

# Fish Bone as a Source of Raw Material for Synthesis of Calcium Phosphate

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Fish bone is rich in calcium carbonate, which makes it an alternative source of low cost calcium carbonate for the synthesis of calcium phosphate bioceramic for use in bone regeneration. The calcium phosphate bioceramic was prepared by a wet precipitation method with acid and base reactions. The synthesized bioceramic was characterized in terms of X-ray diffraction (XRD), scanning electron microscopy (SEM/EDS), thermogravimetric analysis (TGA), Fourier transform infrared spectroscopy (FTIR), average crystallite size and refinement by the Rietveld method for the quantification of crystalline phases. The results indicated the formation of a biphasic calcium phosphate bioceramic comprising 67.6 % of  $\beta$ -calcium pyrophosphate ( $\beta$ -CPP) and 32.1 % of  $\beta$ -tricalcium phosphate ( $\beta$ -TCP). This biphasic calcium phosphate bioceramic synthesized using fish bone waste presented nanostructured nature with an average crystallite size of 69.58 nm, which is very promising for biomedical applications.

**Keywords:** Calcium phosphate, biphasic bioceramic, fish bone waste, nanocrystallites.

## 1. Introduction

Around the world, bone defects are caused by trauma, excision, mutilation, extraction, or some abnormal developed tissue, resulting in a major global health problem around two millions bone repair procedures performed annually<sup>1</sup>. About thirty years ago, the field of materials engineering developed bone tissue engineering to induce bone regeneration, also called bone grafts, which have been shown to have excellent osteogenic capacity, osteoinduction, and osseointegration<sup>2</sup>. These materials were then called biomaterials or bone substitutes. It is well known that the biomaterials most commonly used for bone substitution are those based on calcium phosphate (Ca-P), such as hydroxyapatite, tricalcium phosphates, amorphous phosphate, dicalcium phosphate, among others. Due to the structural and chemical similarities with bone, calcium phosphate-based biomaterials have good biocompatibility, bioactivity and osteoconductivity with implanted tissue<sup>3</sup>.

One of the forms of calcium phosphate that has a high potential to be used as bone replacement material or bone graft is calcium pyrophosphate (CPP,  $\text{Ca}_2\text{P}_2\text{O}_7$ ). The CPP presents a biological response similar to that of the hydroxyapatite (Hap) for the formation of a new bone. The CPP proved to be more biocompatible to bone tissue than to Hap<sup>4</sup>. In fact, Hap particles have an inhibitory effect on the growth of osteoblast cell cultures<sup>5</sup>. This fact has attracted increasing interest in other calcium phosphate based materials<sup>4,6-9</sup>.

In Brazil, the aquaculture sector has been showing great prominence in the last years in the growth especially in the production of fish (fish farming). Tilapia is the most commonly produced freshwater fish species in the world, intended for fillet production<sup>10,11</sup>. However, activities related to fish production generate a large amount of solid waste in the form of carcasses (fish bones), which is usually discarded in the environment (rivers, landfills, and common garbage) inadequately. This causes pollution to the environment and great risk to public health<sup>12,13</sup>. This type of solid residue is rich in calcium carbonate. Therefore, it has great potential to be reused as an alternative source of calcium carbonate for the production of biocements based on calcium phosphate for regeneration or replacement of the bone tissue in medical and dental applications. In fact, several studies have demonstrated the use of alternative sources with high calcium carbonate content for the synthesis of biocements based on calcium phosphate<sup>14-16</sup>.

The aim of this work is to study the possibility of using fish bone waste (tilapia carcasses) from aquaculture sector in the synthesis of calcium phosphate bioceramic for use as bone regeneration material.

## 2. Experimental Procedure

The fish bone waste sample (tilapia carcasses) was supplied from a local fish market, Muriaé-MG, Brazil. Initially, the waste sample was washed with running water at 70 °C to facilitate the removal of the glued meat to the bone.

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Then, it was dried at 110 °C for 24 h to remove moisture, crushed into powder, and sieved through a 140 mesh sieve (106 µm). Mineralogical analysis via X-ray diffraction indicated that the cleaned tilapia carcasses powder is composed of hydrated hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ). Chemical analysis determined by using an energy-dispersive X-Ray spectrometer (EDX 700, Shimadzu) indicated that the cleaned tilapia carcasses powder is essentially composed of calcium (Ca) and phosphorus (P).

The calcium phosphate bioceramic was synthesized from fish bone waste (tilapia carcasses) by the acid-base precipitation method<sup>17</sup>, by using the chemical reagents  $\text{HNO}_3$  (Vetec, 65%) and  $\text{Na}_2\text{HPO}_4$  (Sigma-Aldrich, 98%). Initially, the cleaned tilapia carcasses powder was slowly added in  $\text{HNO}_3$  solution (1 M) under constant stirring for 2 h, resulting in the formation of the  $\text{Ca}(\text{NO}_3)_2$  solution. Then,  $\text{Na}_2\text{HPO}_4$  was slowly added by titrating dropwise to the  $\text{Ca}(\text{NO}_3)_2$  solution under constant stirring at 50 °C for 2 h to form the precipitate. The resulting precipitate from the reaction was vacuum filtered, washed with distilled water and dried at 100 °C for 24 h. Finally, the dry precipitate (in powder form) was then calcined at 900 °C for 2 h, by using a heating rate of 10 °C/min.

X-ray powder diffraction analysis (XRPD) of the synthesized sample was done with a conventional diffractometer (Ultima IV, Rigaku) using  $\text{Cu-K}\alpha$  radiation. The XRD result was refined using the Rietveld method<sup>18,19</sup> for the quantitative identification of the crystalline phases formed. The average crystallite size (D) was calculated by using the Scherrer equation according to  $D = 0.9 \lambda / B \cdot \cos(\theta)$ , where  $\lambda$  is the wavelength of  $\text{Cu-K}\alpha$  radiation, B is the full width at half maximum of the calcium phosphate line, and  $\theta$  is the diffraction angle. The morphology of the synthesized powder particles was observed using a scanning electron microscopic (Quanta SEM, FEG 250) attached with an energy dispersive X-ray spectrometer (EDS). Thermogravimetric analysis (TGA) of the synthesized powder was carried out with a TA Instrument SDT-2960, by using a heating rate of 10 °C/min under air atmosphere. For the identification of the functional groups in the synthesized powder, FTIR (Fourier Transform Infrared Spectroscopy) analysis was performed by using a Spectrum 400 FTIR spectrophotometer (Perkin-Elmer) in the range from 400 to 4000  $\text{cm}^{-1}$ .

### 3. Results and Discussion

#### 3.1 XRD characterization

The X-ray diffractogram of the synthesized calcium phosphate powder is shown in Fig 1. It was found that the synthesized powder is a biphasic mixture composed of tetragonal structured  $\beta$ -calcium pyrophosphate ( $\beta$ -CPP,  $\beta\text{-Ca}_2\text{P}_2\text{O}_7$ , PDF Card: 01-071-2123) and rhombohedral structured  $\beta$ -tricalcium phosphate ( $\beta$ -TCP,  $\beta\text{-Ca}_3(\text{PO}_4)_2$ , PDF Card: 01-072-7587), with predominance of the  $\beta$ -CPP phase.

It can also be seen that there was the presence of isolated particles of calcium (Ca) identified by the PDF card: 01-077-7215. Such a presence of calcium is related to the increase in the pH of the calcium nitrate solution formed from the fish bone waste, which liberates free calcium. The XRD spectrum after refinement by the Rietveld method ( $R_{wp} = 8.32$  and  $\chi^2 = 1.01$ ) is shown in Fig. 2, where the amounts of each identified phase are presented. The following values of the formed phases were found:  $\beta$ -CPP (67.6 %),  $\beta$ -TCP (32.1 %), and Ca (0.3 %). This result confirms the predominance of  $\beta$ -CPP and the secondary phase  $\beta$ -TCP, as well as the isolated presence of calcium particles formed.

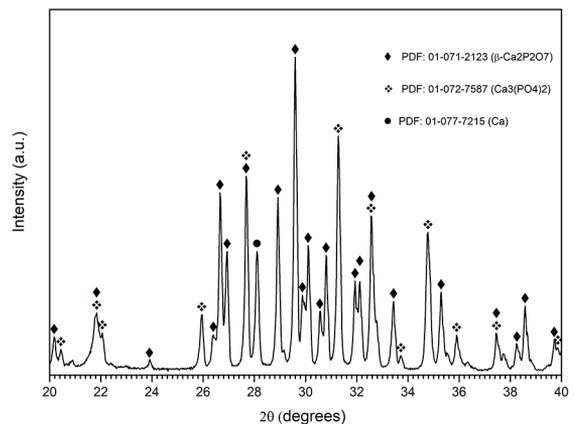


Figure 1. X-ray diffraction pattern of calcium phosphate powder.

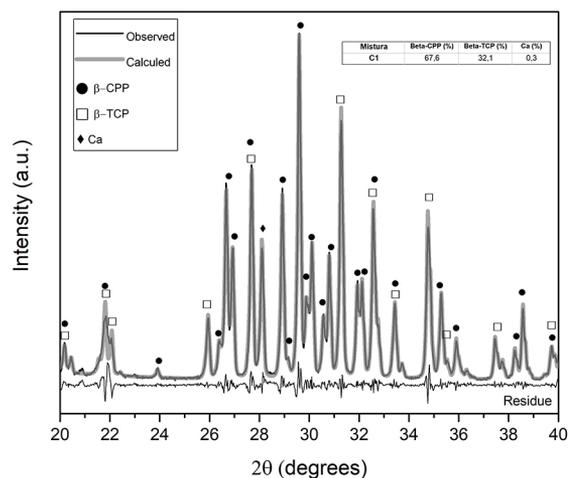


Figure 2. Rietveld analysis pattern for the calcium phosphate powder.

The powder of the synthesized biphasic calcium phosphate mixture has a mean crystallite size obtained by the Scherrer equation within the nanometric range of the order of 69.58 nm. This result is very interesting for biomedical applications, especially in the area of bone tissue regeneration<sup>20</sup>.

### 3.2 Morphological characterization

The morphological characterization of the synthesized calcium phosphate powder is shown in Fig. 3. It can be seen in the SEM image that the biphasic calcium phosphate bioceramic particles are grouped into the formation of highly agglomerated nanoparticle plates. However, the morphology of the crystals has irregular shapes. Through EDS analysis shown in Fig. 4, it can be noted the presence of three main peaks corresponding to the elements Ca, P and O. This result is consistent with the XRD analysis (Fig. 1). The presence of copper (Cu) is related to the metallization technique, while the presence of carbon (C) is due to the carbon ribbon used in the preparation of the sample. The Ca/P ratio of the synthesized bioceramic (Ca = 19.88 and P = 14.61) obtained through SEM/EDS semiquantitative chemical analysis was approximately 1.36. Such a Ca/P value corresponds to an intermediate value between that of  $\beta$ -calcium pyrophosphate (Ca/P = 1) and  $\beta$ -tricalcium phosphate (Ca/P = 1.5).

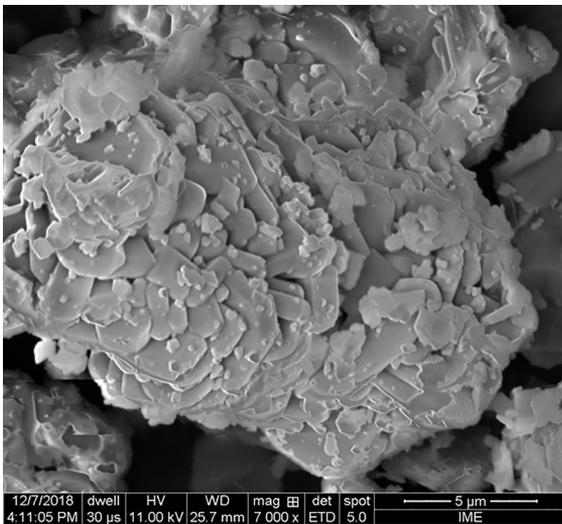


Figure 3. SEM micrograph of the calcium phosphate powder.

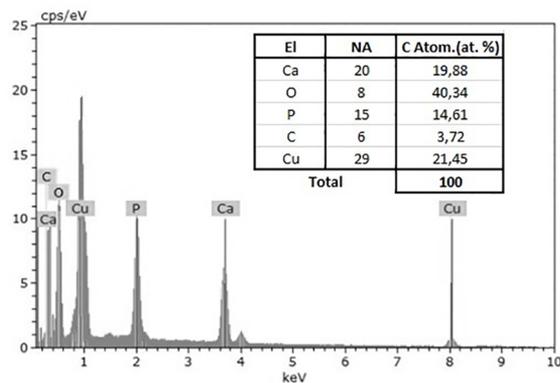


Figure 4. EDS spectrum of the calcium phosphate powder.

### 3.3 Thermal characterization

The Thermogravimetric analysis of the calcium phosphate bioceramic synthesized using fish bone waste (tilapia carcasses) as calcium source is shown in Fig. 5. It was found that the synthesized biphasic bioceramic presented high thermal stability when exposed to temperature up to 1000 °C. The total weight loss was of 1.34 %. It can be seen in Figure 5 the presence of two thermal events. The first thermal event (between ~ 225 °C and 400 °C) totaling 0.71% of weight loss is attributed to the removal of physically adsorbed water on the particles of the synthesized bioceramic. The second thermal event (between ~ 400 °C and 650 °C) totaling 0.63 % of weight loss is probably related to the presence of absorbed ions at sample surface.

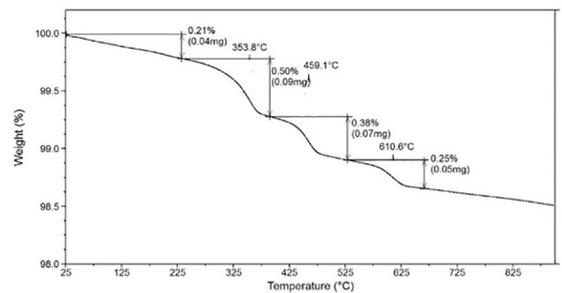
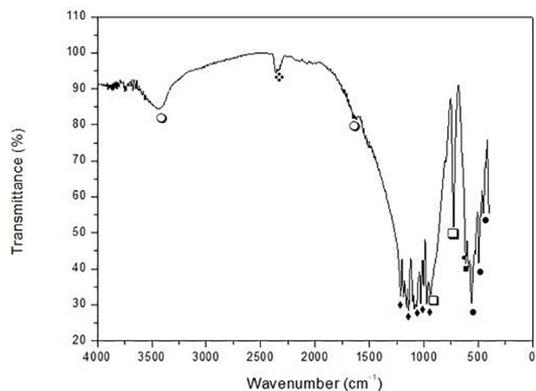


Figure 5. Thermogravimetric analysis of the calcium phosphate powder.

### 3.4 FTIR characterization

The vibrational spectrum in the infrared region (range of 4000 - 400  $\text{cm}^{-1}$ ) of the synthesized calcium phosphate bioceramic obtained by wet precipitation is shown in Fig. 6. The energy bands related to the present vibrational groups are interpreted according to the literature as follows<sup>4,21-22</sup>. The energy bands located at 450, 480, 560, and 620  $\text{cm}^{-1}$  are related to the asymmetric stretching vibrations of O-P-O of the  $\text{PO}_4^{2-}$  groups, while the band at 610  $\text{cm}^{-1}$  corresponds to the O-P-O deformation of the group  $\text{PO}_4^{3-}$ . The bands around 720  $\text{cm}^{-1}$  and 900  $\text{cm}^{-1}$  represent the symmetrical stretching of C-O of the  $\text{CO}_3^{2-}$  groups. The vibrational bands located at 960, 1010, 1030, 1070, and 1140  $\text{cm}^{-1}$  are related to asymmetric stretching of  $\text{PO}_4^{3-}$  or  $\text{PO}_3$  stretch in  $\text{HPO}_3^{2-}$ . Vibrational energy band corresponding to 2360  $\text{cm}^{-1}$  shows the vibration of the  $\text{CO}_2$  grouping, which presence is due to the room atmosphere where the equipment is located. The two weak energy bands located at 3410  $\text{cm}^{-1}$  and 1645  $\text{cm}^{-1}$  correspond to the vibrations of the OH groups (groups of free or hydrogen bonded hydroxyl groups with adsorbed water binding mode), showing the presence of the axial deformation of the group OH.



**Figure 6.** FTIR spectrum of the calcium phosphate powder:  $\text{mH}_2\text{O}$ ;  $\text{vCO}_2$ ;  $\text{wPO}_3^{-2}$ ;  $\text{qCO}_3$ ;  $\text{nPO}_4^{-3}$ ;  $\text{IPO}_4^{-2}$ .

## 4. Conclusions

The experimental results of this work indicated that the fish bone (tilapia carcass) synthesized by wet precipitation method could be a suitable source to produce new nanostructured biphasic bioceramic based on calcium phosphate. X-ray diffraction analysis and refinement by the Rietveld method indicated that the synthesized bioceramic is a biphasic mixture of  $\beta$ -CPP and  $\beta$ -TCP with major proportion of the  $\beta$ -CPP phase. The new biphasic calcium phosphate bioceramic presents irregular morphology, high thermal stability and nanostructured nature with a mean crystallite size of 69.58 nm. In addition, the semiquantitative chemical analysis (SEM/EDS) indicated that the Ca/P ratio was equal to 1.36. Therefore, the fish bone waste (tilapia carcass) has great potential for producing nanopowder of biphasic calcium phosphate bioceramic for biomedical applications, particularly for regeneration and replacement of damaged bone tissue in orthopedics and orthodontics.

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