Advanced Powder Technology 25 (2014) 1661–1666

Contents lists available at ScienceDirect

Advanced Powder Technology

journal homepage: www.elsevier.com/locate/apt

Original Research Paper

A facile hydrothermal method for synthesis of submillimeter-long octacalcium phosphate and hydroxyapatite as drug carriers with sustained release behaviors

Chengfeng Li [a,*], Xiaolu Ge [b], Guochang Li [a], Qiaochun Gao [a], Rui Ding [a]

[a] School of Materials Science and Engineering, Shandong University of Technology, 255049 Shandong, PR China
[b] School of Science, Shandong University of Technology, 255049 Shandong, PR China

ARTICLE INFO

Article history:
Received 15 December 2013
Received in revised form 22 May 2014
Accepted 3 June 2014
Available online 14 June 2014

Keywords:
Octacalcium phosphate
Hydroxyapatite
Hydrothermal method
Fiber
Drug carrier

ABSTRACT

A facile hydrothermal method was employed to synthesize submillimeter-long octacalcium phosphate (OCP) and hydroxyapatite (HAp) crystals as drug carriers. After hydrolysis reaction of urea, calcium phosphate fibers grew preferentially along c-axis due to modulation of crystallization by glutamic acid. With increasing addition of urea, OCP and HAp precipitated with decreased values of crystallite size and aspect ratio. It was found that the conversion of OCP to HAp was completed after hydrothermal treatment at 160 °C for 6 h. The formation of fibrous crystals and adsorption of water-insoluble drugs were accomplished in one-step synthesis process with presence of ibuprofen molecules. The sustained-release behavior of ibuprofen could be described by a Higuchi model. HAp and OCP fibers might be simultaneously used for high-performance reinforcements to improve mechanical properties of composite materials and sustained-release drug carriers to cure postoperative infection of bone replacement.

1. Introduction

In contrast to metal and bioinert ceramic, hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) was native to human body because it was similar with the main mineral component of bones and teeth. Bioactive HAp was then made superior as a biomaterial to facilitate both the integration of osseous tissues with implant and the regeneration of bones. However, post-operative pathologies, like infection or inflammation of bone sites associated with the surgery of bone filling or replacement had severely affected bone healing processes [1–5]. Orthopaedic drug delivery systems of HAp were thus developed through loading drug substances in porous matrix, mesoporous nanoparticles and coating on metal substrates [6–8]. However, it was still challenging to attain a sustained release in a controlled manner because the release rates of drug in all these endeavors were too rapid [9].

Until now, clinical applications of HAp included only coatings, bone fillers and substitutions for bones withstood free or low dynamic loads due to its low value of fracture toughness [10,11]. To improve the reliability of bulk material, HAp whisker or fiber-reinforced composite was developed as a promising material for hard tissue replacement. Its performance was seriously dependent on aspect ratios, surface reactivity and crystallinity of HAp reinforcements [12,13]. And thus, great endeavors were made on synthesis of HAp with high aspect ratio by molten salt reaction [14], high-temperature solid state reaction [15] and hydrothermal processes [13,16–18]. To date, it was still difficult to synthesize well-crystallized HAp with uniform morphology and high aspect ratio (>100) because the crystallization was quite sensitized to preparation conditions [19]. Generally, a few crystal nuclei were generated in a homogeneous condition with low supersaturation and these crystals would grow large. However, the reaction yield of a precipitation reaction was rather limited and the yield percent was only about 10% in our previous report [20].

With the purpose to synthesize calcium phosphate fibers with high values of drug-loading capacity, aspect ratio and yield percent, a modified hydrothermal method was herein employed through the precipitation of calcium phosphate with presence of urea, glutamic acid and hydrophobic drug molecule. Dissociation reactions of these molecules at high temperatures could alter concentrations of all growth units of calcium phosphate, which would dramatically improve the reaction yield and specifically modulate the crystallization behavior. Newly-generated calcium phosphate clusters might be expected to provide abundant binding sites for adsorption and storage of drug molecules. This method

* Corresponding author. Tel.: +86 533 2782232; fax: +86 533 2781665.
E-mail address: cfli@sdut.edu.cn (C. Li).

http://dx.doi.org/10.1016/j.apt.2014.06.001
0921-8831/

had significant advantages, such as ease of handling, low cost, environmental friendliness and promising application in drug delivery [21]. A twofold beneficial effect would be achieved after combining reinforcing properties of bioactive fibrous crystals with a sustained drug release.

2. Methods

2.1. Sample preparation

Products of calcium phosphate were prepared by a modified hydrothermal method as reported previously [20]. Typically, chemical reagents with analytical purity were added to 45 mL of aqueous solution in the following sequence: 0.60 g of L-glutamic acid, 0.38 g of dibasic sodium phosphate ($Na_2HPO_4{\cdot}12H_2O$), 0.07 g of sodium hydroxide (NaOH), 0.05–0.15 g of urea and 0.28 g of Calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$). The transparent reaction solution was sealed in a container and then a hydrothermal treatment was performed at 100 or 160 °C with a dwell time of 24 h. After separation, as-received precipitates were obtained and finally dried for 24 h. For synthesis of drug carriers, reaction solution was prepared by the above process with presence of 0.15 g of urea and 0.40 g of ibuprofen (IBU). It should be mentioned that the hydrothermal treatment at 100 °C was performed in a 250 mL glass bottle sealed tightly with a rubber stopper, while hydrothermal reaction at 160 °C was carried out in a 50 mL Teflon container.

2.2. Characterization

X-ray diffraction (XRD) characterization was performed on a D8 advanced X-ray polycrystalline diffractometer (Cu kα radiation, wavelength: $\lambda = 0.15406$ nm). The crystallite size, t_{hkl} (nm), was calculated from the broadening of XRD peak according to the Scherrer equation:

$$t_{hkl} = k\lambda \cos\theta/\beta_{hkl} \quad (1)$$

where k was the shape factor with a typical value of about 0.89, β_{hkl} was the full-width at half-maximum, and θ was the peak diffraction angle for (hkl) Miller's plane. Crystal morphologies of HAp and octacalcium phosphate ($Ca_8(HPO_4)_2(PO_4)_4{\cdot}5H_2O$, OCP) were characterized by scanning electron microscopy (SEM, FEI SIRION 200). Fourier transform infrared (FTIR, Nicolet 5700, Thermo, USA) spectra were obtained with the wavenumbers recorded from 400 to 4000 cm^{-1} at a 1 cm^{-1} resolution. In order to determine the crystallinity, the infrared spectra splitting factor (IRSF) was calculated by summing the heights of 563 and 603 cm^{-1} peaks and dividing this value by the height of the trough between them. All heights were measured from a base line drawn from approximately 495 to 780 cm^{-1} [22].

2.3. In vitro drug release

Phosphate-buffered saline (PBS, pH: 7.2–7.4) was prepared in deionized water containing 13.7 mM of NaCl, 2.7 mM of KCl, 4.3 mM of Na_2HPO_4 and 1.4 mM KH_2PO_4. 0.0300 g of drug-loaded calcium phosphate precipitates were immersed in 50 mL of fresh PBS at 37 °C under constant shaking (110 rpm). The IBU release medium (1.0 mL) was extracted at given time intervals and replaced by the same volume of fresh PBS preheated to 37 °C. Absorbance of IBU in release medium was measured by a UV–Vis analysis (TU1901) at the wavelength of 222 nm [23]. The measurement was repeated in triplicate.

3. Results and discussion

3.1. Influence of urea addition on the formation of OCP

In reaction solution with absence of urea, dissociation reactions of phosphate ions generated most ions of $H_2PO_4^-$ and rare ions of HPO_4^{2-} and PO_4^{3-} [20]. Calcium phosphate was hardly precipitated even after hydrothermal treatment at 100 °C for 24 h. After addition of urea, hydrolysis of urea at 100 °C facilitated the generation of hydroxyl ion (OH^-) in reaction solutions:

$$(NH_2)_2CO + H_2O \rightleftharpoons 2NH_3 + CO_2 \quad (2)$$

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^- \quad (3)$$

The OH^- ions resulted in the dissociation of $H_2PO_4^-$ ions to yield more HPO_4^{2-} and PO_4^{3-} ions. And thus, OCP would precipitate. It was found that XRD pattern of the sample synthesized at 100 °C for 24 h agreed well with pure phase of OCP (JCPDS No. 26-1065) as shown in Fig. 1. The reaction yield of OCP precipitation increased significantly to almost 85%, which was dramatically higher than that with absence of urea [20].

In FTIR spectra of Fig. 2, all bands derived from PO_4^{3-} groups (560–600 and 1030–1090 cm^{-1}) were clearly visible. The observation of bands at 3354.3 and 1635.3 cm^{-1} was a reflection of water molecule on crystal surfaces. The absorption band at 962.3 cm^{-1} was assigned to antisymmetric stretching vibration of PO_3 in HPO_4^{2-}, indicating the formation of OCP [24]. The stretching bands of CH_3 groups in glutamate were found at 2955.8 and 2887.0 cm^{-1}. The absorption bands at 1405.9 and 873.4 cm^{-1} indicated the entrance of CO_3^{2-} ions into calcium phosphate precipitates [25] because of the formation of CO_3^{2-} ions after the hydrolysis of urea.

Products of OCP exhibited fibrous morphology and uniform length as shown in Fig. 3. Both length and aspect ratio of length/radius decreased with increasing addition of urea, which was similar with the variation of IRSF as shown in Table 1. According to the classical theory of crystallization, the supersaturation degree of OCP increased in reaction solution containing more urea due to the increasing concentrations of HPO_4^{2-} and PO_4^{3-} ions. In this case, the nucleation rate became fast and the growth rate of crystal decreased.

3.2. Conversion of OCP to HAp phase

In order to prepare HAp, hydrothermal temperature should be raised to higher than 140 °C [26]. Herein, reaction solution was firstly treated at 100 °C for 24 h and then followed by a

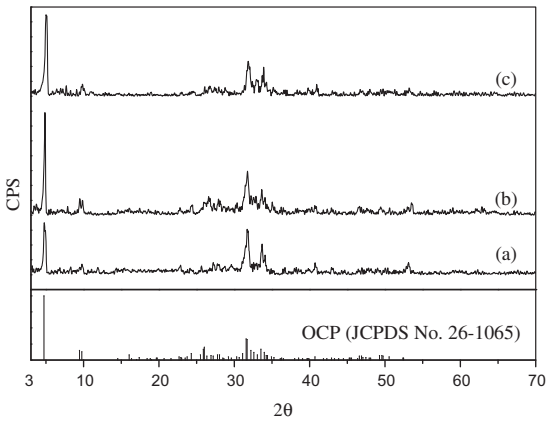

Fig. 1. XRD patterns of samples synthesized at 100 °C for 24 h with different additions of urea: 0.05 g (a), 0.10 g (b) and 0.15 g (c).