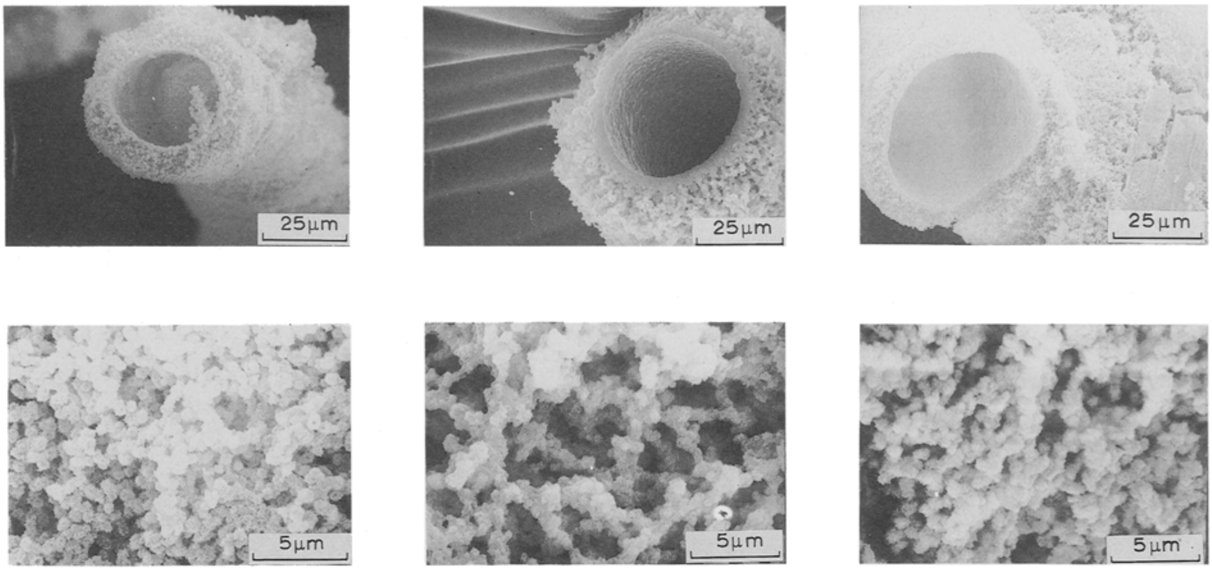
Fig. 5 shows the SEM photographs of cross section and surface of products prepared at pH = 8.47, 9.35 and 10.3. It is seen that the products obtained at pH = 8.47 and 9.35 are very porous. At pH = 10.3, the surface of the product is more dense than inner site, suggesting that the outer surface of the fibre has been peeled off, as has been seen in Fig. 1.

SEM photographs of products obtained at pH = 8.47, 9.35 and 10.3 and heated at 1000 °C for 10 h are shown in Fig. 6. Note that the surface of the product becomes more dense by heat treatment.

4. Discussion

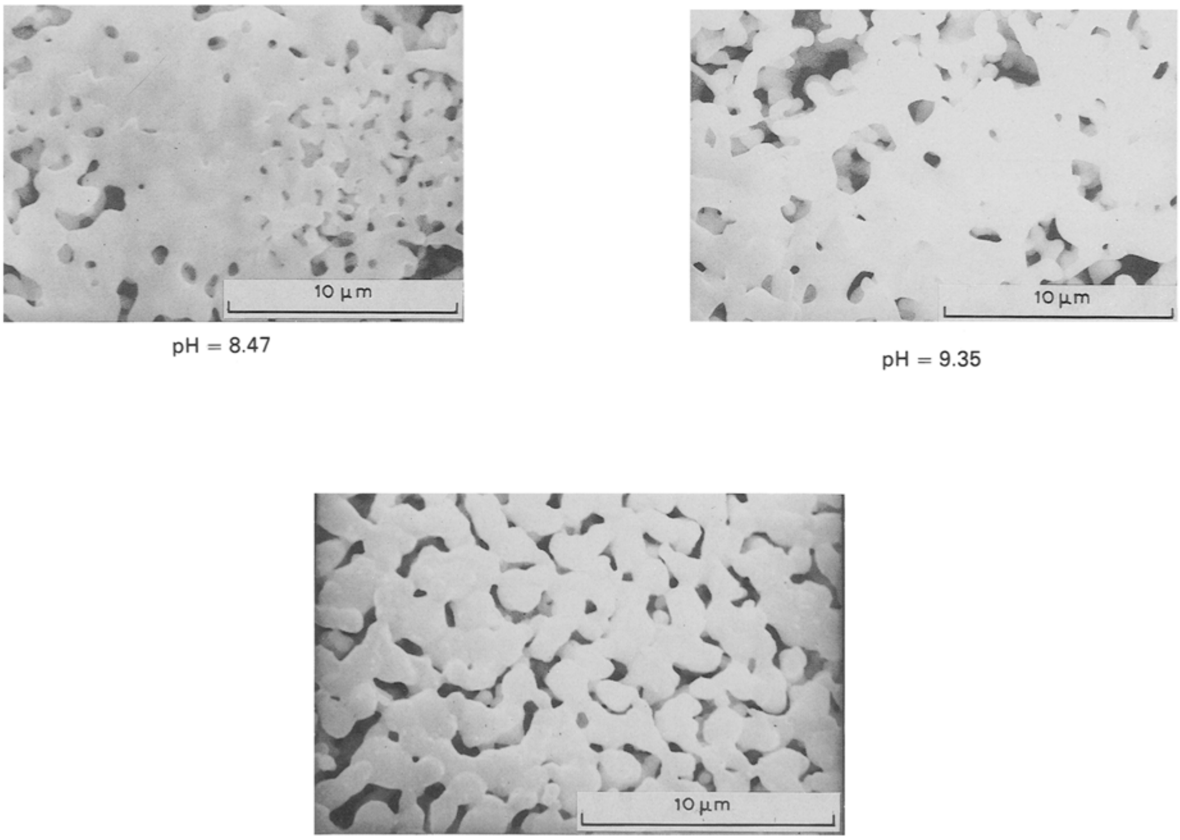
4.1. Relation between pH of the solution and growth rate of fibrous product

The product obtained in the present work changed from zigzag fibre to straight fibre, depending on the pH value of the solution in the pH range from 7 to 10. To confirm the pH range where fibrous HAp is formed in the gel system, $(NH_4)_2HPO_4$ as a PO_4^{3-} source for HAp was replaced by H_3PO_4, and NaOH was used for adjusting pH of the solution. In this case, the



pH = 8.47 pH = 9.35 pH = 10.3

Figure 5 SEM photographs of dried fibrous products obtained at different pHs.



pH = 8.47

pH = 9.35

pH = 10.3

Figure 6 SEM photographs of the fibrous products heated to 1000 °C for 10 h.

fibrous HAp was also obtained in the range $7 < \text{pH} < 12$ (Table III), and straight fibres were grown quickly around pH = 10. When the pH of $(NH_4)_2HPO_4$ solution was varied by using NaOH, the same results were obtained.

In the pH range around 10 where rather straight HAp fibres are grown in a short time, the HPO_4^{2-} ion is predominant when compared with other phosphate species, as shown in Fig. 7 [17]. As mentioned above, fibrous products grown in the early stage of reaction consist of ACP. It has been reported that ACP is usually formed in the pH range of 6.7–12 [18] and just after formation, ACP may have composition of $Ca(HPO_4) \cdot xH_2O$ [19, 20]. Therefore, a rapid growth of fibrous ACP in the pH range around 10 can be explained by the high concentration of HPO_4^{2-} species in the solution. It is considered that, with the increase of ageing time in mother solution, ACP may react with water to change to more apatitic amorphous composition $Ca_3(PO_4)_2 \cdot xH_2O$ [21] followed by

TABLE III Change of the product and its morphology with pH of starting solution (H_3PO_4 was used as a PO_4^{3-} source and pH of the solution was adjusted with NaOH)

pH	H_3PO_4–NaOH	
	Product	Type
6–7	DCPD	Needle
7–8	HAp	Zigzag
8–9	HAp	Zigzag
9–10	HAp	Zigzag, straight
10–11	HAp	Zigzag, straight
11–12	HAp	Straight
12–13	HAp	Gelatinous
13–14	$Ca(OH)_2$	Particle

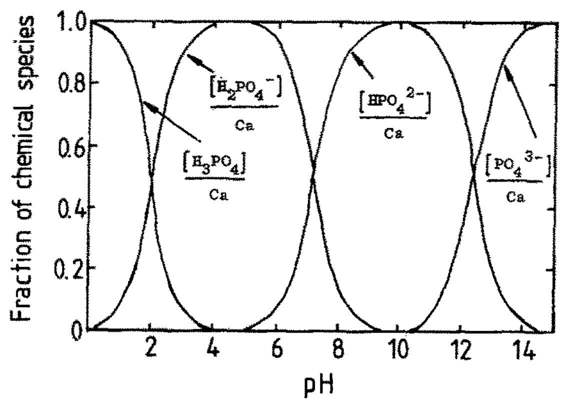


Figure 7 Change of the phosphate species with pH in H_3PO_4 solution.

crystallization to HAp according to the following equation.

$$Ca^{2+} + HPO_4^{2-} + xH_2O \rightarrow Ca(HPO_4)\cdot xH_2O$$

$$2Ca(HPO_4)\cdot xH_2O + H_2O + Ca^{2+}$$
$$\rightarrow Ca_3(PO_4)_2\cdot xH_2O + (x+1)\,H_2O + 2H^+$$

$$2Ca_3(PO_4)_2\cdot xH_2O + 4Ca^{2+} + 2HPO_4^{2-}$$
$$\rightarrow Ca_{10}(PO_4)_6(OH)_2 + 4H^+ + 2(x-1)\,H_2O.$$

Considering these points, the increased growth rate of fibrous HAp around pH = 10 can be interpreted in terms of the increased amount of OH^- and HPO_4^{2-} ions in the solution layered over the gel phase.

4.2. Morphology of the product

The growth mechanism of the fibrous product may be as follows. Ca^{2+} ions included in the agar gel are raised up through the small channels in the gel by the capillary force, and react at the exit-bore with the phosphate species in the solution layered over gel to form gelatinous ACP. Since Ca^{2+} ions are continuously supplied by the osmotic pressure or capillary force, the gelatinous product grows upward, as shown in Fig. 8. The gelatinous gel thus formed is deformable and is elongated by the capillary force acting upwards

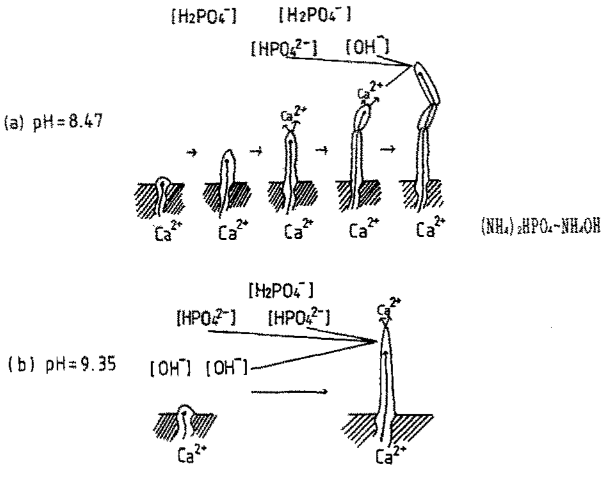


Figure 8 Schematic presentation of the growth of fibrous HAp from the gel phase in the $(NH_4)_2HPO_4$ solution.

until the wall thickness becomes so small that it is broken. The reaction is followed by another formation of oval at the broken part. When the formation rate of gelatinous ACP is small, respective ovals are naturally small and the breakage of the thin-walled oval can take place at unpredictable parts, the zigzag hollow fibres result, as shown in Fig. 8a. On the other hand, when a large amount of ACP is produced, the gelatinous product was greatly elongated before the wall reached critical thickness for the breakage, forming long and straight hollow fibres. This must be the case in the pH range around 10.0. At pH = 10.3, a growth mechanism of product may be different from the case mentioned above because PO_4^{3-} and NH_4^+ is predominant compared with other phosphate species (Fig. 7).

4.3. Relation of Ca/P molar ratio to pH of the starting solution

The Ca/P molar ratio increased with pH (Fig. 4). In the present work, the pH of the solution was varied by adding NH_4OH and HNO_3, so NH_4^+, OH^-, H^+ and NO_3^- ions exist in the $(NH_4)_2HPO_4$ solution. Among these ions, it is only the OH^- ion that can be incorporated in the OH site of HAp. In general, Ca-deficient HAp is represented as $Ca_{10-x}H_x(PO_4)_6\ (OH)_{2-x}\cdot nH_2O$. Incorporating the OH^- ion in the OH site of HAp resulted in an increasing amount of Ca^{2+} or an increase in the Ca/P molar ratio of the products.

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