

Nanoscale Characterization of Hydroxyapatite Particles by Electron Microscopy

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Owing to its chemical similarity to the mineral phase of human bone and to its biological performance, synthetic hydroxyapatite (HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) has long been recognized as an important bone substitute material in orthopaedics, dentistry and artificial implants. HA ceramics have also been studied as drug delivery systems components where an appropriate porosity for ensuring a high ability for carrying and delivering drugs in a controlled manner is required. For some emerging applications demanding nanoscale structured systems endowed with novel potentialities HA nanoparticles may be addressed as alternative components ensuring a large surface area for adsorbing the pharmacological substance of interest. Moreover particle porosity may offer new possibilities for drug or nutrient delivery, or for biocompatible and bioresorbable structural reinforcements in osteologic implants, coatings and adhesives. Mesostructured porous calcium phosphate based systems have been synthesized at macro- and micro-scale, but well established methods for the specific synthesis of porous HA nanoparticles have not been reported so far. Yao et al. reported a successful synthesis of HA mesoporous micron-sized rods 0.5-1 μm in length and 50-100 nm in thickness but having low porosity [1]. A new synthesis challenge for producing nano-sized particles of high surface area and having ordered mesopores is thus addressed.

This work reports the synthesis of mesostructured nano-sized HA particles and examines the effect of the reaction time on the particles characteristics. The necessary supersaturated solution for particle precipitation was prepared by mixing citric acid, calcium nitrate and ammonium hydrogen phosphate as follows: an aqueous citric acid solution (0.6 M) added with small amounts of NH_4OH (25%) for pH adjustment to 8.12 was then mixed with appropriated amounts of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ 0.2 M and of $(\text{NH}_4)_2\text{HPO}_4$ 0.2 M solutions. The resulting homogeneous calcium/citrate/phosphate solution was transferred to a stainless steel autoclave and kept at 180°C during two different times, 1 hour and 24 hours. After cooling the precipitated powders were filtered, washed with distilled water and dried in a desiccator. The precipitated particles were characterized by means of X-ray diffraction (XRD) for crystal phase analysis, high resolution transmission and scanning electron microscopy (HRTEM and HRSEM) for morphology evaluation, energy dispersive spectroscopy (EDS) for elemental analysis, gas adsorption/desorption (BET and BJH methods) for specific surface area and pore size evaluation and small angle XRD for ordered porosity characterization.

The obtained results show that the powders precipitated after 1 and 24 hours (hereafter called nano1 and nano24, respectively) are crystalline hydroxyapatite particles (figure 1a), having a nanometric size (figures 2a and 2c,d). Nano24 are rod like shaped with an average length around 100 nm and ~30 nm width while nano1 seem to display a less compact body with a layered struc-

ture. The corresponding electron diffraction patterns (inset of figures 2a and 2c) confirm the crystallinity of the particles. EDS analysis (figure 2b) showed that nano1 and nano24 elemental profiles consist of calcium, phosphorous and oxygen peaks no other elements being detected except copper from the supporting copper grid thus confirming the high purity of the obtained HA powders. The Ca/P molar ratio in nano1 and nano24 was determined as 1,69 and 1,76 respectively which is closed to the theoretical value of hydroxyapatite (1,67) [2].

N₂ adsorption/desorption isotherms for both type of particles are presented in figures 3. According to IUPAC classification nano1 and nano24 present type IV isotherms which exhibit the characteristic hysteresis loop (HL) of a mesoporous material. The HL corresponding to nano1 (figure 3a) is a typical H3-type isotherm indicative of slit-shaped pores, often found in layered materials [3]. Nano24 (figure 3b) present a H1-type isotherm where pore saturation occurs at 0,8 -1 relative pressure (P/P₀) indicative of tubular pores [3]. The porosity of the precipitated HA particles is also evidenced by TEM results. As observed in figure 4 the HRTEM image of a nano24 particle allows some pores to be detected (indicated by dotted circles and/or arrows). Moreover the lattice fringes are uniformly oriented thereby indicating the particle to be a single crystal. The BET surface area and the average pore size (BJH analysis of adsorption curve) evolved from 81m²/g and 5 nm for nano1 to 60 m²/g and 12.5nm for nano24, respectively whereas the total pore volume remained practically unchanged (figure 3a and 3b). Such pore size increase during precipitation may result from the merging of smaller pores accompanying the particle structure evolution as reported for other oxides systems [4]. Nevertheless the deep understanding of the mechanisms underlying HA porosity evolution demands more detailed studies which are now in progress.

The low-angle XRD patterns of nano1 and nano24 are shown in Figure 1b. A broad peak centred at 2 θ =2,23deg is observed for nano1. According to Bragg's equation this low-angle peak indicates a long-range ordered pore structure where the distance d between neighbouring pore centres is 39,6 Å [5]. This value is in good agreement with the average diameter of 5nm estimated from adsorption isotherm. The low-angle peak is no longer detected after 24 hours of precipitation which might indicate a pore expansion to a value d' corresponding to a too low angle (2 θ <1 deg) to be detected by the used equipment. This idea is in line with the pore size evolution referred above (from 5 to 12.5nm). It may be thus concluded that TEM, N₂ adsorption, low- and wide-angle XRD results confirm that HA nanoparticles with mesoporous structure were obtained by hydrothermal precipitation. These particles are expected to have a wide range of possible applications namely as osteogenesis promoters and/or local suppliers of therapeutic species.

References

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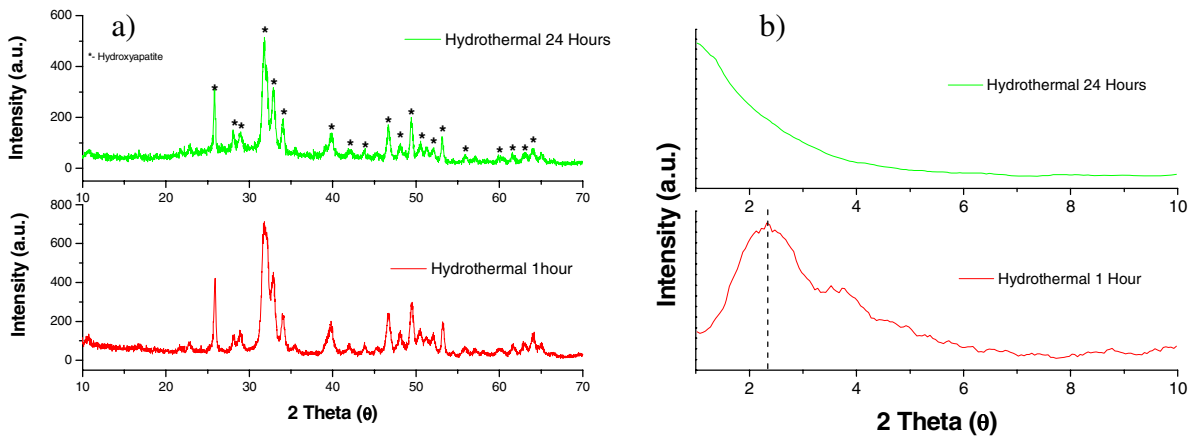


Figure 1- (a) Wide-angle and (b) low-angle XRD patterns of the Hap nanoparticles precipitated during 24 Hours (—) and 1 hour (—)

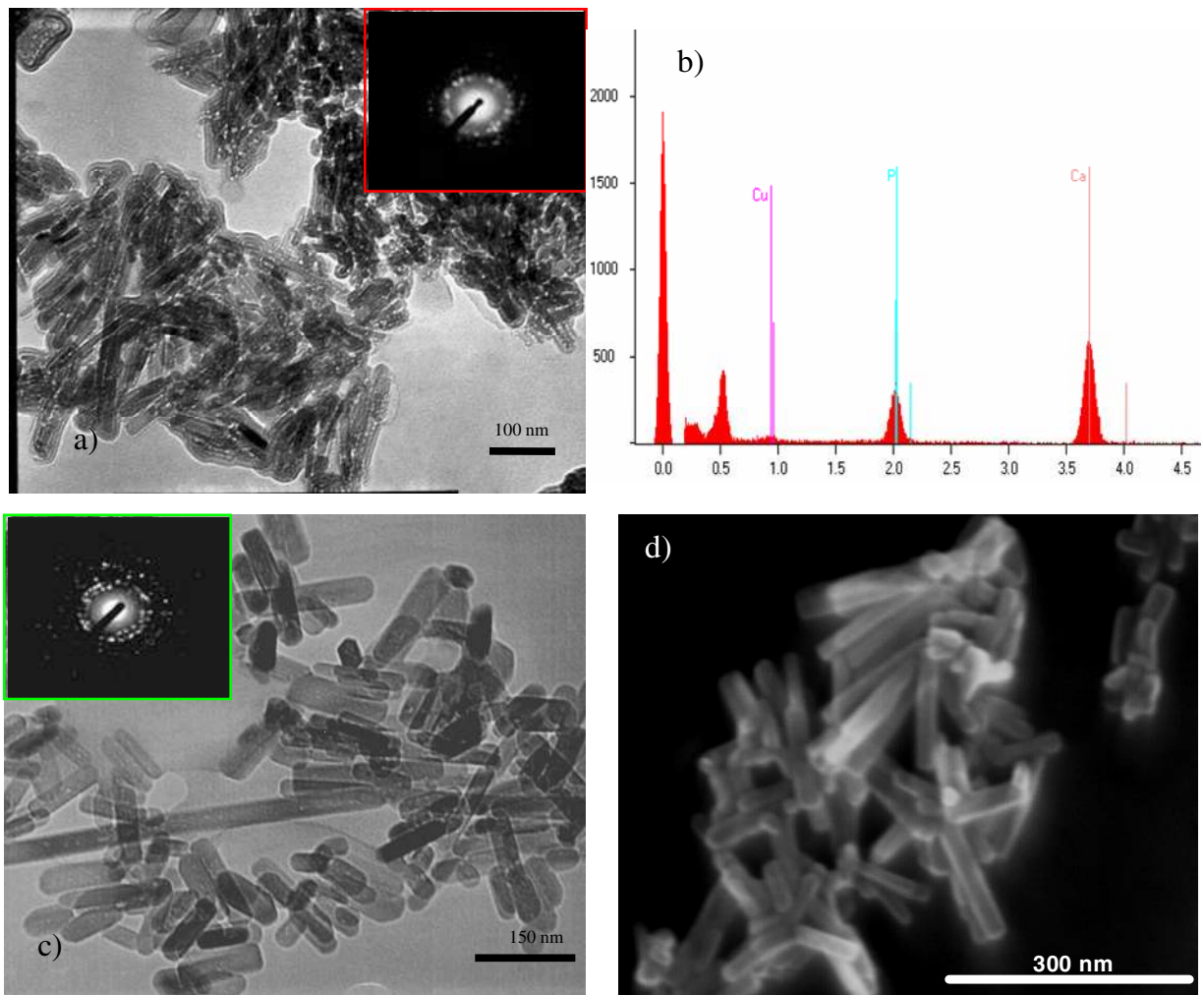


Figure 2- TEM and HRSEM images and corresponding electron diffraction pattern of: (a) nano1; (c, and d) nano24. (b) EDS spectrum corresponding to nano1.

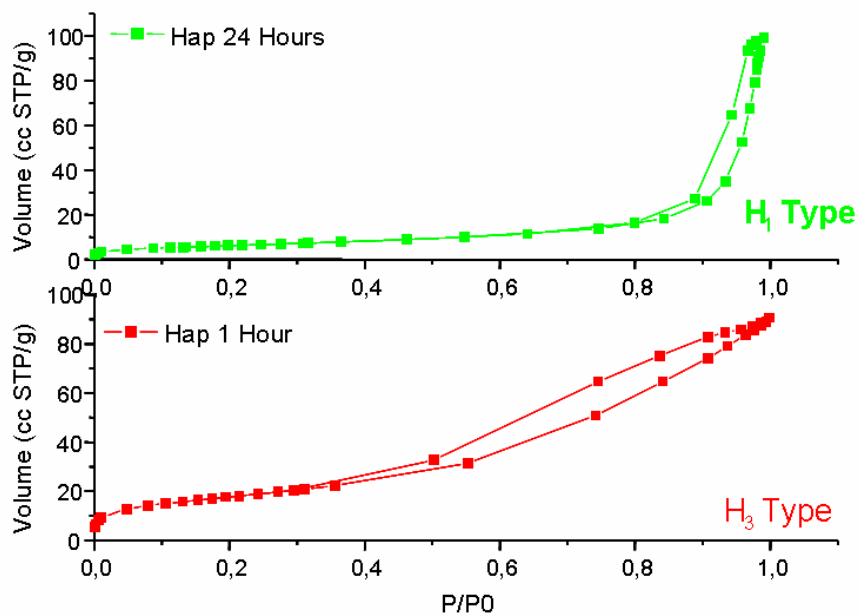


Figure 3- N₂-gas adsorption-desorption isotherms of Hap nanoparticles synthesized during 1 hour (-■-) and 24 hours (-■-).

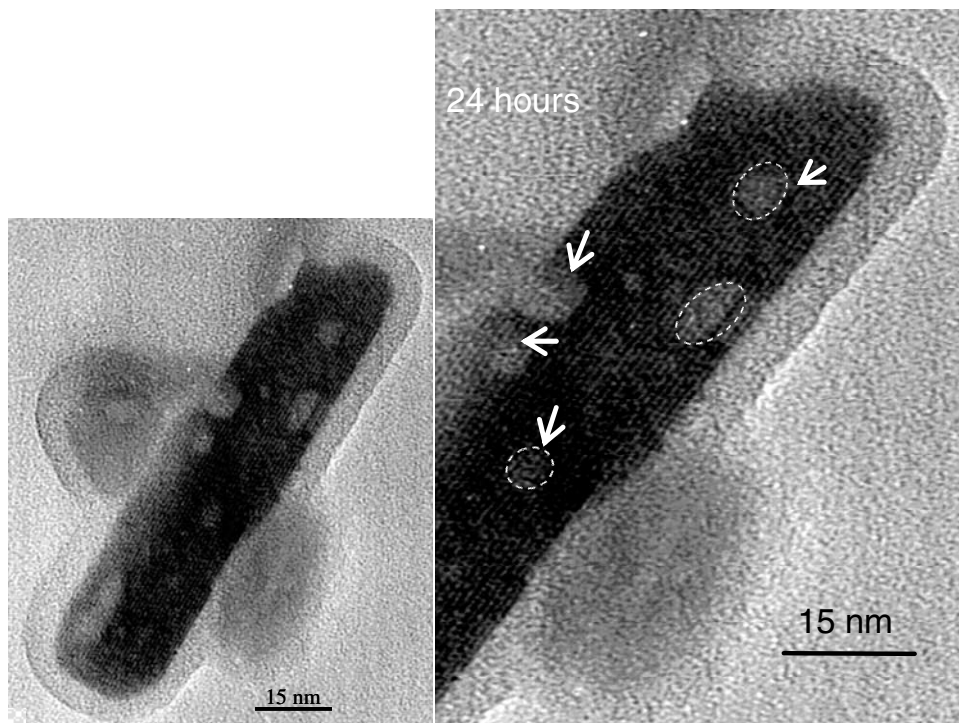


Figure 4- HRTEM image of a nano24 particle (on the left) and amplified region (on the right) showing some pores highlighted by dotted circles.