PARTICLES DIMENSIONAL ANALYSIS AND MICROSCOPIC CHARACTERIZATION OF HYDROXYAPATITE POWDER

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Abstract: The studies conducted for this paper we chose the wet precipitation synthesis method at room temperature to obtain hydroxyapatite powder. Size particle analysis of hydroxyapatite was made in isopropyl alcohol. The surface study on nm scale and the surface topography were evaluated by using AFM with tapping mode. The SEM pictures of the grown spherulite crystals shows many spherical agglomerations and few crystallites of 0.1 μm in size with pores.

Keywords: *SEM and AFM microscopy, hydroxyapatite powder, wet precipitation.*

1. Introduction

A challenging problem in health care is the regeneration of partially or fully lost organs.

There are two types of regeneration: physiological and reparative.

- Physiological regeneration is a natural process of disintegration and restoration of molecules, cells, and tissue (e.g., bone).
- Reparative regeneration in the broad sense is the restoration of parts of body lost as a result of traumas, healing of defects of tissue and organs, etc.

The use of calcium-phosphate-based biocompatible materials close in composition to the inorganic component of bone tissue contributes to the creation of favorable conditions for the reparative functions of bone tissue.

In the past several decades, a number of implant materials based on calcium hydroxyapatite ($Ca_{10}(PO_4)_6(OH_2)$, calcium phosphate (β-$Ca_3(PO_4)_2$, ceramics of these phosphates, bioglasses, and composites have been used in orthopedics, neurosurgery, and dentistry.

In the case of fractures, the restoration of bone tissue is a complicated biochemical process, which includes the synthesis of proteins and nitrogen-containing polysaccharides and deposition of calcium salts and is accompanied by hypermetabolism.

A serious problem related to the biocompatibility of materials is the creation of implants that would ensure the delivery and in situ release of a therapeutic agent with osteoinductive and anti-inflammatory effects [1].

Hydroxyapatite surface coating applications include metallic orthopedic and dental implants where osseointegration HA promotes both processes and reduce the release of metal ions acting as a physical barrier, preparation for the replacement of bone fragments. [2].

2. Materials and methods

The synthesis methods of hydroxyapatite are many, the literature abounds in this area. The studies conducted for this paper we chose the wet precipitation synthesis method at room temperature.

Figure 1 shows the flow of technology of wet precipitation of hydroxyapatite powder by Sung [3].

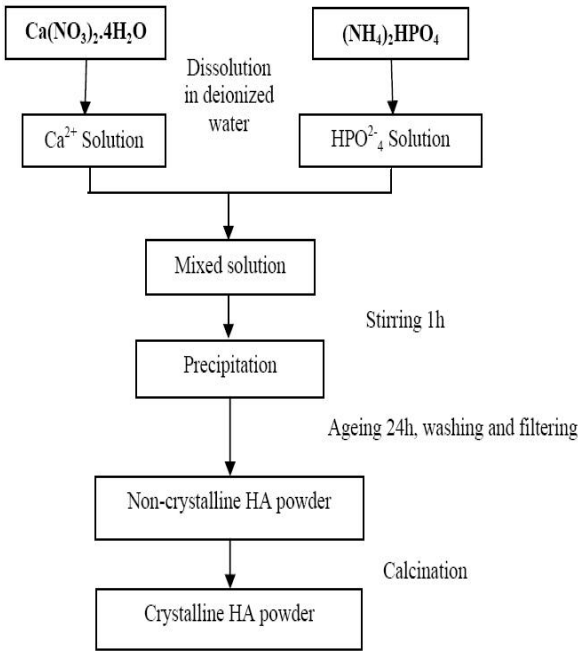

Figure 1: *Modified chemical precipitation route for HA powder preparation. (Adapted from Sung) [3]*

Chemical reagents used in this paper: Ca $(NO_3)_2.4H_2O$ and (NH_4) $2HPO_4$ were separately dissolved in deionized water. He added $Ca(NO_3)_2$ in aqueous solution (NH_4) $2HPO_4$, agitated vigorously for about 1 hour at room temperature and a precipitate was obtained milky, somewhat gelatinous, which was then shaken for 1h.

The mixture was sintered at 1000^0C for 24 hours. After having been washed and filtered. After filtering the sticky product was compacted dry oven at 800^0C. Dried powder was crushed in a mortar with pestle and then calcined in an alumina crucible at three different temperatures 800^0C, 1000^0C and 1200^0C for 1h.

Table 1

Method	Sample	Temp. (^{O}C)	Concentr. (M)
Reflux after mixing	HA1	800	0.1:0.06
Reflux after mixing	HA2	1000	0.1:0.06
Reflux after mixing	HA3	1200	0.1:0.06

3. Results

The objectives of this work were:

- to synthesize biocompatible hydroxyapatite;
- to analyze the size particle of hydroxyapatite;
- to characterize by SEM and AFM microscopy hydroxyapatite powder obtained by wet precipitation.

3.1. Size particle analysis of hydroxyapatite in isopropyl alcohol

Determination of average particle size and particle size distribution will be done using a laser diffraction particle size analysis by Malvern Mastersizer Type S-type. It offers a wide range of measurement in the range 0.05 to 3500 microns, excellent data reproducibility and flexibility in handling evidence.

In the case of HA-A powder disperses not enough to be mechanically made a DLS measurement type. Apparent dispersions are composed of a particulate phase and no detectable perfectly clear, that the precipitating phase.

Precipitation is too fast and does not allow the measurement.

So they resorted to grinding (in agate) to precipitate directly in the presence of alcohol. After the samples were ultrasonic in an ultrasonic bath for 5 min (35kHz). Dispersions have developed an opalescent appearance of white precipitate respectively.

After several minutes, large aggregates have settled, and then were collected samples from the metastable phase (upper).

That is metastable for about an hour the precipitate as well. The average size (math) is about 1800nm (1,8 microns).

But the physical interpretation is quite different, namely: Record several populations of particles.

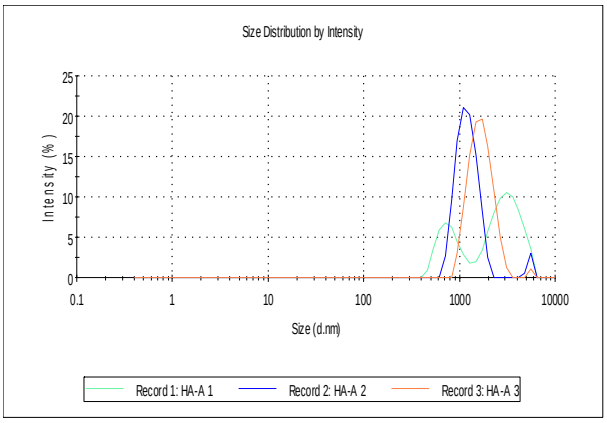

Figure 2: *Size distribution of the three HAp samples after intensity*

Considering the first instability manifested by a set of measurements to another (see the three sets of measurements per color), but also change the number of population (number of peaks) is very likely that the system be reviewed format of elementary particles in different forms of aggregation result from the process of sedimentation. The system contains over 6000 nm particles (the ultimate maximum).

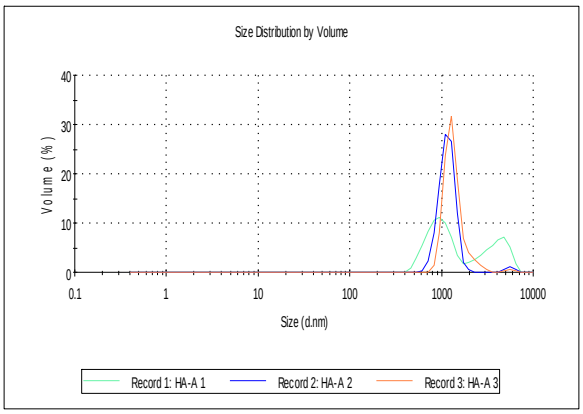

Figure 3: *Size distribution of the three HAp samples by volume*

In the figures 2, 3 and 4 are illustrated the distributions of the three Hap samples after grading intensity, volume and number respectively.

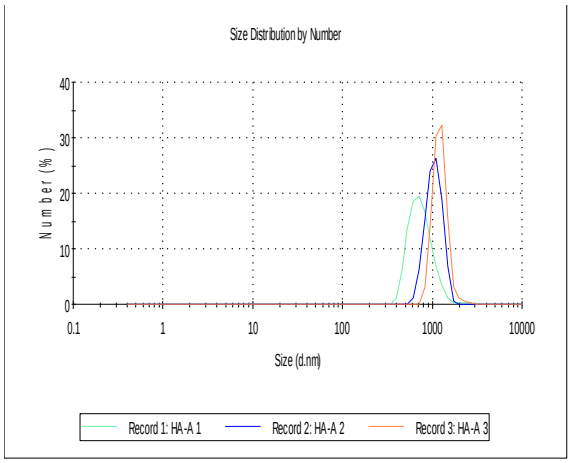

Figure 4: *Size distribution of the three HAp samples by number*

After global analysis of the system (above) has developed software filtering of unit interval analyzed in view of highlighting the possible elementary particles between 0.6 - 100nm which in the global analysis cannot be registered because of the large aggregates shield. Such particles have emerged about 40 nm single mode. These in a different associations with a different kinds of aggregates generates large majority in the global analysis above.

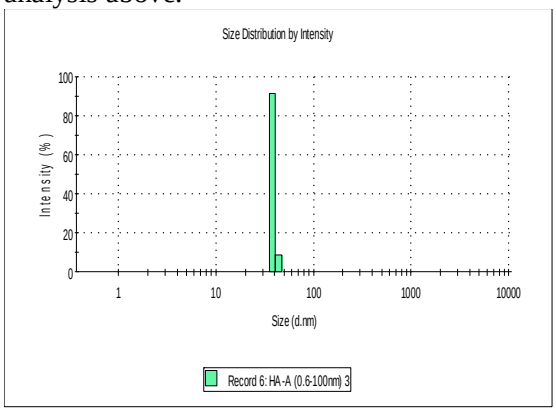

Figure 5: *Size distribution of the HA-A samples by intensity*

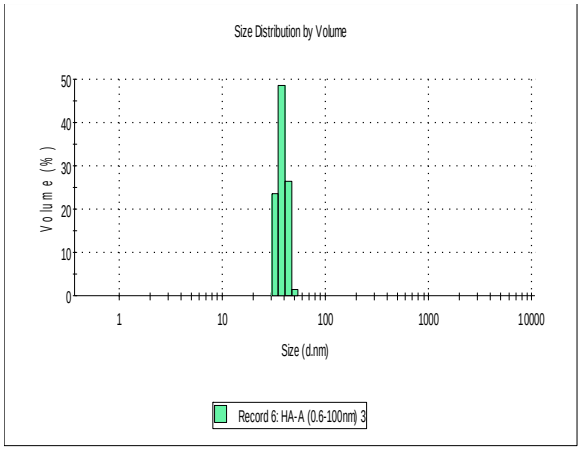

Figure 6: *Size distribution of the HA-A samples by volum*

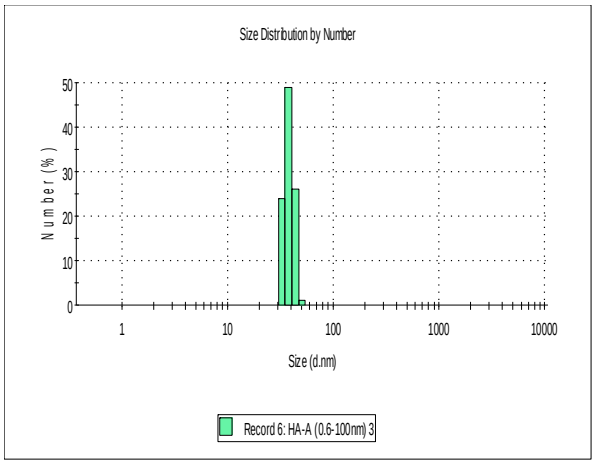

Figure 7: *Size distribution of the HA-A samples by number*

For HA-A has conducted a global analysis. Reproducibility is better between the sets of

measurements, but measured particles seem more independent particles and not aggregates.

3.2. SEM microstructural characterisation

Microstructural observations were performed with a Quanta 200 scanning electron microscope equipped with software analyzer for quantitative elemental analysis. For analysis by scanning electron microscopy, hydroxyapatite powders were placed in LR White resin.

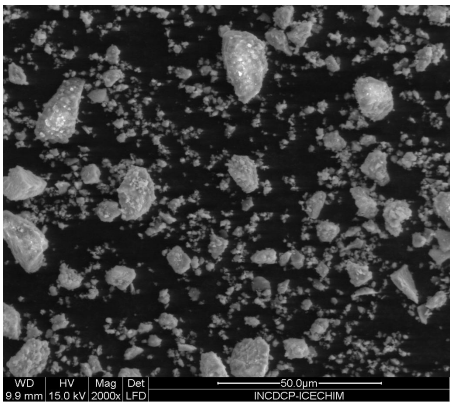

Figure 8: *Electron microscopy SEM for Hap powder calcined at 1000^{0}C, 2000 X.*

In Figure 8 shows electron microscopy of hydroxyapatite sample calcined at 1000^{0}C with 2000x magnification and in the next figure same sample but at a 1000x magnification. We can distinguish micro pores (<10 µ m), allowing diffusion of ions and fluid macro pore (100-600µm) to allow cell colonization. The micro pores give the ceramics osteoconductive properties.

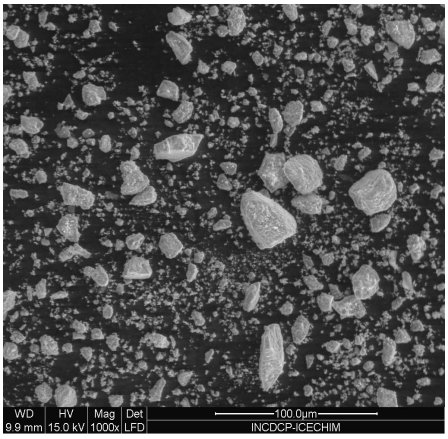

Figure 9: *Electron microscopy SEM for Hap powder calcined at 1000^{0}C, 1000 X*

SEM images of crystals grown shows several spherical clusters and few crystals of 0.1µm.

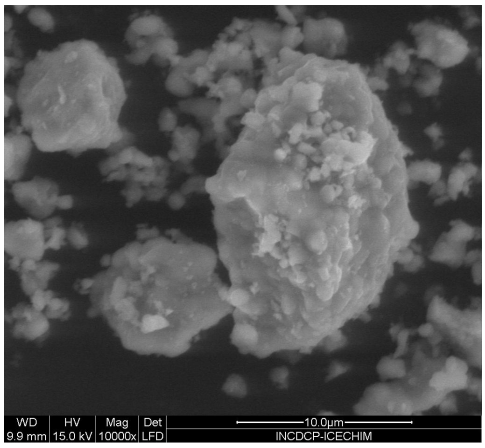

Figure 10: *Electron microscopy SEM for Hap powder calcined at 1000^{0}C, 10000 X*

Subgrains size it is about 70 nm which corresponds to the synthetic HA powder size [5]. For a good correlation, SEM images show several clusters of high spherical spherulites and crystallites less than 0.1 µm in size.

3.3. AFM microstructural characterisation

Investigations by Atomic Force Microscope (AFM) were performed with an Agilent 5500 SPM system, described by PicoSPM controlled by a MAC Mode module and interfaced with a controller PicoScan from Agilent Technologies, Tempe, AZ, USA.

All AFM measurements (256 samples / line × 256 lines) were made by scanning the surface at a rate of 0.8 - 1.2 lines per second and were conducted at room temperature, the mode of palpation.

For investigation with the atomic force microscope, HA solutions were prepared fresh before each experiment by suspending an appropriate amount of each sample in ethanol. Scanning movement is led by a piezoelectric scanner which scans the tip in a raster pattern on the sample (or scans the sample on top).

AFM revealed a rough surface architecture for HA, the predominant size of grains being in the range of 90 - 100nm.

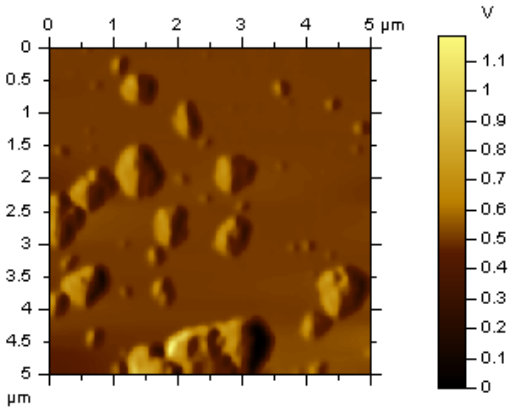

Figure 11: *AFM image of the HA powder calcined at 1000^{0}C*

AFM is conducted in an ambient gas or liquid medium and ethanol in our case. AFM shows the architecture for the HA surface, the grain is large in the range 70-100nm. Crystal size and size distribution reached critical nucleus depends on the size, in conditions of super saturation, rather than a standard crystal growth, because aggregation of very small particles was observed.

Atomic force microscopy (AFM) in comparison with the (SEM) can be measured in all three dimensions (x, y, and z) with a single scan. 3-D surface topography was recorded on an area of 0.5-0.5 mm^2.

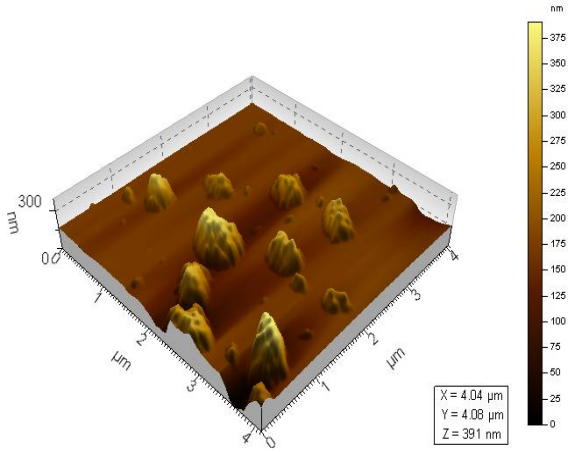

Figure 12: *3-D representation of the HA crystals obtained by wet precipitation*

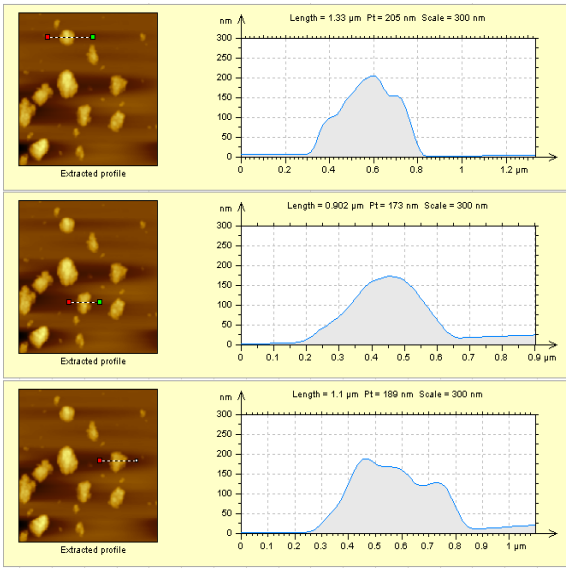

Figure 13: *AFM analysis of hydroxyapatite powder*

4. Conclusions

Hydrothermal techniques give hydroxyapatite powders with a high degree of crystallinity and better stoichiometry having a wide distribution of crystal sizes. Nanometer sized crystals can be obtained at temperatures lower than 100 °C with precipitation techniques.

The chosen methods was the wet precipitation because the wet-chemical precipitation route is the most talented route owing to its ease in experimental operations, low working temperature, high percentages of pure products and inexpensive equipment requirement.

In the SEM images of HA, was depicted small crystals (<100 nm) in the agglomerated particles and the uniform grain size with a narrow size distribution corresponding to an improved crystallinity of HA powders, especially for that sample after calcination at 1000°C for 1h. For a good correlation, the SEM pictures of the grown spherulite crystals show many spherical agglomerations and few crystallites of 0.1 µm in size.

The determination of size distribution of the grown spherulite and sintered HA materials were carried out by atomic force microscopy.

At higher temperature the deagglomeration of bulk phases and agglomeration of nano phases leads to the nano crystalline HA in this present study.

The crystal size distribution attained depends on the size of the critical nucleus under the supersaturation condition, rather than on standard

crystal growth, since the aggregation of very small particles was observed.

In 3-D graphical representation of hydroxyapatite crystals (Fig. 13) can be seen on the grain growth direction z due to the sintering process (wet precipitate) and particularly the calcination temperature.

One of the main attractions at the AFM is the ability to show high-resolution surfaces in the liquid insulation. Since AFM does not rely on the conductivity, and image scanning mechanism will not be disturbed by the presence of liquid

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