HYDROTHERMAL CRYSTALLIZATION OF MICROMETRIC HYDROXYAPATITE FOR BIOMEDICAL APPLICATIONS

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Abstract
The paper presents the synthesis of hydroxyapatite by chemical reaction of calcium nitrate tetrahydrate and diammonium hydrogen phosphate, followed by hydrothermal crystallization at 200-230°C and 24/72 hours. The obtained white powders were characterized by X-Ray Diffraction, Scanning Electron Microscopy and Atomic Force Microscopy, revealing the formation of crystalline hydroxyapatite with microsized lamella shaped crystals, when the crystallization took place in acidic environment.

Introduction
Synthetic calcium phosphates are widely used biocompatible materials due to their similarity with the natural hydroxyapatite (HA, $Ca_{10}(PO_{4)6}(OH)_2$), present in the hard tissues of the human body. Among the calcium phosphate compounds, HA presents excellent properties as a bioactive material for human implants [1]. HA has been synthesized by a variety of methods like precipitation, hydrolysis, sol-gel, solid state reactions, etc [2-4]; among these methods, in recent years, the hydrothermal method [5, 6] has gained an increasingly important role due to a series of advantages, like: a) relatively low processing temperatures (typically below 350°C), comparing to calcination where high temperatures are required; b) short time, comparing to the sol-gel method; c) the autoclave is a closed system, so outside influences, like airborne impurities, are eliminated; d) there is no need for organic additives (which might alter the biocompatibility properties). Hydrothermal method seems to offer a better control over particles size, chemical composition and morphology, and thus its use has continuously extended over the last decades [7]. Most of the studies of HA synthesis aim at fabricating nanoparticles; the aim of the present study was to achieve microparticles of HA, to be used for future construction of biocomposites.

Experimental
The synthesis was performed usinganalytical grade reagents, namely calcium nitrate tetrahydrate $Ca(NO_3)_2.4H_2O$ (Sigma Aldrich) and diammonium phosphate $(NH_4)_2HPO_4$ (Reactivul Bucuresti) as Ca and P ions precursors, dissolved in distilled water so as to yield 0.05 M solutions. The Ca/P molar ratio of 10/6 was achieved by mixing the appropriate amounts of precursor solutions. The P precursor was added dropwise onto the Ca precursor, under magnetic stirring, followed by 30 minutes stirring. Then the reaction mixture was poured into a teflon liner, which was introduced into a steel autoclave and placed in the oven at temperatures ranging between 200 and 230°C and times of 24 and 72 hours, in order to follow the influence of time and temperature on the crystallization product. Since the pH of the reaction media is acidic, in the range 5-6 (measured with a pH-meter), in order to observe the difference introduced by a pH change, one synthesis was performed with pH adjustment, using ammonia (the pH value was brought to 8).
After the time spent in the oven, the reaction product was cooled to room temperature naturally, extracted and washed with distilled water for 5-6 times, until pH has returned to neutral, and dried at 60°C for 4 hours.

The resultant white powder was subjected to physico-chemical characterization by means of x-ray diffraction technique, using a PANalytical X'Pert Pro MPD Diffractometer with Cu anode, working parameters 45 kV and 30 mA. Images of the samples were obtained using a PANalyticalInspect S scanning electron microscope coupled with an energy dispersive X-Ray analysis detector (EDX) at 3000x magnification (powder samples were supported on glass holders and coated with Ag). Atomic force microscopy was performed on a MultiView 1000 scanner from Nanonics Imaging Ltd., in intermittent mode, using 20 nm radius tip doped with chromium, for the HA1 sample, scanned on a 10x10 μm scale. The analyzed sample was diluted in ethanol and dropwise added to the glass substrate

Results and discussion

The xrd results are presented in Figure 1, where the names of the samples are correlated with experimental parameters as follows:

Name	Synthesis (pH, temperature (°C), time(h))
HA1	5.35; 200; 72
HA2	5.83; 230; 24
HA3	8; 220; 24

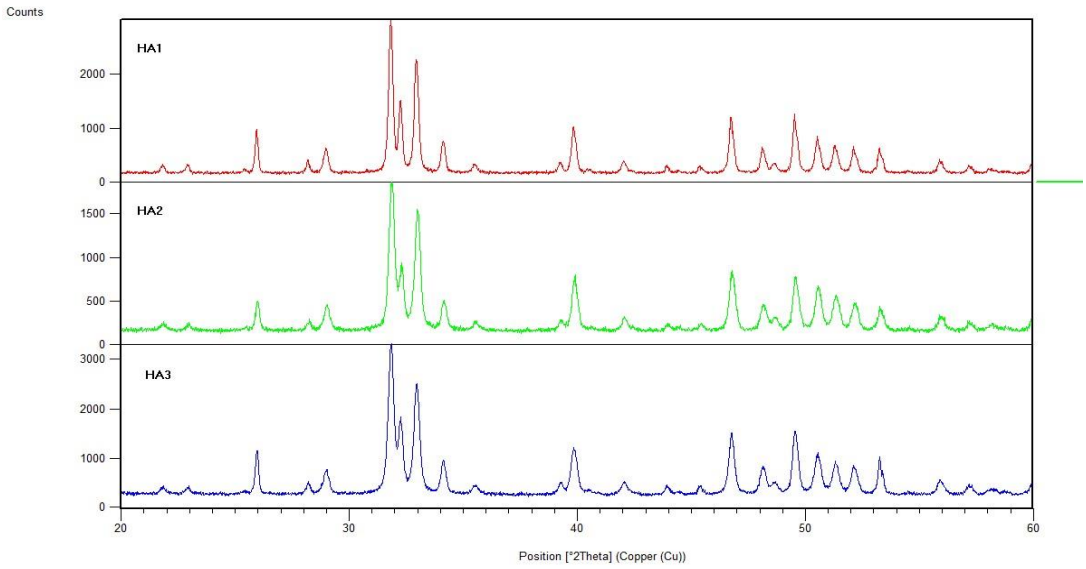

Figure 1. X-ray diffractograms of HA samples

As can be seen in the diffractograms, the samples are crystalline and have been identified as hydroxyapatite; no other crystalline phase is present in any of them. Comparing the peaks intensities, one can conclude that increased time of hydrothermal crystallization (72h, compared to 24h) implies a better crystallization of the samples, and the pH increase also leads to an increase in the crystallinity degree.

The SEM images of the crystalline HA samples are presented in Figure 2. Synthesized in acidic condition,HA1 and HA2 samples present an elongated morphology, plate-like (lamellas). The crystals in the HA1 sample are micrometric-sized (10-20 μm) with uniform distribution of dimensions (most of the crystals appear to be broken, probably during the washing and drying procedures). The 30 degrees difference in the processing temperature between samples HA1 and HA2 seems not to matter; instead, the time difference of 48 hours seems of great importance, since the distribution of the crystals in the HA2 sample belongs to a smaller range; just a small number of them appear in the range of tens of microns. Most of

the crystals appear as wires with lengths of a few microns (≤5μm); some of the crystals appear as agglomerations. The pH difference is too small to count for such a big difference in the sample morphologies. This statement seems to be in agreement with the XRD results, where HA2 sample is a little less crystalline than HA1 sample, which means it needs a longer time to complete the crystallization process.

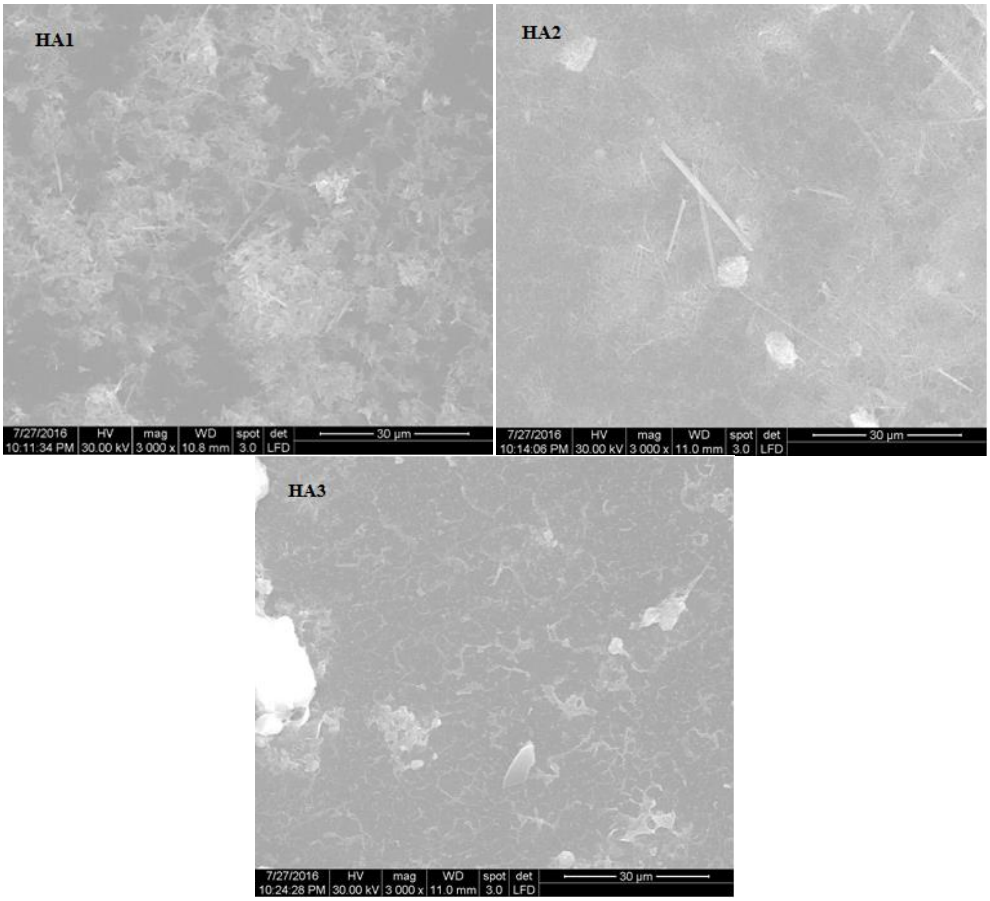

Figure 2. SEM images of HA1, HA2 and HA3 samples

Regarding the sample HA3, the SEM image presents agglomerations of very small crystallites. Correlating this image with the XRD result, one can conclude that sample HA3 contains agglomerated nano-crystallites which, even if very well crystallized, are highly compacted (literature mentions this problem in fabricating nano-HA). The "slice" that appears on the image of the HA3 sample comes from an impurity, because it is an isolated appearance. Some results of the morphologic analysis by AFM of the HA1 sample are presented in Figure 3: image(left), measured profile (center) and 3D image (right). The AFM results confirmed the presumption of micrometric lamellas formation; the left image shows broken lamellas, which is in agreement with the SEM image for the HA1 sample, and could also be observed in the AFM 3D image (right). The profile (center image) from the selected area was recorded along the green line in the left image. The measured base-width of the crystal was around 700 nm and the maximum height of the measured lamella was around 25 nm. The measured profile is actually a reprezentation of the lamella half-cross-section, with relatively sharp lateral facets and waved top. Even if the top surface appears as smooth on the SEM images, the AFM shows that the actual surface is rather rough, which is a quality required for a bioceramic to be used in medical implants. The AFM confirms the formation of a crystalline material, with crystalls in the micrometer-domain.

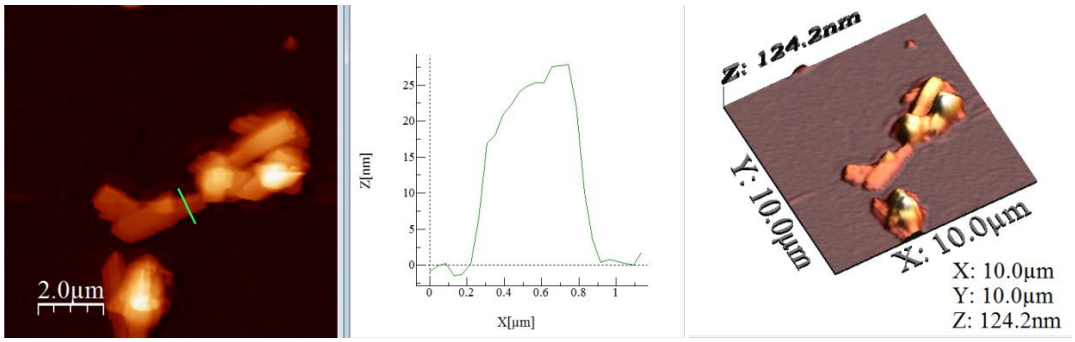

Figure 3. AFM results of the HA1 sample: image(left), profile (center) and 3D image (right)

From this study of HA synthesis and crystallization, we can conclude that variations of temperature in the range 200-230 do not introduce much difference in the results. Instead, the change of pH from 5-6 to 8 will introduce a big difference, and so is the variation in the crystallization time, from 24 to 72 hours.

Conclusions

The conclusions of this study are:

1. Crystalline rough hydroxyapatite can easily be fabricated by a facile one-step hydrothermal route
2. Temperatures in the range 200-230°C are to be used
3. pH in the range 5-6 is to be used, if micrometric crystals are desired
4. longer crystallization times lead to longer crystals (72h results are promising)
5. potential future investigation directions may regard lower temperatures crystallization (100-200°C) and/or longer time (>72h) studies

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