

Strontium-substituted calcium phosphates prepared by hydrothermal method under linoleic acid–ethanol solution

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Received 11 June 2009; received in revised form 9 July 2009; accepted 1 October 2009

Available online 3 November 2009

Abstract

Strontium-incorporated calcium phosphates show potential in biomedical application, particularly the doped strontium may help to new bone formation. In this study, the particle was synthesized by hydrothermal treatment at 120 °C for 15 h. It was found that the phase purity and aspect ratio was significantly affected by the addition of linoleic acid due to reduction of solution pH. In particular, strontium played important role in the transformation of minerals. Below 20% solution of calcium substituted by strontium, only apatite was formed; above it, a mixture of apatite and DCPA was detected, and α -SrHPO₄ was formed if all calcium solutions were replaced by strontium. Meanwhile, the adsorption of fatty acid on crystal was detected by FTIR. In summary, the incorporated fatty acid not only affected the morphology, but also its composition.

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Keywords: A. Powders: chemical preparation; D. Apatite; E. Biomedical applications

1. Introduction

Poly(methyl methacrylate) (PMMA) has been widely used for bone cement to affix implants and remodel lost bone for several decades due to good biocompatibility, ease of handling, good biomechanical strength and cost-effectiveness. However, the disadvantage is frequently mentioned due to poor adhesion to bony surfaces. The attempts to increase its bioactivity have been extensively reported such as the addition of bioactive materials [1,2]. In particular recently, the incorporated strontium is showing great potential, such as coating on titanium [3], bone cement filler [4] and toothpaste [5]. It was found that strontium could enhance preosteoblast differentiation, inhibit osteoclast differentiation, and thus reduce osteoclast function [6], thus an oral drug – strontium ranelate (SrR) has been confirmed to be particularly effective in the treatment of osteoporosis for postmenopausal women [7].

As the main composition of bone and teeth, hydroxyapatite (HA) and related calcium phosphates play a special interest in orthopaedics treatment as bone filler and substitution materials. Therefore, the incorporation of strontium is particularly important to develop novel bioceramics for biomedical application, which can be prepared by sol–gel [8], hydrothermal synthesis [9], solid reaction [10] and wet method [11]. The substitution of calcium by strontium is expected to be easy on the basis of chemical similarity, but the distortion of crystal structure may be occurred due to larger ionic radius of strontium, thus the solubility increases attributing to the decrease of crystal stability [12], particularly, the release of strontium is expected to stimulate new bone formation.

In human body, essential fatty acids are primarily used to produce hormone-like substances that regulate a wide range of functions, including blood pressure, blood clotting, blood lipid levels, immune response and inflammation response to injury infection. Thus, the incorporation of unsaturated fatty acid may decrease the risk of inflammation in orthopaedic treatment [13,14]. In particular, two of them linoleic acid (LA) and alpha-linolenic acid (ALA) cannot be produced by human itself, but are widely distributed in plant oils [15]. In addition, fatty acids

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with different chains geometry have been recently reported to significantly affect particle morphology and phase purity via adjusting synthetic temperature and linoleic acid/ethanol ratio [16]. Thus, the present aim of this work was to study the nucleation of calcium phosphates affected by strontium using hydrothermal treatment, linoleic acid was used to adjust the synthetic conditions. The composition, constituent and morphology of the precipitations were characterized by XRD, SAED, EDX, SEM, TEM and FTIR.

2. Experimental procedures

1.25 g of octadecylamine, 25 ml linoleic acid and 25 ml of ethanol were firstly mixed by agitation. Theoretical Sr/(Ca + Sr) ratio of 0%, 2%, 5%, 20%, 40% and 100% was prepared by dissolving required calcium nitrate/strontium nitrate into 17.5 ml deionized water. And then, the solution was added to pre-prepared octadecylamine–fatty acid–ethanol mixture. After agitating around 10 min, sodium phosphate solution was added with theoretical (Ca + Sr)/P ratio of 1.67 for apatite. The solution was further agitated rigorously for 10 min, and then hydrothermally treated at 120 °C for 15 h. The precipitates were washed respectively by 70% ethanol and dehydrated absolute ethanol twice. Besides, pure HAp was prepared as comparison without treatment by fatty acid, no other condition was changed. All chemicals used here are ACS grade or Wako grade I or above.

The composition and structure of prepared solids were respectively determined by X-ray diffraction (XRD; Model D/max 2550 V, Rigaku, Tokyo, Japan), using Cu K α ($\lambda = 1.5406 \text{ \AA}$) radiation in step-scan mode ($2\theta = 0.02^\circ$ per step) and selected area electron diffraction (SAED; Technai G2 20 TEM, FEI, Hillsboro, OR, USA). Standard JCPDS: HAp (72-1243), CaHPO $_4$; DCPA (77-0128) and α -SrHPO $_4$ (33-1335) were used to compare. Element analysis by energy-dispersive X-ray analysis (EDX) (LEO 1530 FESEM, Oxford Instruments, Oxford, UK) was used to calculate the substitution degree of calcium by strontium in the pre-

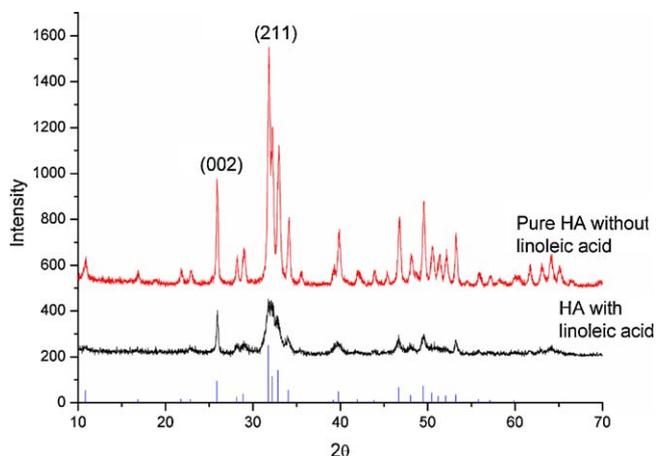


Fig. 1. X-ray diffraction patterns of prepared HA with and without control by fatty acid. Standard JCPDS: HA (72-1243) used to compare.

cipitates. The particle morphology was respectively observed by scanning and transmission electron microscopy (FESEM, LEO 1530; TEM, Technai G2). The functional group was analyzed by Fourier transform infra-red (FTIR; PerkinElmer, USA).

3. Results and discussion

Well-crystalline HA was prepared by hydrothermal treatment at 120 °C for 15 h, but the crystallinity became poorer with the addition of linoleic acid (Fig. 1). The standard XRD pattern of HA (JCPDS 72-1243) gives the ratio of the peak intensity of (0 0 2) reflection relative to that for the (2 1 1) reflection as 0.40. However, the ratio determined here (Fig. 1) gives the ratio respectively as 0.42 (without linoleic acid) and 0.87 (with linoleic acid). The observed change in the ratio was attributed to the growth of crystal with preferred alignment along (0 0 2) reflection, indicating as high aspect ratio (length/diameter) of the particles (Fig. 2b). But no preferred alignment along (0 0 2) reflection was found in the precipitates without

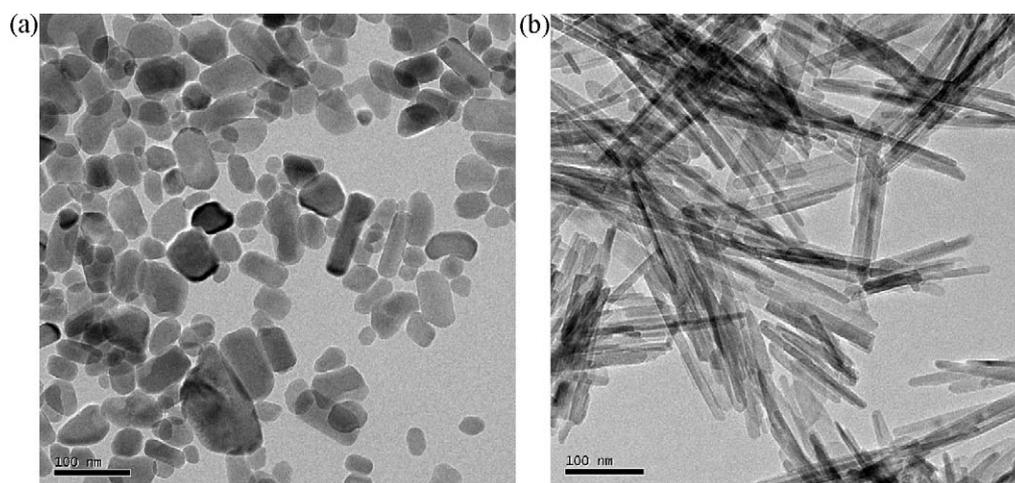


Fig. 2. TEM morphology of the prepared HA (a) without treatment by fatty acid and (b) treated by fatty acid.

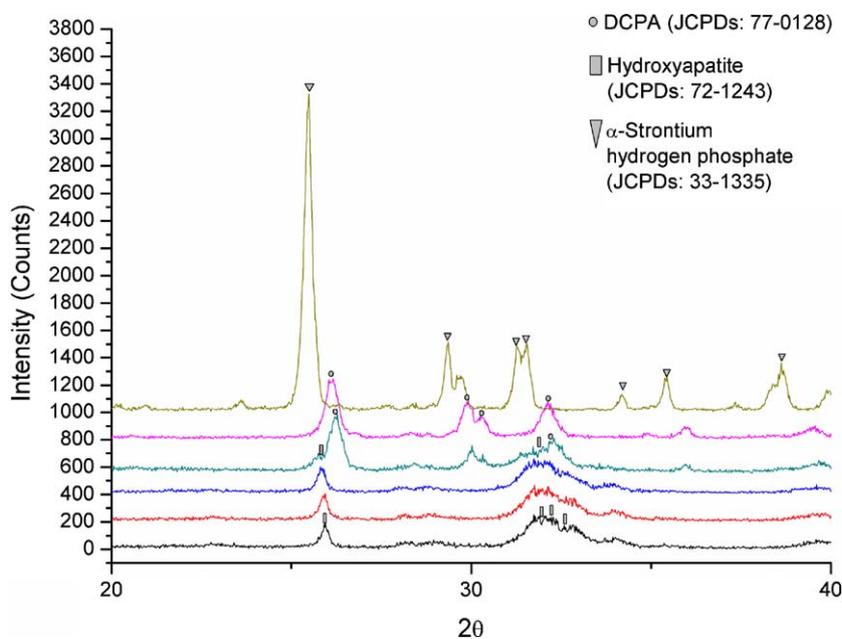


Fig. 3. X-ray diffraction patterns of prepared strontium-substituted calcium phosphates.

treated by linoleic acid (Fig. 1), indicating as fine particles with lower aspect ratio (Fig. 2a). Although HA is considered as thermodynamic stable phase above pH 4.2, nucleation is favored at higher pH. However, it was found the solution pH was dramatically affected by the addition of linoleic acid (down to ~ 5.80), controlled by the slow hydrolysis of linoleic acid, thus indicating as larger length and aspect ratio of the crystals and less crystallinity. Therefore, it is obvious that the super-saturation degree of solution can be controlled by the activity product of ions, which in turn depends on solution pH.

Furthermore, XRD peaks slightly deviated from the standard HA JCPDS card with the increase of strontium concentration in solution (Fig. 3), possibly due to the incorporated strontium ion, because the ionic radius of strontium (113 pm) is appreciably larger than that of calcium (99 pm). Meanwhile, preferred alignment along (0 0 2) reflection was also detected, where peak intensity of (0 0 2) reflection relative to that for the (2 1 1) reflection was respectively as 0.91 and 0.88 when partial solution calcium was substituted by strontium (at 2% and 5%) (Figs. 3, 4b, 4c, 5b and 5c).

However, when 20% solution calcium was substituted by strontium, not only poor-crystalline apatite, but also a well-crystalline DCPA was detected in the precipitates. It was found that the crystallinity of apatite decreased with the increase of strontium content, where the peaks were partially overlapped by the sharp peaks of DCPA (Fig. 3). Thus, as indicated in their morphology as two phases coexisted: DCPA (plate-like) and Sr–HA (whisker-like) (Figs. 4d and 5d). Although apatite seems to be the most stable phase at current pH, nucleation difficulty leads to formation of poor crystallinity particle. Thus metastable phase was formed.

When 40% solution calcium was substituted by strontium, only well-crystalline DCPA was found by XRD, no apatite peaks were detectable (Fig. 3). However, the morphology indicated two phases coexisted: large plate-like crystal and small whisker-like particles (Figs. 4e and 5e). The formation of large crystal is consistent with XRD pattern as DCPA. However, the morphology of small particles is similar to above crystals, indicating as apatite, although XRD peaks were not readable. SAED (Fig. 5e) confirmed that the small particles were apatite, the two bright rings indicating as (0 0 2) and (2 1 1) reflection. Here, it needs to be pointed out that amorphous phase was normally determined by XRD due to lack of long-range order. However, the difficulty in the determination of calcium phosphates lies in the small nano-scale and poor crystallinity, the check of SAED is therefore necessary.

Solution pH plays an extremely important role in the transformation of minerals. HA is reported to be the most stable phase above pH 4.2, although recently found less soluble at lower pH than any other calcium phosphates, in particular widely believed DCPA, only HA is considered as the thermodynamic phase [17]. In addition, the initial pH of the solution before hydrothermal treatment was around 11.65 without the addition of fatty acid, thus it was previously reported that pure Sr–HA can be formed even the substitution degree reaching 60% and 100% [12]. However, the solution pH measured in this study was found to be dramatically decreased to ~ 5.80 due to the incorporation of fatty acid, here no significant change of pH was detected by the addition of different amount of strontium. Therefore, it is believed that the formation of DCPA is just a kinetic issue; the nucleation of apatite seems to be difficult with the increase of strontium content at lower

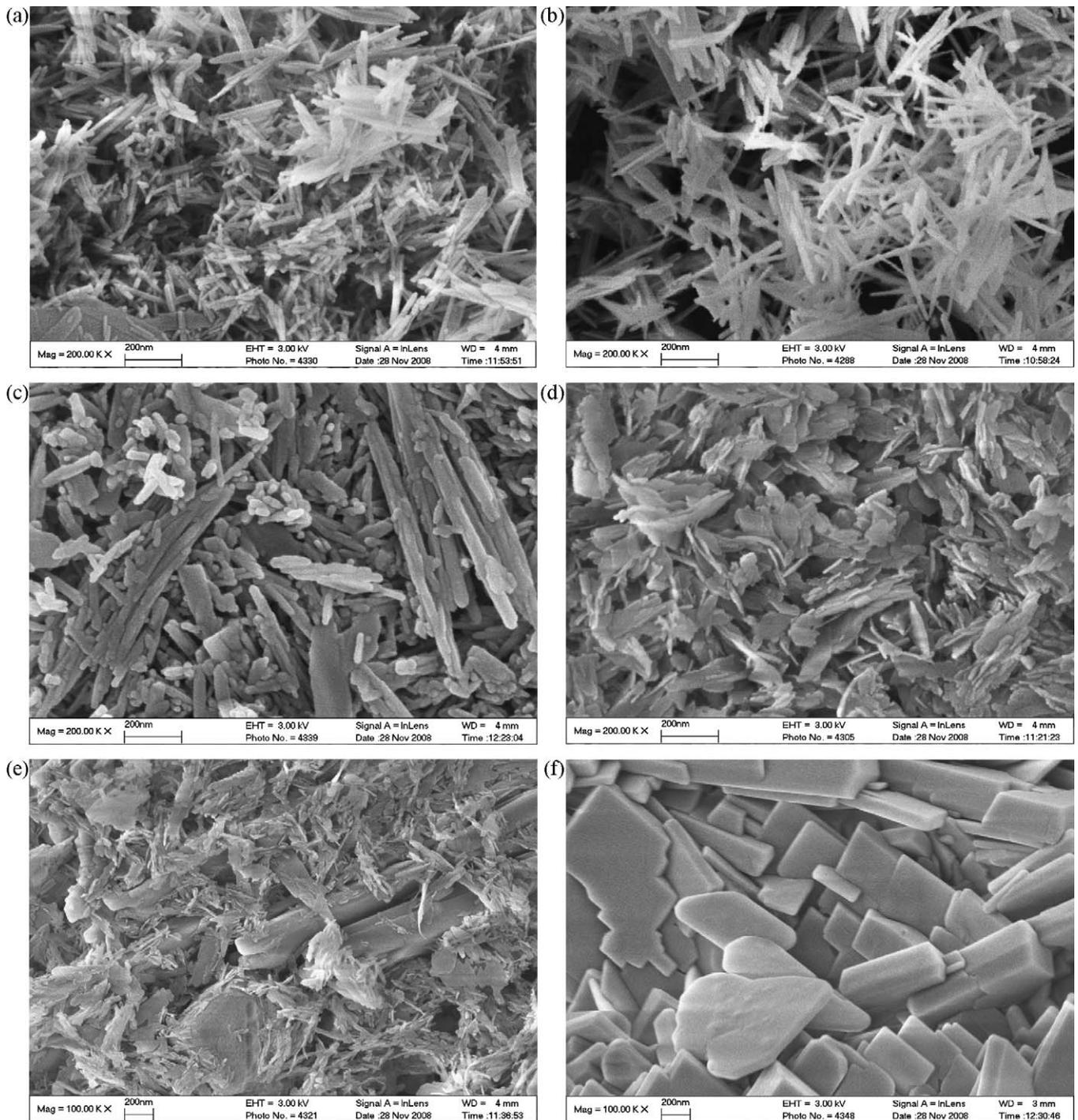


Fig. 4. SEM morphology of the prepared solids with theoretical substitution degree: (a) 0%, (b) 2%, (c) 5%, (d) 20%, (e) 40% and (f) 100%.

pH range, in particular more stable phase HAP was also found.

Furthermore, no apatite-like phase was detectable when all solution calcium was substituted by strontium, the only identified phase was α -SrHPO₄ (Fig. 3), indicating as large plate-like crystal (Figs. 4f and 5f). Therefore, the incorporated strontium significantly affected the nucleation of calcium phosphates. In addition, due to chemical similarity, the

substitution of calcium by strontium seemed to be easy, thus the calculated substitution degree was close to the theoretical values, as shown in Table 1. Therefore, the formation of Sr-HA appears to depend on a threshold substitution degree of strontium at $\sim 20\%$ due to solution pH was adjusted by fatty acid.

Besides, the absorption of $-\text{CH}_3$ and $-\text{CH}_2$ at 2922 cm^{-1} and 2924 cm^{-1} respectively indicated linoleic acid was

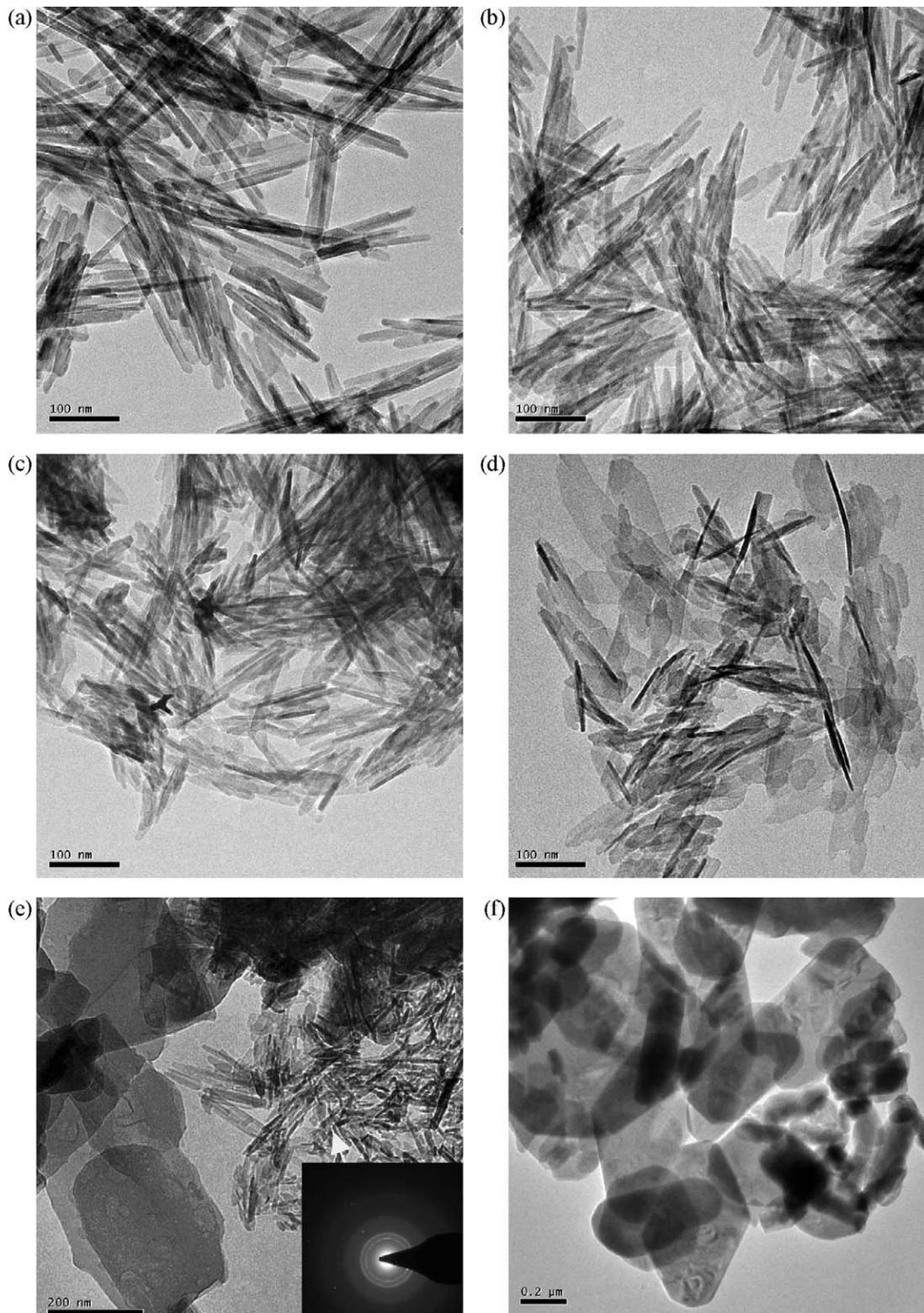


Fig. 5. TEM morphology of the prepared solids: (a) 0%, (b) 2%, (c) 5%, (d) 20%, (e) 40% and (f) 100%. SAED used to identify the composition of small crystals in (f).

chemically adsorbed on the particle surface (Fig. 6), thus it verified that the prepared crystal was indeed coated by linoleic acid. In addition, according to the solubility diagram of Ca–P system [17], DCPA is more soluble than HA. Therefore, it is believed that α -SrHPO₄ also dissolves much

faster than Sr–HA due to chemical similarity, but the dissolution behavior, bioactivity and related biocompatibility, particularly the cytotoxicity arisen by rapid release of strontium has to be extensively studied, which will be included in the next step.

Table 1

Element analysis of Ca and Sr in the precipitates by EDX. Calculated Sr/(Ca + Sr) ratio was used to compare the theoretical values (mean \pm SD, $n = 5$).

Theoretical solution	Ca	Sr	Calculated Sr/(Ca + Sr) Sr/(Ca + Sr) (%)
0	8.32	N/A	N/A
2	8.61	0.16	1.82 \pm 0.17
5	10.56	0.64	5.71 \pm 0.33
20	10.98	3.01	21.51 \pm 1.78
40	8.43	5.39	39.00 \pm 3.42
100	N/A	10.13	N/A

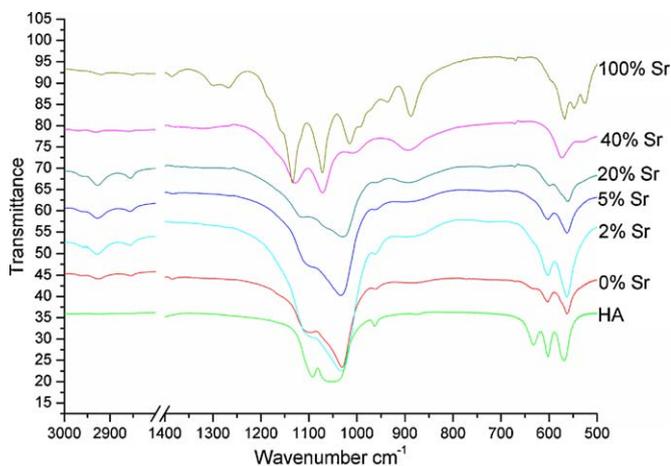


Fig. 6. FTIR spectra of as-prepared solids.

4. Conclusion

Fatty acid (linoleic acid) significantly affected the prepared crystal not only in morphology, but also its composition. The substitution degree at \sim 20% of solution calcium by strontium seemed to be a threshold leading to the phase transformation, below this value, pure Sr–HA was formed; above this value; two phases coexisted (DCPA and Sr–HA) at both 20% and 40%, and only phase of α -SrHPO₄ at 100%.

Acknowledgement

This research was supported by Hong Kong Innovation Technology Commission ITF-GHP 009-06 and RGCHKU 714707E.

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