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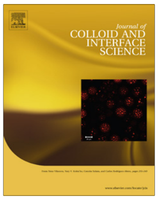

Hydrothermal synthesis of hydroxyapatite nanorods in the presence of sodium citrate and its aqueous colloidal stability evaluation in neutral pH

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ABSTRACT

In this paper, colloidal hydrophilic hydroxyapatite nanorods were synthesized in the presence of sodium citrate via thermal-decomplexing method. The influence of sodium citrate/Ca ions molar ratio (CT/CM) on the synthesis of HA nanorods was characterized in term of structure, size, morphology, and colloidal stability through TEM, XRD, Zeta potential, DLS and long-term standing test. The results show that increasing CT/CM would evidently facilitate high aspect ratio and enhance colloidal stability of nanorods. When the CT/CM is above 1/3, well crystallized HA nanorods with mean aspect ratio up to 5 would be obtained and kept colloidally stable for over two months in neutral pH (7.3–7.5). The origin responding to the results is that increasing CT/CM would enhance the inhibiting growth effect of sodium citrate and increasing absorption density of citrate ions on the particle surface. This work provides new insights into the role of citrate on both the crystallization and tailoring of colloidal stability. Moreover, the synthesized HA nanorods are promising materials for use as carriers for local targeted drug delivery systems as well as building blocks for the preparation of nanostructured scaffolds for cells in bone tissue engineering.

1. Introduction

Hydroxyapatite is the most stable calcium phosphate phase in physiological conditions and the model compound used to denote the mineral component of bone and dentin because of its excellent biocompatibility and biological activity and strong ion exchange capacity [1]. Artificial HA (hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$), possessing extremely similar properties as the natural one, is widely used in a range of fields, such as bone tissue engineering [2,3], heavy metal ions absorption [4,5], fluorescent materials [6,7], protein separation [8,9] and drug carriers [10,11]. In which well-crystalline monodisperable HA nanorods with good aqueous colloid stability in neutral pH are always pursued as desirable basic units for biology-related applications.

In this context, many attempts have been made to develop synthesis strategies in response to that purpose such as hydrothermal method [12,13], chemical precipitate method [14,15], wet chemical method [16,17], and sol–gel method [18,19]. Among these methods, hydrothermal method has raised up much interest owing to its good repeatability, wide range of reaction temperature, simplicity of operation; and still for this method, addictives such as polymers [20], surfactants [21], small molecules [22,33] are necessary to tailor the morphology of HA and enhance the colloidal stability. Small molecules in particular like sodium citrate and some amino acids which are widely distributed in organisms are preferentially favored, owing to their excellent biocompatibility, strong ability of binding with calcium ions, great dispersing ability [10,28,31,33].

Actually early than 1970s, it had been recognized that amino acids and sodium citrate could interfere with the HA crystal thickening owing to the strong interaction between COO– group and Calcium ions [23]. Nanosized hydroxyapatite could be easily synthesized from homogeneous calcium/citrate/phosphate solutions. Rosanna Gonzalez-McQuire and her coworkers [25] synthesized hydroxyapatite nanorods in the presence of glycine, alanine, serine, lysine and arginine at 80 °C and pH 9. The obtained particles are rod-like with less than 80 nm in length and 5 nm in width and colloidally stable. Wang and her coworkers [34,35] evaluated HA nanoparticles of morphology through a hydrothermal method with aid of sodium citrate at temperature from 40 to 200 °C. Li and her coworkers [30] adopted a two-steps method for synthesizing long-term stable hydrocolloids of hydroxyapatite with the aid of citrate ions.

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The effect of citrate ions on HA crystal growth would be summarized as follows [28,33]. On the one hand, crystal nuclei process of HA would be affected by complexion between with citrate ions and calcium ions which reduce the binding rate between calcium ions and phosphate ions. On the other hand, citrate is fundamentally different from carbonate, fluoride, hydroxide, or phosphate ions, in that it is too large to be incorporated into the apatite crystal lattice. Therefore, bound citrates should remain an interfacial component on the HA crystal surfaces, which lead the crystal negatively charged. Besides, the spacing of the terminal COO– groups in citrate matches well with the lattice parameter *c* in HA. Two of the three carboxylate carbons are slightly closer to the particle surface than are methylene and quaternary C, whose similar distances of 0.4 nm show that the long axis of most citrate molecules is tilted slightly to the surface. The spacing of COO– groups in citrate matches that of calcium ions along the *c*-axis in apatite. The area density of citrate on bone apatite is around 1/2 nm^2, which is sufficient to make the nanocrystal surface less hydrophilic. Thus, it is proposed that citrate ions adsorbed on the HA crystal could reduce the diffusion velocity of phosphate ions on *c*-axis direction. Thus, it is proposed that citrate not only favors the formation of rod-like morphology of nano-sized HA but also could provide sufficient electrostatic repulsion.

Inspired by literatures mentioned above and our previous work [36–38], a systematical evaluation on the sodium citrate/Ca molar ratio (CT/CM) on particle morphology, crystallinity and colloid stability of hydrothermal synthesized colloidal HA nanorods were conducted. A proposed mechanism of these effects was also discussed.

2. Materials and methods

2.1. Materials

Calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$, AR), sodium phosphate tribasic dodecahydrate ($Na_3PO_4{\cdot}12H_2O$, AR), sodium citrate tribasic dihydrate ($C_6H_5Na_3O_7{\cdot}2H_2O$, AR) and ethanol absolute (C_2H_6O, AR) were supplied by Sinopharm Chemical Reagent Co. Ltd. All chemicals were used as received without further purification and deionized water was used throughout.

2.2. Methods

Colloidal HA nanorods were prepared by a hydrothermal method. In a typical experiment, $C_6H_5Na_3O_7{\cdot}2H_2O$ solution with a fixed concentration (0–0.0133 M, 10 ml) was slowly added in 10 min to the $Ca(NO_3)_2{\cdot}4H_2O$ aqueous solution (0.01 M, 15 ml) with continuous stirring. Then, $Na_3PO_4{\cdot}12H_2O$ aqueous solution (0.006 M, 15 ml) was progressively added within 15 min to the mixture under vigorous stirring. After that, the as-obtained mixed solution was transferred to a Teflon-lined stainless steel autoclave with 50 ml capacity. Then the autoclaves underwent hydrothermal treatment at 150 °C for 24 h. After the hydrothermal treatment, the autoclaves were cooled down naturally and the resulting product was purified by a three cycle of centrifugation-washing (3700*g*) process with deionized water and ethanol. Finally, part of purified product was redispersed in deionized water to form aqueous dispersion with a fixed concentration, and the left was freeze-dried into powder form for characterization.

2.3. Characterization

The dried powder was characterized by X-ray diffraction with a Philips Analytical X-ray diffractometer (PANalytical B.V. X'Pert PRO) using a Cu Kα radiation (1.5406 A) source at 60 kV and 60 mA from 5° to 80° with a scan rate of 0.5°/min. Fourier transform infrared spectroscopy (FT-IR) was acquired from KBr pellets with a Nicolet 8700 FTIR spectrophotometer in the range of 400–4000 cm^{-1}. TGA measurements were performed with a DSC/TGA thermal analysis system (SDT Q600, USA). The mass loss from 5 to 10 mg of dried sample was monitored under N2 at temperatures from 30 to 800 °C at a rate of 15 °C/min. The morphology of the products was inspected using a Transmission Electron Microscope (TEM, FEI Tecnai G2/F20) with accelerating voltage of 200 kV. Samples of colloidal HA nanoparticles were drawn by pipette and 1–2 droplets were placed onto the carbon side on holey carbon-coated Cu TEM-grids. The TEM-grids were placed on a glass petri-dish with filter-paper to absorb excess liquid leaving only a thin layer of particle suspension behind, which was allowed to dry in air for about 5 min before the TEM-grids were transferred for individual storage in a BEEM embedding capsules. The mean size (including diameter and length) is derived from statistical analysis of more than 300 nanorods in the TEM image using Adobe Photoshop software CS2 edition. Zeta potential and particle size distribution were performed by Dynamic Light Scattering (Malvern Zetasizer (nano series) nano- zs 90). Particle size distributions were measured in disposable polystyrene cuvettes containing particle dispersions (0.01 g/ml) after equilibration for 2 min at 25 °C. The data were recorded and calculated using the Dispersion Technology Software v. 5.0 (Malvern Instruments). These measurements were conducted three times for each sample.

For Zeta potential measurement, samples (1% particle dispersion) were located in clear disposable zeta cell and results were automatically generated with the help of software. pHs of samples, modified by NaOH or HCl (0.01 M), were detected with a Sartorius PB-10 pH meter (Sartorius AG, Amtsgericht, Goettingen, Germany) at the temperature of 25 °C. No additional electrolytes were added for these measurements.

Besides, the colloidal stability of dispersions was evaluated by observing the behavior of the sedimentation after a period time using a digital camera (Xiaomi, MiTwo, China).

3. Results and discussion

3.1. Effect of sodium citrate on crystallinity of HA

Particle morphology, crystallinity and colloidal stability are very important factors for the applications of HA nanorods [39]. In that context, it is very necessary to establish a clear relationship between experimental conditions and particle morphology, crystalline and colloidal stability.

The crystalline structure of the obtained samples synthesized with different CT/CM is shown in Fig. 1. All diffraction peaks of the XRD pattern can be easily indexed to a pure hexagonal phase of HA which is in good agreement with the reported data (JCPDS files, PDF No. 86-740) and literatures [32,33,40]. It should be noted that the intensity and integrity of peaks of HA decrease with the rise of CT/CM, which indicates that the citrate ions would affect interfering crystal growth of HA strongly upon CT/CM and such interference would be strengthened along with the increase of CT/CM.

3.2. Effect of sodium citrate on morphology

To visually observe the effect of sodium citrate on the morphology of HA nanorods, TEM was used to examine the morphology difference of HA synthesized with different CT/CM as shown in Fig. 2. On the whole, the obtained HA keeps rod-like among all the CT/CM investigated. The size of HA nanorods is 24 nm in mean diameter and 70 nm in mean length without addition of sodium citrate. When CT/CM increases to 1/3, the size of HA nanorods is 13 nm in mean diameter and 49 nm in mean length. Further increase of