

EFFECT OF SYNTHESIS TEMPERATURE ON MORPHOLOGY AND STRUCTURAL CHARACTERISTICS OF HYDROXYAPATITE WHISKERS

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Synthesis temperature shows important effects on the hydrolysis rate of acetamide (AA) additive and the driving forces for the formation of nuclei and the growth of hydroxyapatite (HA) crystals from aqueous solution. Variation in the temperature in the present work affected neither the constitution & crystallinity of the products nor the crystal growth habit along the c-axis. However, the morphology of products greatly depended on the synthesis temperature and was correlated with the starting pH of the solution. Higher initial pH only provided a basic reference for quickly obtaining supersaturation for the formation of HA nuclei. Lath- or branch-like HA often appeared in the whiskers at high initial pH, being independent of the synthesis temperatures. Neither high nor low temperatures were beneficial to the preparation of long HA with high aspect ratio. 180 - 200°C seemed to be an appropriate synthesis temperature to prepare long HA whiskers from a solution with Ca/P 1.67 and starting pH 3. The Ca/P ratio of products increased with the temperature, and the whiskers obtained at low temperature had high calcium deficiency.

INTRODUCTION

Hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, HA) is one of the most bioactive and biocompatible materials, and has been accepted and used widely and successfully to repair bone and tooth defects [1]. However, inferior mechanical properties and reliability make HA ceramics unsuitable for load-bearing situations. Moreover, composites filled with HA particles have been found to fall short in terms of mechanical strength and durability [2, 3]. To satisfy the physico-chemical demands for clinical applications, reinforcement of HA ceramics and composites have acquired a great deal of importance in past decades. Although reinforcement by whiskers or fibers has been considered as an effective way of improving the mechanical properties [4], most available ceramic, metal and polymer fibrous materials are at least bio-inert, easily provoking a fibrous tissue capsule of a variety of thicknesses when implanted in the body, decreasing the biocompatibility and bioactivity [5-8]. With this view, HA whiskers or fibres appear to be one of the most promising candidates to reinforce the mechanical and biological properties of biocomposite materials. So far, HA whiskers or fibrous HA have been prepared by a variety of methods, such as growth in gel [9], solid-state reaction [10], wet chemical precipitation [11, 12], dissolution-precipitation [13], hydrothermal synthesis [14, 15], molten salt synthesis [16] and electrospun [17].

Among these methods, wet chemical synthesis has been considered as a major route widely-used due to low cost and simplicity. However, whiskers with controlled morphology and composition are difficult to prepare due to the sensitivity to the preparation conditions [18, 19], and the whiskers prepared by most method mentioned above are generally Ca-deficient, with low aspect ratio [11, 12]. Therefore, preparation of long HA whiskers with high aspect ratio is of potential significance for the development of candidate biomaterials for large stress-bearing dental restorations and hard tissue replacement.

Nucleation and crystal growth of whisker-like and fibrous HA from aqueous solution greatly depend on the properties of starting solution and can be controlled by a slow release of Ca ions or by the control of reaction pH value [11, 20-22]. However, the products obtained usually have a large variation in length and aspect ratio, depending on the synthesis methods, concentration of each reactant and temperature. In the case of hydrothermal synthesis, although aqueous solutions containing Ca (or Ca-chelate) and PO_4 could yield whisker-like HA at 120 - 200°C in the presence of additives, for examples of urea, KOH, K_3PO_4 and EDTA [15, 20, 23-25], the outcome was mainly fibrous HA, with low aspect ratio and short length; the nucleation and growth of HA crystals were found to be affected by the synthesis temperature. Unfortunately, despite much effort to improve the quality of HA whiskers, no more

relevant work concerning the effect of the synthesis temperature have been reported. To prepare long and uniform HA whiskers with high aspect ratio, high purity and good crystallinity, acetamide (AA) was introduced as hydrothermal homogeneous precipitation agent to control the nucleation and growth of HA whiskers at low degree of supersaturation in our previous works [26, 27]. The morphology and structural characteristics of the whiskers were strongly affected by the preparation conditions. The purpose here is to elucidate the effect of synthesis temperature on the morphology and growth characteristics of HA whiskers using such additive.

EXPERIMENTAL

Preparation

Aqueous solutions containing Ca and PO₄ were prepared by dissolving analytical grade reagents Ca(NO₃)₂·4H₂O and (NH₄)₂HPO₄ based on 0.05 mol·l⁻¹ Ca and 1 mol·l⁻¹ acetamide (99 %, Alfa Aesar, Heysham, Lancashire, England). Aqueous calcium and phosphate solutions with a fixed Ca/P ratio of 1.67 were used in the first group. The starting pH of each solution was adjusted to 2, 3, or 4 using 0.1 mol·l⁻¹ HNO₃ or 1:1 NH₄OH. After processing at 140, 160, 180, 200 and 220°C for 10 h, respectively, as reported previously [27], the mixture was cooled naturally over 12 h to the ambient temperature. The product was then filtered and washed quickly with deionized water four times, and dried in air at 80°C.

Characterization

The constitution and crystallinity of the product was characterized using X-ray diffraction (XRD) (X'Pert Pro, PANalytical BV, Almelo, The Netherlands) and Fourier-transform infrared spectroscopy (FTIR) (FTS-165, Bio-Rad Inc., Hercules, CA, USA). Morphology and microstructure were observed using scanning electron microscopy (SEM) (Model XL30CP, Philips Electron Optics, Eindhoven, The Netherlands). Transmission electron microscopy (TEM) observation, selected-area electron diffraction (SAED) patterns and high-resolution TEM (HRTEM) images of HA whiskers were performed on a Tecnai G2 20 S-TEM (FEI, Philips, Hillsboro OR, USA) to identify the growth direction of whiskers. XRD

pattern processing software (MDI Jade 5, Materials Data Inc., Livermore, Livermore, California, USA) was used for phase identification and lattice parameter calculation. Image processing software (QWin, Leica Microsystems Imaging Solutions Ltd., Cambridge, UK) was used to determine the aspect ratio of the whiskers and *d*-spacings. The degree of crystallinity (*X_c*) of the HA whiskers was evaluated by the following equation according to the examined XRD diffractograms [28]:

$$X_c = 1 - (V_{112/300}/I_{300})$$

where $V_{112/300}$ is the intensity of the hollow between (112) and (300) peaks and I_{300} is the intensity of the (300) peak. In addition, the Ca/P ratio was determined by energy dispersive X-ray spectroscopy (EDX) using a field emission-scanning electron microscope (FE-SEM) (LEO 1530, Oxford Instruments, Abingdon, UK). The EDX results were believed to be accurate to about 5 %. The pH of the solution was measured with a combination electrode at room temperature (25 ± 2°C). After processing, the pH of the filtrate was measured as representing the final pH of the synthesis solution.

RESULTS

Constitution and structural characteristics

Figures 1 and 2 show the XRD patterns and FTIR spectra of samples of the HA prepared at various temperatures. The products showed similar XRD patterns and FTIR spectra, as reported in previous studies [26, 27]. All XRD peaks matched well those of the reference diffraction pattern (JCPDS PDF 9-432) for synthetic HA, and the products were identified by XRD as Ca-deficient HA, with a Ca/P of 1.59 - 1.62 (Table 1). No other phase than HA was detected. The lattice parameters calculated from the XRD patterns varied: for *a* from 9.430 to 9.435 Å, and for *c* from 6.8817 to 6.9013 Å, coinciding well with those calculated from the reference (JCPDS PDF 9-432). Both *a* and *c* values were slightly greater than for stoichiometric HA. Increasing the synthesis temperature affected neither the lattice parameters nor the crystallinity, but giving rise to an increase in the final Ca/P ratio of the product. For specimens of the products prepared at 160 - 220°C, the XRD intensity

Table 1. Summary of Synthesis parameter and characteristics of the products. All at 180°C, for 10 h; starting pH 3 and Ca/P 1.67, and [AA] = 1 mol·l⁻¹.

Temperature (°C)	Final pH	Ca/P ratio of product	Crystallinity (%)	Lattice parameter	
				<i>a</i> (Å) (<i>a</i> _{obs.} ± S.D.)	<i>c</i> (Å) (<i>c</i> _{obs.} ± S.D.)
140	5.09	1.59	96.87	9.433 ± 0.003	6.895 ± 0.004
160	5.29	1.61	96.44	9.435 ± 0.003	6.901 ± 0.006
180	5.67	1.62	96.09	9.434 ± 0.004	6.896 ± 0.002
200	5.55	1.61	95.96	9.434 ± 0.002	6.898 ± 0.004
220	5.53	1.61	96.17	9.430 ± 0.005	6.882 ± 0.003

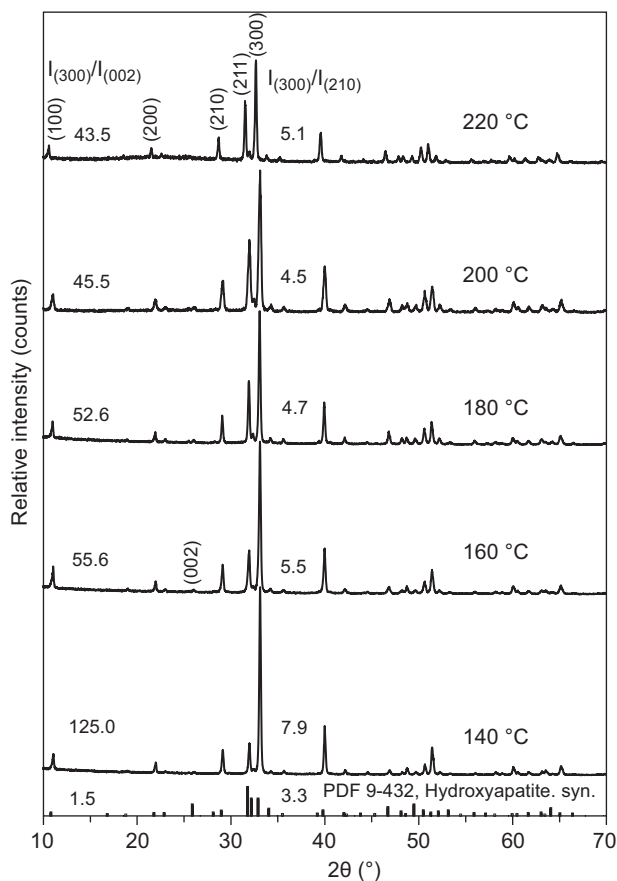


Figure 1. XRD patterns of HA prepared at various temperatures using solutions with a fixed Ca/P 1.67 and a starting pH 3.

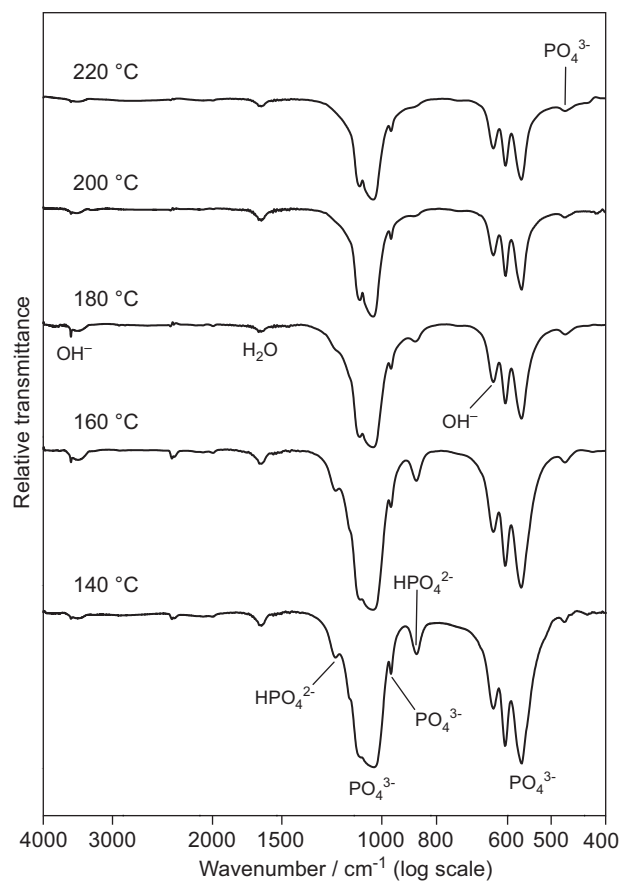
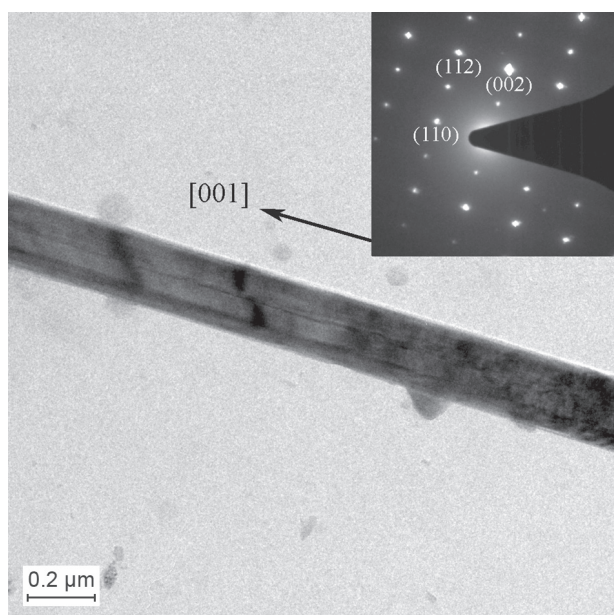
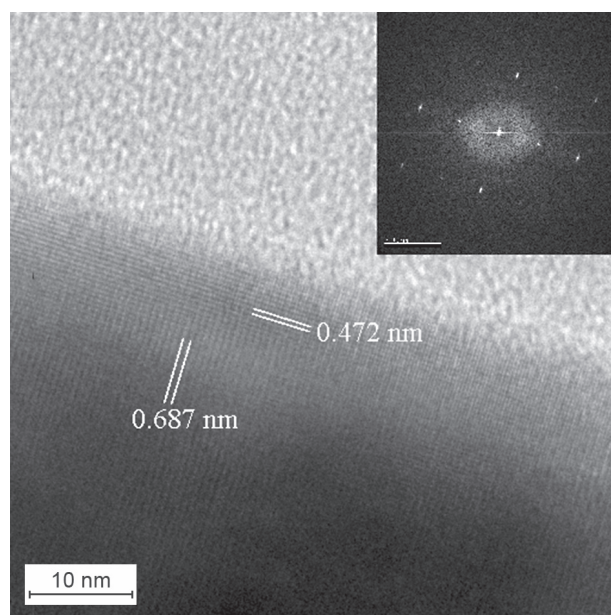


Figure 2. FTIR spectra of HA prepared at various temperatures using solutions with a fixed Ca/P 1.67 and a starting pH 3.



a) TEM



b) HRTEM

Figure 3. TEM images of HA whiskers prepared at 140°C using a solution with a Ca/P ratio of 1.67 and pH 3: a) TEM, low magnification and SAED pattern (insert), and b) HRTEM image and Fourier transform pattern (insert).

ratios, $I_{(300)}/I_{(002)}$ and $I_{(300)}/I_{(210)}$, varied from 43.5 to 55.6 and from 4.7 to 5.5, respectively, and the Ca/P ratio of the products ranged from 1.61 to 1.62. These slight differences indicated that the synthesis temperature did not affect the crystal growth habit along the *c*-axis of HA crystals and the crystallographic characteristics. Further increase in temperature over 180°C did not show any obvious effect on the constitution or crystallinity of whiskers. However, for the samples prepared at 140°C, the intensity ratios for both $I_{(300)}/I_{(002)}$ and $I_{(300)}/I_{(210)}$ were higher than at other temperatures, and the product formed at such low temperature had slightly higher Ca-deficiency, with a Ca/P ratio of 1.59.

All the above findings could be also confirmed by the FTIR and TEM analysis. The characteristic bands at 1092, 1034, 602 and 564 cm^{-1} for phosphate group, and for hydroxyl at 3570 and 633 cm^{-1} , were similar in each [29]. However, the absorption band at 871 cm^{-1} , attributed to the symmetrical stretching vibration of HPO_4^{2-} group, was obviously visible at low temperature, and their intensity was gradually decreased with increasing the temperature. The bending mode of HPO_4^{2-} group at 1208 cm^{-1} only appeared in the spectra for specimens prepared at 140 - 160°C [29-31]. Although the products obtained at low temperature had high Ca-deficiency, the preferred growth orientation along the *c*-axis did not show any change (Figure 3). Strong reflection spots were identified as (002), (110) and (112) in the SAED pattern, and the whiskers had high purity and crystallinity [12]. The longitudinal direction of the whiskers was parallel to the [001] direction. The calculated *d*-spacings for the (002), (110) and (112) lattice planes gave 3.426 Å, 4.721 Å and 2.774 Å, respectively; and the interfacial angles of $\alpha_{(110), (002)}$ and $\alpha_{(112), (002)}$ ranged 89.94° and 36.27°. They matched well those calculated from the reference (JCPDF 9-432), which suggested that the prepared whiskers had the same crystallographic characteristics as normal HA crystals, despite the strongest XRD peak intensity appeared for the (300) lattice plane, rather than (211) as is usual for HA. Moreover, the HRTEM image showed a clear atomic arrangement and two lattice periodicities, the *d*-spacings for the (001) and (110) lattice planes gave 0.687 nm and 0.472 nm, respectively, being coincident with the XRD analysis results.

Effect of initial pH

The variation of length and width of the products prepared at various temperatures and initial pHs is shown in Figure 4. The length increased initially with the temperature, and then decreased for temperatures > 180°C; the whiskers prepared at pH 3 showed a high mean value in length. For whisker width, it increased with both the initial pH and the temperatures for samples prepared at the pHs 2 and 3. Long and uniform whiskers with high aspect ratio were obtained at 160 - 200°C and pH 3 or at 180°C and pH 2 (Figure 5), giving a mean

length of 94 - 116 μm and an aspect ratio of 89 - 103. However, for the pH 4, quick growth in width was clear when the temperature was at 180°C, and the both the length and width decreased with further increase in temperatures, lath- or branch-like HA was often found to accompany the whiskers, being independent of the synthesis temperatures. In addition, when the preparation was conducted at 140°C, a few irregular particles or rod- or lath-like HA were found to appear in the products; at 220°C, some aggregations composed of small needle-like particles or irregular plate-like particles were found in the products.

DISCUSSION

The effect of temperature on nucleation and crystal growth could be expressed through two aspects. The rate of nucleation and crystal growth benefits from increase in synthesis temperature: higher temperature, greater driving force. On the other hand, the rate of hydrolysis of AA is increased at a high temperature, which in turn made the solution pH increase more quickly. Here, these two changes led to a high supersaturation in a short period at high temperature, and more nuclei were formed initially. More [Ca], $[\text{PO}_4]$ and $[\text{OH}]$ would be needed to satisfy the growth of large numbers of nuclei. A conflict occurred between the formation rate of nuclei and their growth; the crystal growth was limited by the concentration of each nutrient component. The supply of Ca and PO_4 and OH became the rate-determining factor for crystal growth. As shown in Table 1, the final pH of synthesis solution increased firstly with the temperature and then decreased when the temperature was over 180°C, being regardless of the starting Ca/P ratio and initial pH. Accompanying the decrease of the final pH, the length of the whiskers also decreased (Figure 4), which was supposed to be caused by more nuclei formed in the solution and shortage in OH ions supply at high temperature. At low temperature, the crystals grew in a relatively-low pH due to slow hydrolysis rate of additive. The supply of each nutrient component could meet well the crystal growth, the length increasing with the temperature. However, the morphology of products was easily affected by the solution pH, and a few irregular particles accompanied the whiskers for the preparation at 140°C. Meanwhile, high H^+ activity could be the driving force for introducing hydrogen ions into the Ca-sites of HA crystals, resulting in the formation of Ca-deficient HA [32]. Therefore, the whiskers obtained at low temperature showed appreciable HPO_4^{2-} bands in the FTIR spectra.

On the other hand, hydrothermal homogeneous precipitation of HA whiskers here is realized by the control of the solution pH value. The rate of nucleation and growth of crystals intimately depend on the degree of supersaturation, which directly relates with the [Ca]

and $[\text{PO}_4]$ in the starting solution and the hydrolysis rate of AA. Since the total amount of Ca and PO_4 and the AA concentration were fixed throughout the whole experiments, the change of the starting pH gave rise to the variation of the degree of supersaturation, eventually affecting the nucleation and growth of HA crystals.

For solutions with a given starting Ca/P, higher initial pH provided a basic reference for quickly obtaining supersaturation for the formation of HA nuclei. Higher initial pH, more nuclei in the solution. Thus, the length varied with the initial pH at the examined temperatures, giving a high value at pH3 (Figure 4). In the case of

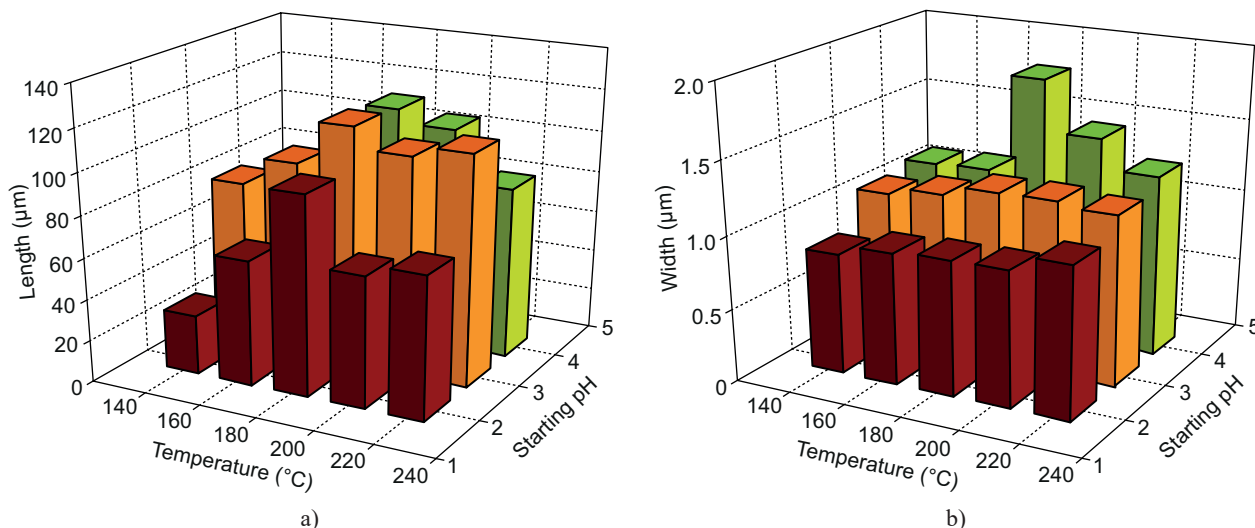
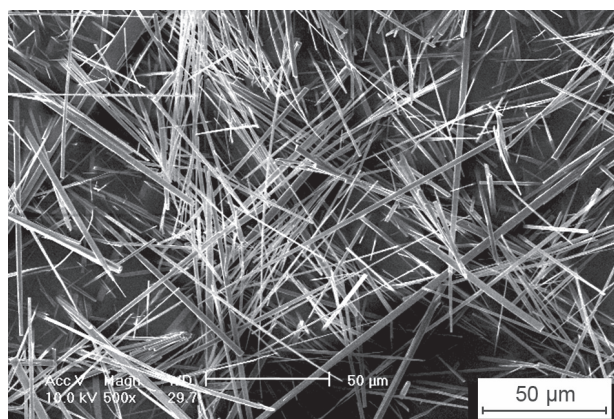
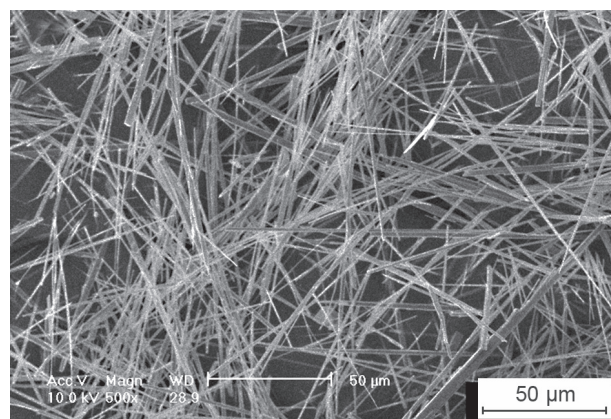


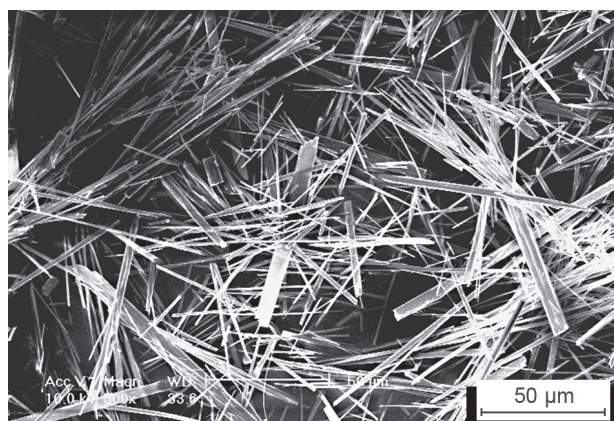
Figure 4. Variation of length and width of HA prepared at 140, 160, 200 and 220°C under various starting pHs.



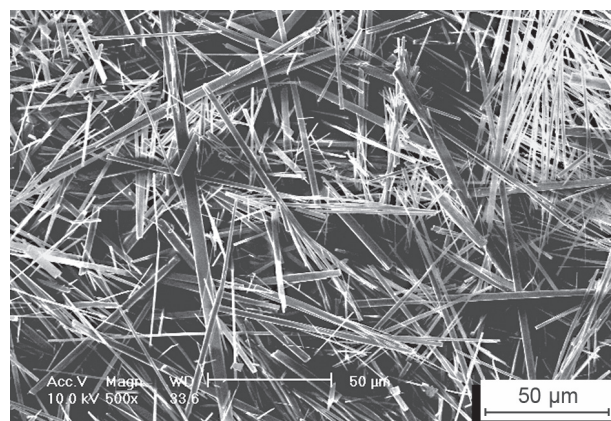
a) pH 3 and 200°C



b) pH 2 and 180°C



c) pH 4 and 140°C



d) pH 4 and 220°C

Figure 5. SEM images of HA prepared using various starting pHs and temperatures at Ca/P of 1.67: a) pH 3 and 200°C, b) pH 2 and 180°C, c) pH 4 and 140°C, d) pH 4 and 220°C.

the initial pH of 4, since the competition with hydrogen ions for adsorption of Ca on {100} faces became less intense due to the electrostatic attraction by PO₄, the incorporation of more Ca on {100} faces would accelerate the growth along the a-axis of the crystal. Thus, lath- or branch-like HA often appeared in the whiskers, being independent of the synthesis temperatures, which was coincident with our previous research [27].

Summary

Pure Ca-deficient HA whiskers were prepared at temperatures from 140 to 220°C. The temperature studied here did not affect the constitution and crystallinity of the products as well as the crystal growth habit along the c-axis. However, the Ca/P ratio of the products increased with the temperature, and the whiskers obtained at low temperature had high calcium deficiency. Whisker morphology greatly depended on the synthesis temperature and was correlated with the starting pH of the synthesis solution. Higher initial pH only provided a basic reference for quickly obtaining supersaturation for the formation of HA nuclei. Lath- or branch-like HA often appeared in the whiskers at high pH, being independent of the synthesis temperatures. Neither high nor low temperatures were beneficial to the preparation of long HA with high aspect ratio. 180 - 200°C seemed to be an appropriate synthesis temperature to prepare long HA whiskers using a solution with Ca/P 1.67 under a starting pH 3.

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