

Constitution and morphology of hydroxyapatite whiskers prepared using amine additives

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Abstract

Currently-available HA particle-reinforced composites are not suitable for use in large stress-bearing restorations and biomedical substitution due to low strength and poor durability. HA is the most promising because of good biocompatibility and bioactivity, and since reinforcement efficacy is greatly affected by length and aspect ratio, the preparation of long HA whiskers could be important for biomedical applications. Acetamide, formamide, and carbonyl diamide were tested as precipitation agents to modify crystal growth habit. The morphology and composition of the whiskers showed a sensitive dependence on the additive used. Carbonyl diamide and formamide led to contaminated HA; crystals were needle-like but contained carbonate or formate, respectively. In comparison, acetamide showed a low hydrolysis rate under hydrothermal conditions, giving rise to a rapid growth of HA crystals at a low supersaturation. Furthermore, the hydrolysis products of AA affected neither the composition nor the growth habit of the HA.

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1. Introduction

Hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, HA) is the main constituent of human bone and tooth tissue (enamel, dentine, cementum) and has been considered as one of the most bioactive and biocompatible materials for dental and biomedical application.^{1–3} Unfortunately, general clinical applications of synthetic HA are limited to small and unloaded implants, powders, coatings and low-loaded porous implants due to poor flexural strength and fracture toughness.¹ Moreover, HA particle-filled composites have also been found to fall short in terms of mechanical strength and durability,^{4,5} while reinforcement by whiskers or fibres has been considered as an effective way of improving mechanical properties. Compared with most available bio-inert whiskers or fibres, the most promising reinforcements seem to be calcium phosphate-based due to their good biocompatibility and bioactivity.^{6,7} Thus, HA whiskers combine both reinforcement and bioactivity, and may

be effective in improving both mechanical performance and adhesion to bone and implant surfaces, extending the use of such “biocomposites” to large stress-bearing restorations and bone-substitution as well as the preparation of bioactive scaffolds with large porosity for tissue engineering.

Control of crystal constitution and morphology is of critical importance for such applications. To realize this purpose and elucidate the mechanism of HA growth, a great deal of attention has been paid to the effects of various additives on the nucleation and growth of HA crystals.^{8,9} Non-collagenous matrix macromolecules have been suggested to be involved in the control of nucleation and growth of the mineral phase *in vivo*.^{10–14} However, most are effective inhibitors of HA growth due to their preferential adsorption on specific crystallographic faces of a growing crystal, blocking active growth sites. Although some substances such as sodium cholate, colloidal potato starch and ethanol were found to favour the precipitation of fibre-like HA, the composition and morphology were easily affected by the synthesis conditions, it being difficult to obtain homogeneous and pure HA.^{15–17} It is therefore of great importance to find an appropriate additive for the preparation of whiskers with controlled composition, constitution and morphology.

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Wet chemical precipitation with a low reaction rate is a commonly-used and important procedure for producing uniform HA whiskers or fibrous HA.^{18–21} Although the nucleation and growth of HA can be easily controlled by the slow release of Ca^{2+} ions from Ca complexes, or by the control of the pH value through the hydrolysis of carbonyl diamide (CA) (urea), its structure and properties are very sensitive to the preparation conditions:^{22,23} whiskers are short, have small aspect ratio, and show relatively inferior crystallinity and thermal stability.^{24,25} Furthermore, the poor dispersibility of these whiskers due to entanglement or agglomeration becomes a problem when mixing with matrix materials, and the reinforcing efficacy is low due to the low aspect ratio and short length.^{26,27} Amide additives can be hydrolysed in both acidic and basic aqueous solution to release ammonia, and so may be used to control solution pH and modify growth habit.^{28,29} The preparation of HA by homogeneous precipitation at 95–100 °C using acetamide (CH_3CONH_2) (AA) and formamide (HCONH_2) (FA) has been reported, but their slow hydrolysis gave large needle- or rod-like, well-crystallized HA particles, rather than whiskers.^{30,31} Both crystallinity and morphology depended on the kind and concentration of the amide as well as ageing time.²⁹ Since hydrolysis is accelerated at high temperature, the rate of change of pH affects the degree of supersaturation with respect to HA. Such a regime is worth exploring as a mean of preparing suitable HA whiskers.

Hydrothermal homogeneous precipitation is a useful method of preparing large and non-aggregated crystals and whiskers with high crystallinity, controllable aspect ratio, high purity and low dislocation density.^{32,33} However, until now, no relevant work concerning the hydrolysis of such additives, and the characterization of the resulting HA whiskers, seems to have been conducted at above 100 °C.³⁴ The aim of this paper is to report comparison of the effects of AA, FA and CA on the morphology and composition of the product so as to find the most appropriate additive for the preparation of long HA whiskers.

2. Materials and methods

Commercial analytical grade reagents, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (AnalaR, BDH, Poole, England) and $(\text{NH}_4)_2\text{HPO}_4$ (AnalaR, BDH), were employed as starting materials, and acetamide (99%, Alfa Aesar, Heysham, Lancashire, England), formamide (AnalaR, BDH), and carbonyl diamide (AnalaR, BDH) were used as additives to control the pH of the synthesis solution and modify crystal growth habit. 0.5–1.5 mol/L additive (as amide, $-\text{NH}_2$) was dissolved in an aqueous solution containing 84 mmol/L Ca and 50 mmol/L PO_4 (i.e. total, analytical concentrations), and the pH adjusted to 3.00 using 0.1 mol/L HNO_3 or 1:1 ammonium hydroxide. After processing at 180 °C for 5–45 h, the mixture was cooled naturally over 12 h to ambient temperature (~ 25 °C). The product was then filtered off, washed quickly with deionized water (Milli-RO, Milli-Q; Millipore, Bedford, MA, USA) four times, and finally dried in air at 80 °C.

To check the effect of the presence of the acetate arising from the hydrolysis of the AA on the morphology of whiskers, experiments were also performed at 180 °C for 10 h using solu-

tions containing 42 mmol/L Ca and 25 mmol/L PO_4 and 1 mol/L AA, with the inclusion of 20, 40 or 60 mmol/L acetate as acid (AnalaR, BDH), again adjusted to start at pH 3.00.

The products were characterized using X-ray powder diffraction (XRD) (X'Pert Pro, PANalytical BV, Almelo, The Netherlands), Fourier-transform infrared spectroscopy (FTIR) (FTS-165, Bio-Rad, Hercules, CA, USA) and scanning electron microscopy (SEM) (XL30CP, Philips Electron Optics, Eindhoven, The Netherlands). XRD pattern-processing software (MDI Jade 5, Materials Data, Livermore, CA, USA) was used for phase identification and lattice parameter calculations. Four or five SEM micrographs of non-overlapping fields were used to select randomly more than 80 whiskers (showing the whole length), and their length and width were determined using image processing software (QWin, Leica Microsystems Imaging Solutions Ltd., Cambridge, UK). Aspect ratio, length/width, was then calculated for each. Transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) observations, and selected-area electron diffraction (SAED) patterns (Tecnai G2 20 S-TEM, Philips, Hillsboro OR, USA) of whiskers were also taken. The Ca/P ratio was analysed by energy dispersive X-ray spectroscopy (EDX) using a field emission-scanning electron microscope (FE-SEM) (LEO 1530, Oxford Instruments, Abingdon, UK). EDX results were believed to be accurate within $\sim 5\%$. The pH of the solution before the synthesis was measured with a combination electrode (GK2401C, Radiometer Analytical, Copenhagen, Denmark) at room temperature (25 ± 2 °C). After processing, the pH of the filtrate was measured as representing the final pH of the synthesis solution.

3. Results

Figs. 1 and 2 show SEM images of the products, and the systematic variation of the mean length, width and aspect ratio of whiskers against the concentration of amine additives, respectively. The morphology of the HA showed a dependence on the additive used. Increasing [AA] increased whisker length and decreased width, giving a higher aspect ratio than for those prepared using FA and CA. Uniform whiskers with mean length 48–62 μm and aspect ratio 55–87 were obtained for [AA] = 0.75–1.25 mol/L. However, with [AA] > 1.5 mol/L or < 0.5 mol/L, small plate- or petal-like particles were found in the product (Fig. 3). Using FA, the mean length and aspect ratio of the products varied from 26 to 39 μm and from 29 to 41, respectively. For [FA] > 0.75 mol/L, the crystals were needle-like. Although the HA was whisker-like for [FA] = 0.5–0.75 mol/L, the aspect ratio was ~ 40 (Fig. 4). With CA, crystals were short and needle-like, with low aspect ratio. [CA] had no obvious effect on length and aspect ratio, which ranged from 18 to 27 μm and from 15 to 29, respectively; but small and irregular particles were found to accompany the needle-like HA at high [CA].

The products were identified as HA by XRD and FTIR as previously reported.³⁴ All XRD peaks of the products for each additive matched well those of the JCPDS PDF 9-432 diffraction pattern for synthetic HA, showing good crystallinity and high phase-purity. The strongest peak intensity appeared for the (3 0 0) lattice plane, rather than for the (2 1 1) as is usual, and

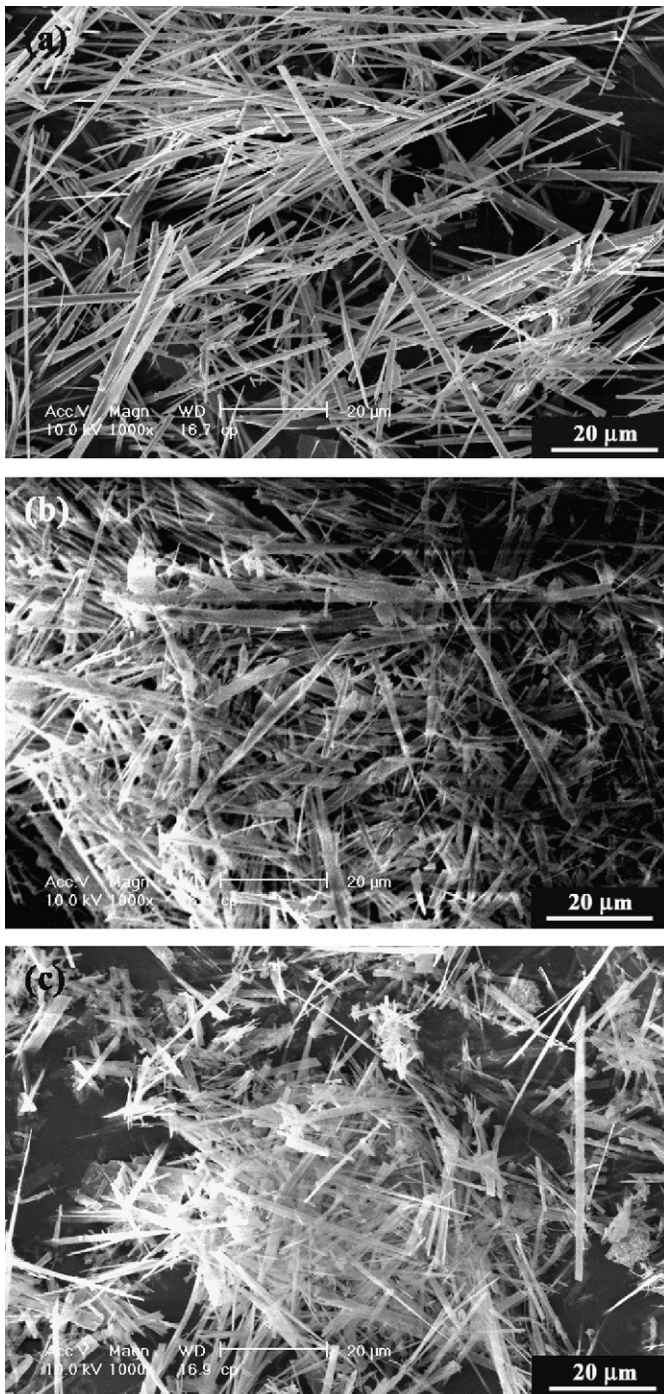


Fig. 1. SEM images of HA prepared at 180 °C for 8 h with (a) 1 mol/L AA, (b) 1 mol/L FA, and (c) 0.5 mol/L CA.

the peak intensity ratio $I_{(300)}/I_{(002)}$ was higher than that of the reference pattern. Crystals were obviously elongated along the c -axis, and this was also confirmed by the TEM analysis results. As shown in Figs. 5 and 6, spots in the SAED pattern near the (000) plane were identified as (002), (110) and (112), which were taken from $[-220]$ zone axis; they matched the vector relationship of the crystal planes. The strong reflections indicated high purity and crystallinity.²⁵ The HRTEM images showed a clear atomic arrangement; the d -spacings for the (001) and (110)

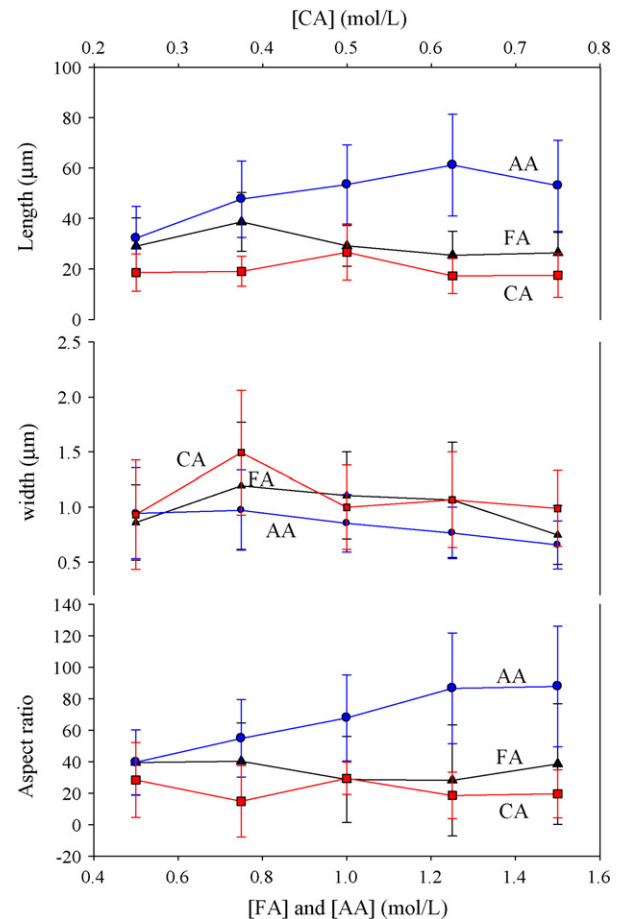


Fig. 2. Variation of length, width and aspect ratio of HA with additive concentration at 180 °C for 8 h.

lattice planes were 0.685 and 0.471 nm for AA and 0.682 and 0.474 nm for FA, respectively, in agreement with the XRD analysis results. The longitudinal axis of the whiskers was parallel to the [001] direction. The HA prepared using both AA and FA had an obvious preferred growth orientation along the c -axis, as is usual.^{21,35}

The positions of characteristic bands at 1094, 1038, 963, 602 and 563 cm^{-1} , and bands at 3571 and 633 cm^{-1} , in the FTIR spectra, assigned as stretching and bending of phosphate and the stretching mode of hydroxyl group of HA, respectively,³⁶ were similar in each. Since the synthesis was conducted in a moderately acidic solution, the symmetrical stretching vibration band of HPO_4^{2-} group at 872 cm^{-1} was present.³⁷ However, bands at 1421 and 1457 cm^{-1} , attributed to the CO_3^{2-} group, only appeared in the case of CA.³⁶ Distinct bands at 1543, 1384 and 1343 cm^{-1} , attributed to the formate ion (HCOO^-), were found in the spectra of samples prepared using FA.³⁰ The whiskers had slight calcium deficiency and ion substitution when CA and FA were used.

Fig. 7 shows the changes with ageing time of the length and aspect ratio of the whiskers: AA gave a higher aspect ratio and length than FA; duration >10 h had no obvious effect on these characters for either. The peak intensity ratio $I_{(300)}/I_{(002)}$ in the XRD patterns for AA was also found to increase with the

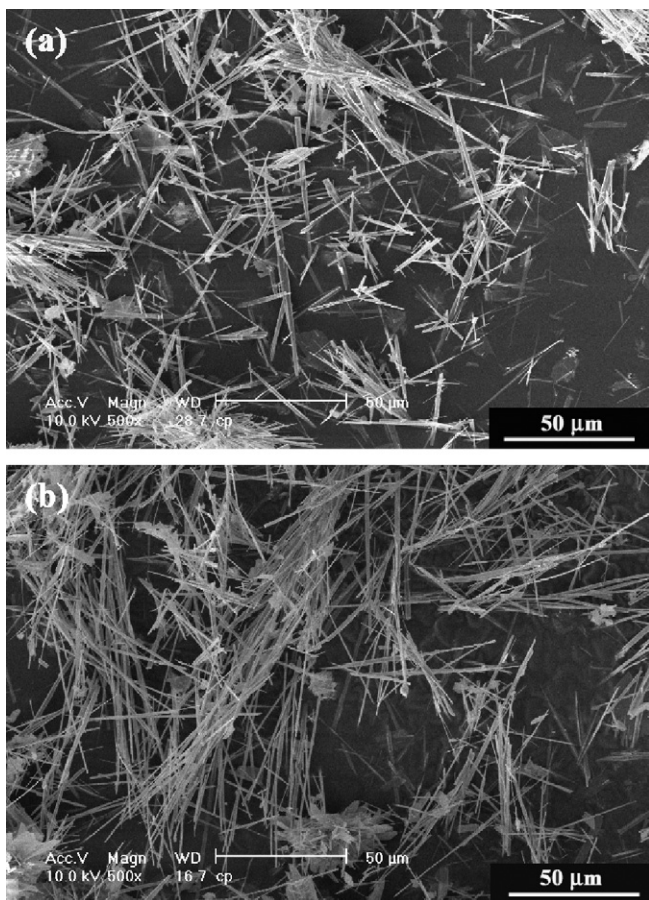


Fig. 3. SEM images of HA prepared at 180 °C for 8 h with (a) 0.5 mol/L AA and (b) 1.5 mol/L AA.

duration initially, but at >10 h the change was slight. The ratio $I_{(300)}/I_{(002)}$ with FA seemed unaffected by duration from 5 to 45 h.

4. Discussion

Generally, mineral precipitation occurs from supersaturated solution. The rate of both nucleation and subsequent growth of crystals intimately depend on the degree of supersaturation, and this can be controlled through the activity product of ions (IP) of the solution.^{15,16} For HA, the IP can be simply expressed by:

$$IP = (f_2[Ca^{2+}])^5 (f_3[PO_4^{3-}])^3 (f_1[OH^-])$$

where f_i is the activity coefficient of the i th ion. Because the Ca and PO_4 are distributed across a number of solution species, in part dependent on protonation of the phosphate, IP greatly depends on the pH of the solution. The hydrolysis of the selected amines may be represented by^{29,38}:

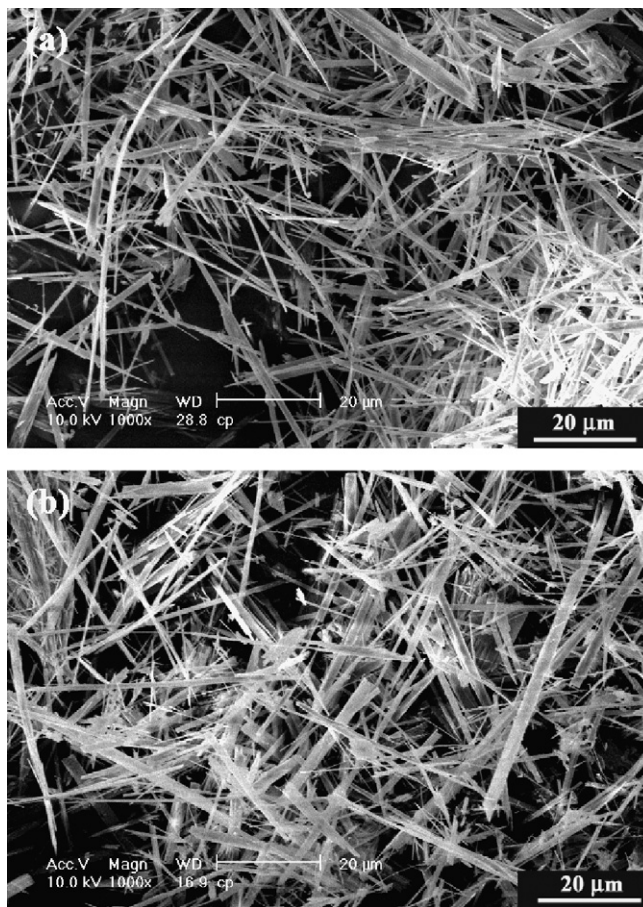
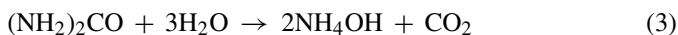
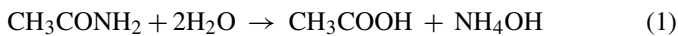


Fig. 4. SEM images of HA prepared at 180 °C for 8 h with (a) 0.5 mol/L FA and (b) 0.75 mol/L FA.

The released ammonia raises the pH of the solution, the extent of which is mainly controlled by the hydrolysis rate and amine concentration.

The hydrolysis characteristics of these additives under hydrothermal conditions, conducted in an autoclave at 80–200 °C, using solutions containing 0.5 mol/L of the additive (as amide, $-NH_2$) at initial pH 3, had been examined previously: pH increased steadily with time, the rate being accelerated at higher temperature.³⁴ CA hydrolysed rapidly beginning at ~ 80 °C; the pH quickly rose above 9.4 and the processing (final, plateau) temperature had no obvious effect on pH when this was above 160 °C. In comparison, FA and AA showed relatively low hydrolysis rates; the pH only rose to 5.0–5.5 when the temperature was raised to 160 °C. The pH gradually rose with increasing temperature, and the hydrolysis reaction occurred continuously with time at the design temperature. High supersaturation was rapidly attained when CA was used, resulting in more nuclei, so that the length and aspect ratio of the crystals were smaller than for AA and FA. Thus, despite being widely-used in the preparation of HA whiskers by conventional wet chemical precipitation,^{20,25,28} CA is seen to be inappropriate to prepare HA whiskers under hydrothermal conditions. However, the hydrolysis of AA and FA, in contrast with CA, was more moderate: a rapid rise in pH could be avoided so that the low supersaturation of the synthesis solution may be main-

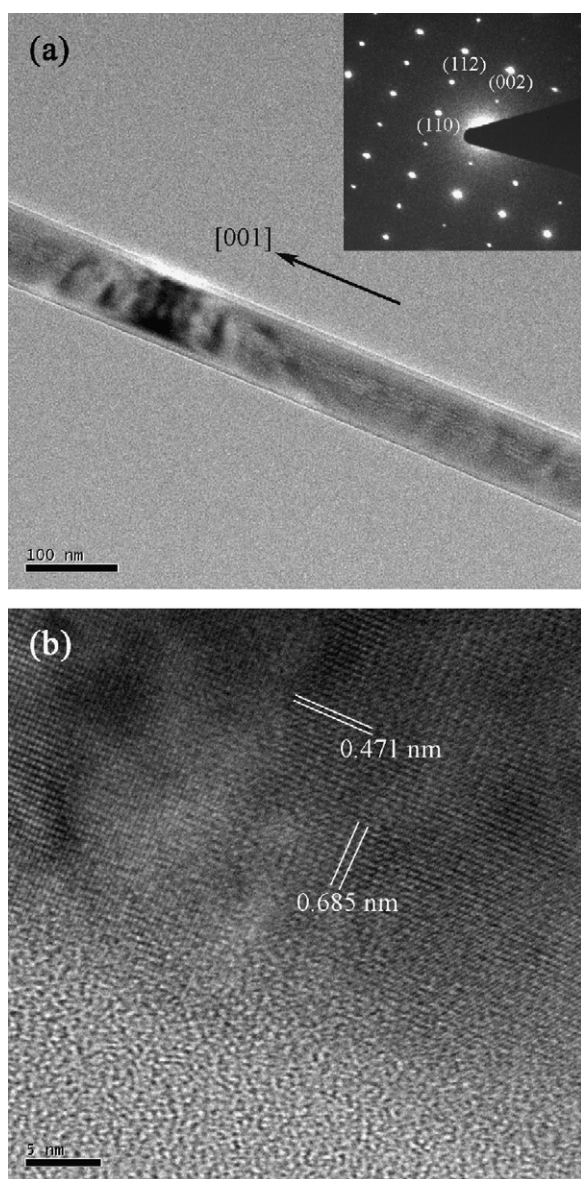


Fig. 5. TEM images of HA whiskers prepared at 180 °C for 8 h using AA. (a) TEM, low magnification and SAED pattern (inset) and (b) HRTEM.

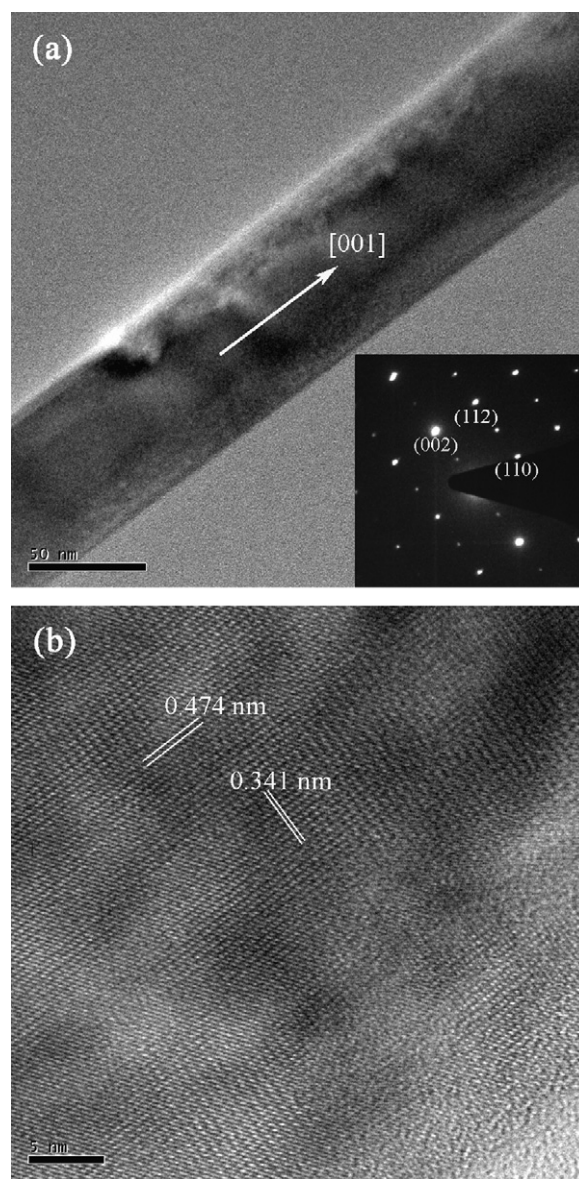


Fig. 6. TEM images of HA whiskers prepared at 180 °C for 8 h using FA. (a) TEM, low magnification and SAED pattern (inset) and (b) HRTEM.

tained. Nevertheless, because of a greater hydrolysis rate of FA than AA at all temperatures, and especially at high temperature, the greater number of nuclei formed initially affected crystal growth and morphology. Thus, the HA prepared using FA was needle-like, being shorter and wider than with AA, which gave long whiskers with uniform morphology and high aspect ratio. Although AA has seldom been used in this context,^{29,30} it seems now to be the agent of choice in an hydrothermal environment.

The constitution of HA precipitated from aqueous solution has proved to be sensitive to the synthesis conditions. Yasukawa et al.^{29,31} reported that the formic and acetic acids produced by the hydrolysis of FA and AA affected the formation and transformation of HA precursors at 95–100 °C. Irregularly-shaped and needle-like particles were formed together. However, by keeping [AA] or [FA] low in the present study, maintain-

ing low supersaturation, the precipitate was free from such precursors. However, various substitutions, which cause distortion of the structure of the HA, can also occur.³⁶ CA forms NH_4^+ and CO_3^{2-} ions, the latter being easily incorporated into the structure in the position of OH (A-type) or PO_4 (B-type),³⁶ low pH being said to favour the OH substitution.³⁹ However, this did not occur here. The bands at 1421 and 1457 cm^{-1} in the FTIR spectra indicated that only B-type substitution occurred, and this resulted in calcium deficiency and contraction in the *a*-axis⁴⁰ (Table 1). In the case of FA, the resulting formate (HCOO^-) ions have similar shape and dimensions to carbonate, and again can be located in both PO_4 and OH sites. This also expands the unit cell in the *a*-axis and causes Ca-deficiency.³⁰ For AA, no evidence of acetate was detectable in the FTIR spectra, constitution or crystallinity of the HA.

Table 1
Effect of additive on some characteristics of the HA prepared at 180 °C for 8 h.

Additive	Starting conditions				Final pH	Ca/P of product	Lattice constants	
	[Ca] (mmol/L)	Initial Ca/P	Initial pH	[additive] (mol/L)			<i>a</i> and <i>b</i> (Å)	<i>c</i> (Å)
AA	83.5	1.67	3.00	1.0	5.48	1.61	9.435	6.887
FA				1.0	4.91	1.55	9.442	6.879
CA				0.5	8.50	1.61	9.427	6.880

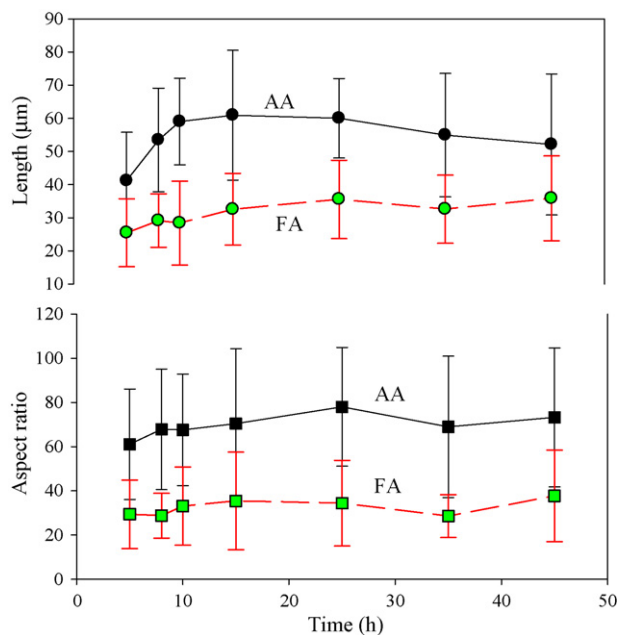


Fig. 7. Variation of length and aspect ratio with duration of HA prepared at 180 °C using 1 mol/L AA and FA.

In essence, the hydrolysis of the amide additive is to raise the pH by generating weak acid anions. This then offsets the fall in pH that precipitation of HA causes. Thus, the efficiency of obtaining HA whiskers from buffer solutions of calcium acetate and phosphoric acid was improved by up to about 30 times compared with a non-buffer solution at pH 4–5 at 90 °C.⁴¹ However, given the contamination problems arising from formate (FA) and carbonate (CA), and although no contamination from acetate (AA) was detected,^{23,29} it was necessary to check whether it had any detrimental effect on crystal growth. As shown in Fig. 8, no obvious morphological effect was present; mean length, width and aspect ratio varied in the ranges 113–116 μm, 1.17–1.20 μm, and 99–103, respectively. The slopes of the linear regression lines against the concentration of acetate were not significant ($p > 0.05$). Thus, the hydrolysis products of AA not only did not contaminate the product,²⁹ it had no influence on the whiskers in either morphology or crystal growth habit, while the hydrolysis behaviour favoured rapid crystal growth. AA was therefore concluded to be the most appropriate of those additives tested.

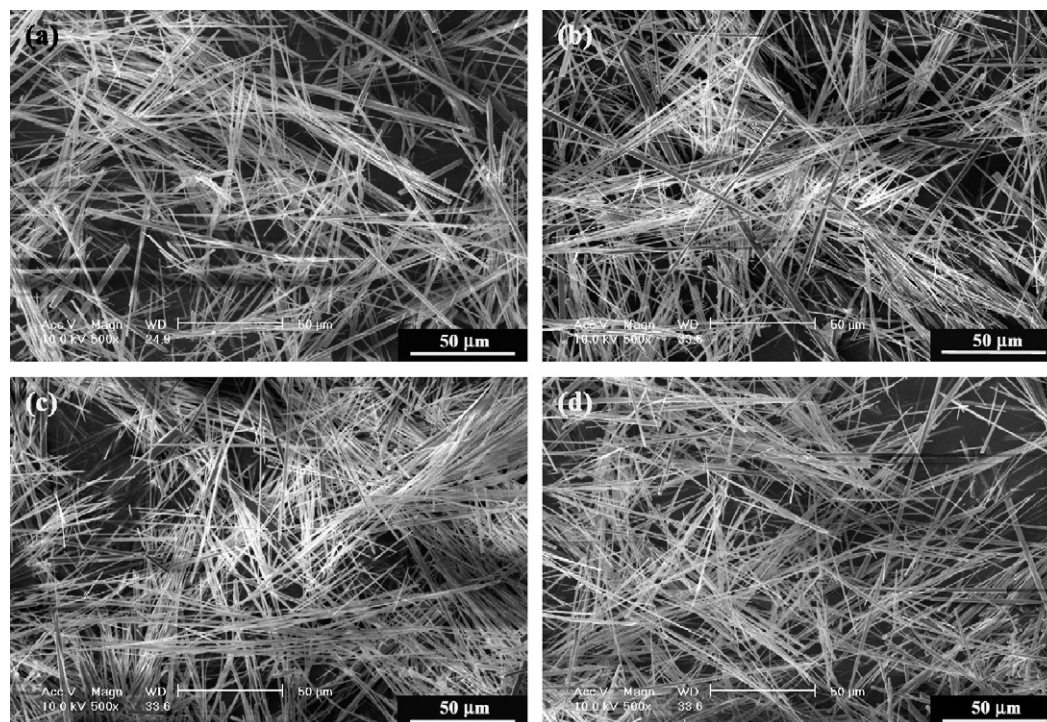


Fig. 8. SEM images of HA prepared at 180 °C for 10h using solutions containing added acetate: (a) 0 mmol/L, (b) 20 mmol/L, (c) 40 mmol/L, and (d) 60 mmol/L.

5. Summary

The morphology and constitution of HA whiskers prepared under hydrothermal conditions was affected by the choice of amine additive due to the differing hydrolysis rates and products. Compared with FA and CA, AA showed a low hydrolysis rate, the pH rising slowly, giving smaller numbers of nuclei, and facilitating whisker growth at a low supersaturation. The hydrolysis products of AA affected neither the constitution, the crystallinity nor the crystal growth habit of the HA. Long and uniform whiskers with high aspect ratio and good crystallinity were thereby prepared successfully. With CA and FA, the HA had a needle- or rod-like morphology, and although at low [FA] whisker-like HA could be obtained, both length and aspect ratio were low. Even so, the HA was then contaminated with carbonate and formate, respectively.

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