SPRAY DRYING OF AMORPHOUS CALCIUM PHOSPHATE

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Abstract. Hydroxyapatite is the major mineral constituent present in the body, in the teeth, in the bones and in other hard tissues, and this is why it is largely used as a substitute for the bone tissue implant in regenerative medicine. Wet synthesis is one of the processes most used. In this process, a product suspended in aqueous medium was obtained. The separation by filtration or evaporation of the final product has been employed. The aim of this study was to characterize the samples of the amorphous calcium phosphate spray dried. Two synthetic variants were studied. One without ultrasound and the other one with ultrasound. The obtained material was dried by spray drying. The obtained samples were characterized by X-ray Powder Diffraction, FTIR spectroscopy and Scanning Electron Microscopy. Particle size by scanning electron microscopy was determined. The calcium content by atomic absorption spectrometry was determined. Being the particle size smaller and homogeneous mainly in the samples to which ultrasound was applied. On the other hand, in other characterizations no differences between the dried methods were observed. Then it was concluded that the spray drying process does not affect the quality of the product.

Key Word: Amorphous calcium phosphate, Spray drying, Biomaterials, Particle size, Ceramics

INTRODUCTION

Calcium phosphates are widely used in medical, orthopedic and dental applications due to the compatibility of the synthesized material with the inherent properties of calcium phosphates present in the body, in the teeth, in the bones and in other hard tissues. Hydroxyapatite, Ca₁₀(PO₄)₆(OH)₂, forms between 30% and 70% of the mass of bones and teeth, being the main mineral constituent of these structures. In regenerative medicine it is used as a substitute for bone tissue implant (Perez, 2012). Several hydroxyapatite synthesis methodologies in the literature were reported. Solid-state reaction, precipitation methods, sol-gel hydrothermal, electrophoretic deposition methods among others have been used. The use of ultrasound or microwave during the synthesis process has been reported (Giardina et al, 2010; Zhang et al, 2007; Gedanken, 2004; Kim et al, 2000; Lopez et al, 1998; Jarcho et al, 1976).

Wet synthesis is one of the most used processes. In this process, a product suspended in aqueous medium was obtained. The separation by filtration or evaporation of the final product has been employed. Spray drying is a type of continuous dryer used to dry various materials where the solution or suspension to be dried is sprayed into a hot air stream and it is circulated through a chamber (Murtaza et al, 2012). The aim of this study was to characterize samples of amorphous calcium phosphate (ACP) spray dried.
MATERIALS AND METHODS

Synthesis of ACP (Ca$_3$(PO$_4$)$_2$.n H$_2$O).
Obtaining ACP was performed using the wet method (Perez, 2012). Calcium hydroxide solution was heated to 80 °C. Then, under constant stirring the phosphoric acid solution was added. Two variants of synthesis were studied. In one variant the synthesis process was performed without ultrasound. In the other variant, the synthesis process was performed with ultrasound. Equipment Sonus Vibra Cell, USA. (Pulse: 15s (on time) and 3s (off time); Amplitude: 30%) was used.
After the reaction was completed, both suspensions were atomized (Equipment Büchi 191, Switzerland) at a pressure of 600 L/h and a flow rate of 10 ml/min, while the inlet and outlet temperatures of the nozzle were adjusted to 160 °C and 70 °C, respectively. The samples obtained were characterized.

X-ray powder diffraction studies.
The XRD spectra were recorded at room temperature (25 °C) with a SIEMENS D5000, DIFFRAC PLUS XRD diffractometer (Germany) with BRAGG-Brentano geometry, Cu Kα radiation (λ=0.154 nm), Flicker detector and graphite monochromator. The scattering angle range from 4° to 80° with 2θ step interval of 0.02° was used. ACP samples were cut into small pieces and laid on the glass sample holder, analyzed under plateau conditions. An operating voltage of 40 kV and current of 30 mA was utilized, and the intensities were measured in the range of 5° < 2θ < 30°. Peak separations were carried out using Gaussian deconvolution. The d-spacings were calculated using the Bragg equation. Crystallographic search match software was used to identify the crystal structure of samples.

FTIR spectroscopy.
FTIR spectra of the samples were measured on a FTIR - VERTEX 70 / BRUKER spectrometer (Germany). A total of 64 cumulative scans were taken, with a resolution of 4 cm$^{-1}$, in the frequency range of 4000 to 400 cm$^{-1}$, in transmission mode.

Scanning Electron Microscopy.
Scanning electron microscopy (SEM) imaging of ACP was carried out using a FEG-MEV; JEOL 7500F scanning electron microscope (Germany). The equipment was operated at an acceleration voltage of 2 kV. The samples were coated by carbon evaporation (Baltec SCD 050 Sputter Coater, USA). Particle size by scanning electron microscopy was determined. Five SEM images at magnifications X100000 were evaluated. In each images, five random measurements of the particle size were performed. With the data obtained, the mean particle size was determined.

Calcium determination.
The calcium content by atomic absorption spectrometry was determined. Spectr AA-50 (VARIAN, USA) equipment (calcium hollow cathode lamp, air-acetylene gases, 422.7 nm wavelength and 10A current) were used. Standard calcium solutions used for calibration curve (between 2 and 14 ppm) contained weighed amounts of calcium carbonate (NIST SRM 915b dried at 250 ºC for 2 h) and La$_2$O$_3$ in the concentration of about 5.8%. Mix and bring to volume. Determine the calcium concentration in the equipment.
Statistical analysis.
The batch comparison was performed using an analysis of variance (ANOVA). The results were considered significant at p < 0.05.

RESULTS AND DISCUSSION
Mineral formation of apatites is complicated by the possibility of formation of different phases of calcium phosphates during the process. Other co-precipitant phases such as dicalcium phosphate dihydrate (Brushite, DCPD) and octacalcium phosphate (OCP). This occurs due to the initial precipitation of DCPD and/or OCP with subsequent transformation to a more apatitic phase. DCPD and OCP have been implicated as potential precursors of apatite formation (Johnsson et al, 1992; LeGeros et al, 1984).

Figure 1 shows the XRD patterns of untreated and treated with ultrasound sample. Four crystalline phases were detected in both samples. Octacalcium phosphate (OCP), hydroxyapatite (HA), calcium hydrogen phosphate (DCPD) and calcium oxide (CaO), characteristic of ACP type apatite (Perez, 2012). The main indices (h k l) for ACP are indicated on the spectra according to the International Centre for Diffraction Data (PDF 18-0303).

![Figure 1: X-ray diffractogram. a: Rx analysis of ACP synthesized without ultrasound and b: Rx analysis of ACP synthesized with ultrasound](image-url)

In the sample without ultrasonic treatment intense peaks corresponding to the OCP phase are observed. It is also observed the presence of less intense peaks corresponding to the DCPD phase. On the other hand, in the sample treated with ultrasound a decrease in the intensity of the peaks corresponding to the OCP phase was observed. Meanwhile, the intensity of the HA and DCPD peaks increases.

It is also known that the direct precipitation of HA depends on the availability of PO₄³⁻, Ca²⁺ and OH⁻ ions in the solution, as well as the agitation method used. The latter is a key factor to facilitate the dissolution process of sample and thus the formation of HA (Giardina et al, 2010). Ultrasonic treatment is a more efficient method than the mechanical stirring facilitating the process of dissolution of the solids. Ultrasonic treatment stimulates the reactivity of the chemical species involved in the synthesis process. Studies reported on the sonochemical synthesis of HA from H₃PO₄ and Ca(OH)₂ assume that in the first 15 min of the reaction the formation of CaHPO₄...
2H₂O (DCPD) predominates as an intermediate formed by the typical acid-base reaction (Kim et al., 2001).

On the other hand, the heating of the sample to 80 °C during the synthesis process in association with the ultrasonic radiations, accelerate the process of dissolution of the sample. In our opinion, under these conditions, the formation of the DCPD phase was favored as the intermediary of the reaction. This may explain the results observed in the XRD where in the sample treated with ultrasound the predominant precursor species was DPCP.

Figure 2 shows the FTIR spectrum of the synthesized samples. The bands at 1120 and 1026 cm⁻¹ (stretching vibration) and the bands at 559 and 515 cm⁻¹ (bending vibration) are due to the presence of phosphate groups. The spectra also show a peak at 890 cm⁻¹ (stretching vibration) is due to the presence of PO₄³⁻ ions. The peaks characteristic of the presence of the OH group of the hydroxyapatite (approximately 3500 and 650 cm⁻¹) are not perceptible in the spectrum, which does not mean that it is not present in the structure of the hydroxyapatite. It has been reported that the infrared spectrum is not definitive to determine the presence of OH groups in the structure of the hydroxyapatite due to the widening of the phosphate bonds particularly in uncrystalline materials (Taylor et al., 2003).

Figure 2: FTIR spectra. a: FITR analysis of ACP synthesized without ultrasound and b: FITR analysis of ACP synthesized with ultrasound

Peaks at 1408, 1343 (asymmetric stretch vibration) and 882,1 cm⁻¹ (out-of-plane bend vibration) characteristic of the CO₃²⁻ group were observed. These peaks indicate the presence of type A and type B carbonated hydroxyapatite in the samples. Differences between the samples were not observed.
SEM analysis showed for both samples the formation of spherical groupings characteristic of spray drying (Figure 3). These spheres are composed of agglomerated particles of different sizes. The agglomeration during the spray drying process can be defined as the association of smaller particles into clusters due to bringing independent particles into contact with one another (Murtaza et al, 2012). In the case of the sample obtained by the synthesis without ultrasound, the average particle size was 85,1 ± 6,9 nanometers (261 to 32 nm). While in the sample obtained by the ultrasound synthesis, the mean particle size was 36,1 ± 1,1 nanometers (58,7 to 21,1 nm) (Figure 4). The statistical analysis applied to compare the samples (ANOVA) showed significant differences between them (p = 0.0156) for a level of 0.05.

![Figure 3: Results of analysis by scanning electron microscopy. ACP synthesized without ultrasound (a: X 25000; b: X 50000 and c: X 100000). ACP synthesized with ultrasound (d: X 25000; e: X 50000 and f: X 100000)](image)

It is known that ultrasonication is an effective way to break aggregates and reduce particle size. The process of cavitation in aqueous medium favors the formation, growth and rupture of microbubbles causing a decrease of the particles. On the other hand, this process stimulates the reactivity of the chemical species involved, resulting in the effective acceleration of heterogeneous reactions between liquid and solid reactants (Hazar et al, 2012).
In this study the results showed that the use of ultrasound during the synthesis process significantly decreases the particle size of the material. In addition, greater uniformity was achieved in the sample. In addition, a small particle size such as that achieved by the use of ultrasonics generated particles with a larger surface area, which favors the drying of the sample during the spray-drying process.

The results of the determination of calcium by atomic absorption (calibration curve: \( y = 0.0046X + 0.00, R^2 = 99.7\% \)) showed calcium values of 67.3 ± 3.6 and 69.4 ± 2.4 for ACP synthesized without ultrasound and ACP synthesized with ultrasound, respectively. Statistical analysis using the parental sample test showed that at the 0.05 level the mean population difference was not significantly different with the test difference (\( p = 0.8201 \)). This result confirmed that the calcium content in the sample was not affected by the use of ultrasound.

CONCLUSION
The results of this study demonstrate that the spray drying process allows to obtain ACP powder with adequate quality, obtaining better results with the process of synthesis done with application of ultrasound.

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REFERENCIAS


