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Hydroxyapatite micro- and nanoparticles: Nucleation and growth mechanisms in the presence of citrate species

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Abstract

Hydroxyapatite (HAP) particles with different morphologies were precipitated from homogeneous calcium/citrate/phosphate solutions at physiological temperature. Small variations of the starting solution pH in the range $7.4 < \text{pH} < 8.5$ made it possible to switch the precipitated particle morphology from a micrometric bundlelike to a nanometric needlelike shape. The role of the existing citrate species as calcium chelates is here discussed within the framework of particle nucleation and growth mechanisms. While temperature-dependent calcium citrate complex (Cacit) stability is here suggested to control the free calcium availability and thereby the nucleation rate, the adsorbed citrate species are proposed to control the nanoparticle stability. Moreover, an attempt to detail the role of citrate species in the ordered aggregation of HAP nuclei leading to the observed peanut and bundlelike microparticles morphology is also presented.

Keywords: Hydroxyapatite; Nanoparticles; Microparticles; Stabilization; Aggregation; Self-organization

1. Introduction

The major inorganic constituent of bones and teeth is a calcium phosphate phase with a composition similar to that of synthetic hydroxyapatite (HAP; $Ca_5(PO_4)_3OH$). This similarity is on the basis of the excellent biological properties of HAP-based materials: bone bonding capability, osteoconductivity, and biocompatibility [1]. Along with composition, the morphological characteristics of HAP particles, such as shape, size, and size distribution, play an important role in the mechanical, chemical, and biological properties of HAP-based materials.

There is a great deal of interest in the study of the solution crystallization pathways of HAP, both from a technological point of view, and from a fundamental perspective aiming at understanding some of the biomineralization processes. It is usually accepted that the precipitation mechanism of HAP follows a nucleation/aggregation/growth sequence of events [2]. In accordance with the prevailing mechanism, HAP particles with different sizes and morphologies can be obtained: monodisperse particles with sizes ranging from nanometric to micrometric, as well as irregular and ordered aggregates.

For those precipitating systems where particle growth occurs typically through an aggregation mechanism, as in the case of HAP particles precipitated from calcium/citrate/phosphate solutions at >85 °C [2], the increase of HAP particle size results preferentially from the aggregation of smaller precursor units, typically nanometric nuclei, rather than from normal growth. As observed by Macipe et al. [3], micrometric HAP particles are replaced by nanosized ones with needlelike morphology and length 30–60 nm if a restrained aggregation mechanism takes place during particle precipitation.

The mode in which aggregation takes place plays an important role in determining the particles' final shape and size [4,5]. The formation of a series of particle shapes that comprises needle shape followed by peanut- and dumbbell-like shape and finally spherulites was reported for an aggregation-mediated mechanism. Busch et al. [6] suggested the aggregation of nanocrystals by a fractal growth mechanism guided along electrical field lines to explain this morphological evolution for the formation of fluorapatite notched spheres in the

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presence of collagen. Such an electrical field results from the adsorption of polar collagen molecules onto the surface of the fluorapatite particles, which originates a strong (intrinsic) permanent dipole [6]. The assembly of small units into complex shapes as peanut-, dumbbell-, or spherulite-like shapes was also reported for other systems with particular additives including CdS in the presence of poly(styrene sulfonate sodium) and cetyltrimethylammonium bromide [7], hematite (α-Fe_2O_3) in the presence of sulfate ions [8], and $BaSO_4$ in the presence of double-hydrophilic block copolymers [9], as well as the aggregation of ZnO, Bi_2S_3, MnO_2, and $La(OH)_3$ nanorods directed by linear PEG into spherical globules [10]. The anisotropic adsorption of the additive species has been suggested to guide particle aggregation into these particular shapes.

Parallel to these findings, a crystal-growth-mediated mechanism has also been proposed to explain the formation of micrometric particles having monorod, bowknot-like, or flowerlike morphologies from calcium/EDTA/phosphate nanostructured HAP particles [11]. According to the authors, the preferential adsorption of OH^- ions from solution ($pH > 9$) onto specific nuclei facets and their shielding effect seem to guide crystal growth along preferential facets [11].

The analyzed reported results thus point out that the differentiated adsorption of solution ions onto particular crystal facets plays a fundamental role in particle growth, either through aggregation or through normal crystal growth. This common feature of the different models is a key issue for explaining the origin of the different particle shapes.

In the present work, HAP particles with sizes ranging from nanometers to micrometers were obtained from calcium/citrate/phosphate solutions by small variations of the solution pH. The physiological temperature (37 °C) was selected for the present studies, thereby introducing a new temperature condition when compared to that used by Macipe et al. [3]. The influence of the experimental conditions, namely the solution pH, on the system supersaturation and its effects on particle nucleation and growth as well as on the adsorption process of citrate species were studied. Moreover, an attempt to detail the role of the adsorbed citrate species in tailoring the particle size and shape is also presented here.

2. Experimental

2.1. Chemicals

For the preparation of the calcium/citrate/phosphate solutions, the following chemicals were used without further purification: citric acid monohydrate ($C_6H_8O_7{\cdot}H_2O$, Riedel–deHaën, 99.5%), calcium nitrate-4-hydrate ($Ca(NO_3)_2{\cdot}4H_2O$, Riedel–deHaën, 99%), ammonium hydrogen phosphate ($(NH_4)_2HPO_4$, Merck, 99%), and ammonia solution (NH_4OH, Riedel–deHaën, 25%).

2.2. Particle precipitation

The supersaturated solutions used in this work as precipitating media were prepared by mixing citric acid, calcium nitrate, and ammonium hydrogen phosphate as follows: an aqueous citric acid solution (0.6 M) with small amounts of added NH_4OH (25%) for adjusting its pH to a value lying between 7.4 and 8.5 was then mixed with appropriate amounts of $Ca(NO_3)_2{\cdot}4H_2O$ 0.2 M and $(NH_4)_2HPO_4$ 0.2 M solutions. A ratio of 3:1 was selected for the citric acid/calcium nitrate molar proportion (Cit/Ca), as this condition could ensure stable starting solutions at room temperature, not prone to prompt precipitation. For a similar reason, the pH of the starting citric acid solutions was maintained below 8.5; otherwise calcium citrate ($Ca_3(C_6H_5O_7)_2{\cdot}4H_2O$) precipitates promptly at room temperature. The solution was filtered through a 0.45-μm Millipore and then placed in a water bath at 37 °C for 24 h. After this period, precipitation was observed only for those solutions having a pH above 7.4. Therefore, for the goal of studying the influence of the starting solution pH on the characteristics of the precipitated powders, the precursor solution pH was limited to the $7.4 < pH < 8.5$ range. Table 1 summarizes the characteristics of the solutions selected for the present work.

Table 1
Selected experimental conditions for particle precipitation

Sample code	pH of starting solution (±0.02)	Final pH of precipitating medium (after 24 h) (±0.02)
A	7.43	6.94
B	7.55	6.98
C	7.70	7.05
D	7.87	7.14
E	8.15	7.27
F	8.46	7.66

The obtained precipitates were separated by centrifugation in an ultracentrifuge (Model Beckman L8-70M) at 10,000 rpm for 15 min. The supernatant liquid was discarded and the powder was washed and dried in a desiccator.

2.3. Particle characterization

The crystalline phases of the precipitated powders were identified by powder X-ray diffraction (XRD) analysis, using an X-ray diffractometer (Model Rigaku PMG-VH, $CuK\alpha = 1.5405$ Å). The crystallite size was estimated from the X-ray diffractograms using the Scherrer formula [12],

$$D = k\lambda/\beta_{1/2}\cos\theta, \tag{1}$$

where D is the crystallite size (Å) estimated using the reflection (002), k is a shape factor equal to 0.9, λ is the X-ray wavelength (1.5405 Å), θ is the diffraction angle related to the reflection 002 ($\theta = 12.92$), and $\beta_{1/2}$, expressed in radians, is defined as

$$\beta_{1/2} = \left(B^2 - b^2\right)^{1/2}, \tag{2}$$

B being the diffraction peak width at half height and b the natural width of the instrument.

The calcium and phosphorus contents of the precipitated powders were assayed by inductively coupled plasma spectroscopy (ICP) (ISA Jobin Yvon–JY70 Plus). Fourier transform infrared spectroscopy (FTIR) (Mattson galaxy 3020) in transmittance mode was used in the range of 400–4000 cm^{-1}