CHARACTERIZATION OF A CALCIUM PHOSPHATE CEMENT BASED ON ALPHA-TRICALCIUM PHOSPHATE OBTAINED BY WET PRECIPITATION PROCESS

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ABSTRACT

There are several systems of calcium phosphate cements being studied. Those based on alpha-tricalcium phosphate are of particular interest. After setting they produce calcium-deficient hydroxyapatite similar to bone like hydroxyapatite. This work aims to obtain alpha-tricalcium phosphate powders by the wet precipitation process, using calcium nitrate and phosphoric acid as reagents. This powder was characterized by infrared spectroscopy, X-ray diffraction and particle size distribution. In order to prepare the calcium phosphate cement, the powder was mixed with an accelerator in an aqueous solution. The mechanical properties of the cement were assessed and it was evaluated by means of apparent density, X-ray diffraction and scanning electron microscopy. The described method produced crystalline alpha-tricalcium phosphate as the major phase. The calcium phosphate cement showed high values of compression strength (50 MPa). The soaking of the cement in a simulated body fluid (SBF) formed a layer of hydroxyapatite like crystals in the surface of the samples.

Keywords: cement, nitrate, biomaterials, alpha-TCP

INTRODUCTION

During the past 50 years, advances in many specialty bioceramics such as alumina, zirconia, hydroxyapatite, tricalcium phosphates and bioactive glasses have made significant contribution to the development of modern health care industry and have improved the quality of human life. These are the ceramics, which can be used inside the body without rejection to augment or replace various diseased or damaged parts of the musculoskeletal system [1,2]. They are primarily used as bone substitutes in the biomedical industry due to their biocompatibility, low density, chemical stability, high wear resistance, and for calcium phosphates, mainly for their compositional similarity with the mineral phase of bone. But the potential of any ceramic material to be used as an implant in vivo depends upon its ability to withstand complex stresses at the site of application and its compatibility with the biological environment. Calcium phosphates are the materials of choice in both dentistry and medicine. They have been used in the field of biomedical

engineering owing to the range of properties that they offer, from tricalcium phosphates being resorbable to hydroxyapatite being bioactive; they are undeniably the current rage for clinical usage [2-4]. They exhibit considerably improved biological affinity and activity compared to other bioceramics.

Despite the existence of various systems of calcium phosphate cements (CFC) studied, the ones based on α-tricalcium phosphate (α-TCP) are of particular interest due to the formation, during the setting reaction, of calcium deficient hydroxyapatite, similar to bone hydroxyapatite. Hydroxyapatite promotes bone growth where implanted, establishing links of chemical nature between the hydroxyapatite and the bone (bioactivity), allowing the proliferation of fibroblasts, osteoblasts and other bone cells [5-7]. It has been reported that α-TCP is a metastable phase and can only be obtained after heat treatment of β-TCP up to 1250 °C for 15 hours, followed by rapid cooling, resulting in a material composed by α-TCP as major phase [8].

The *in vivo* osteoinductivity and degradability of cements are commonly simulated *in vitro* by immersing them into a Simulated Body Fluid (SBF). Through the studies of the apatite formation and the *in vitro* degradation properties, the *in vivo* osteoinductivity and degradation properties of cements can be predicted [9-13]. Currently, these *in vitro* simulations are typically performed only in non-moving (i.e. “static”) simulated body fluid that contains ions similar to those of blood plasma [9]. Alpha- tricalcium phosphate (α-TCP) are widely used in tissue engineering for the regeneration of bone tissue, due to their biocompatibility and biodegradability [14,15]. However, the brittle nature of α-TCP development confines clinical application to non-load-bearing repair and substitution.

This work aims to obtain and characterize alpha-tricalcium phosphate powders. The powder is obtained by the wet precipitation process, using calcium nitrate and phosphoric acid as reagents. To obtain the powder was not use rapid cooling and reducing the holding times during the calcinations. In order to prepare the calcium phosphate cement the powder was mixed with an accelerator in an aqueous solution, for making specimens. The specimens are soaking in SBF for until 14 days to then assess the mechanical properties.

MATERIALS AND METHODS

The TCP was synthesized in laboratory using calcium nitrate ($Ca(NO_3)_2.4H_2O$) and phosphoric acid (H_3PO_4), according to the following reaction:

$$9\ Ca(NO_3)_2 \cdot 4\ H_2O + 6\ H_3PO_4 \rightarrow\ 3\ Ca_3(PO_4)_2 + 36\ H_2O + 18\ HNO_3$$

The Ca/P ratio was maintained in 1,5 and the reagents concentration was 1,0 M. The solution obtained by mixing the reagents was maintained at a temperature of 90 ± 2 °C for 24 hours. After this period, the solution was dried at 120 ± 2 °C for 24 hours and then calcined at 1500°C for 1 hour.

After the calcination, the reaction product was grounded using mortar and pestle to obtain a fine powder sieved in # 325, resulting in the samples named C.

The evaluation of the phase composition of powder after calcination and the specimens after compression test was performed using X-ray diffraction. Phillips X'Pert MPD diffractometer with a copper tube (Kα radiation = 1.5418 Å) was used for this analysis. The voltage and current used in the tube were 40 kV and 40 mA, respectively. The scanning speed of the goniometer was 0.05 °/s, and the scan interval (2Ɵ) from 10 to 40°.

Cement pastes were prepared by addition of a 2.5% w/v Na_2HPO_4 aqueous solution to the powder. The L/P ratio employed was maintained in 0.3 $mL.g^{-1}$. The samples were conformed in a mold according to ASTM F 451-95 [16]. The mold used was stainless steel containing cavities of 6 ± 0.1 mm in diameter and 12 ± 0.1 mm in height. The demolded samples were kept in a humid environment for 24 hours, and then be immersed in SBF solution for different periods of time.

A liter of simulated body fluid (SBF) was prepared by dissolving NaCl 7.995 g, $NaHCO_3$ 0.353 g, KCl 0.224 g, $K_2HPO_4.3H_2O$ 0.228 g, $MgCl_2.6H_2O$ 0.305 g, $CaCl_2$ 0.227 g, and Na_2SO_4 0.071 g into distilled water. The solution was buffered at pH 7.4 by adjusting the volume amount of Tris (tris-hydroxymethylaminomethane) and HCl at 36.5 °C. The *in vitro* studies were performed by immersing the specimens in a customized static chamber at 37°C. The SBF was replaced daily. The specimens were immersed in SBF for 0, 1, 7 and 14 days, resulting in the samples named C-0D, C-1D, C-7D and C-14D, respectively.

The apparent porosity and apparent density was performed based on ASTM C20 - 00 (2010) [17].

The compressive strengths after SBF immersion were determined using a universal mechanical testing apparatus (Instron, 3369) at ambient temperature. Samples were compressed between platens with a constant deformation rate of 0.5 mm/min. All results were the averages of three measurements.

Scanning electron microscopy (SEM), was performed on the fracture surface of specimens used in compression test, using a JEOL scanning electron microscope, model JSM 6060.

RESULTS AND DISCUSSION

It can be seen from Fig. 1 that the porosity of the specimens manufactured, firstly decreased and then tended to increase up to the degradation time. At the early stage of degradation, apatite formation was faster than the degradation of the samples. Therefore, the formed apatite compensated for the increase in porosity. With increasing degradation time, the degradation and dissolution of α-TCP accelerated the increase in porosity. The apparent porosity of the samples has great influence in the apparent density. As result of lower porosity, can be observed the increased of apparent density of the samples immersed in SBF solution for 7 days.

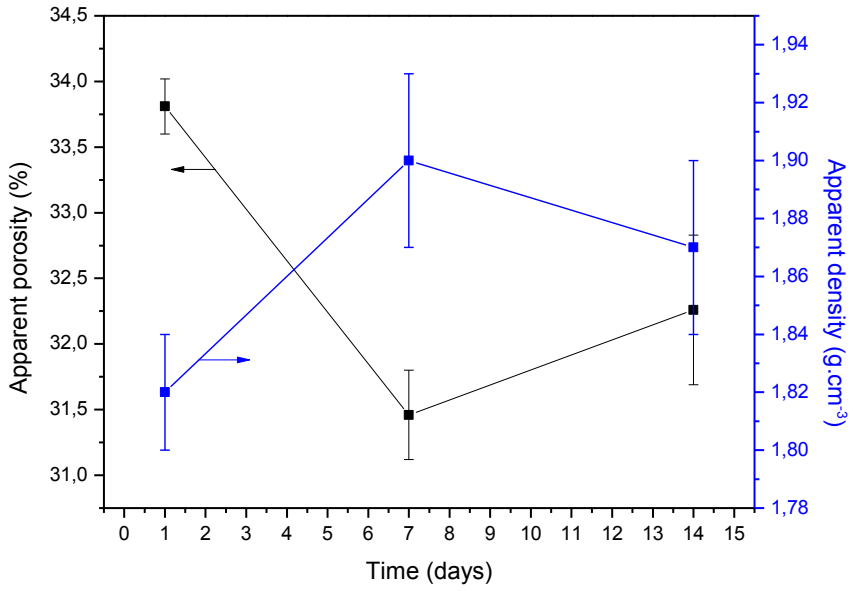


Figure 1. Influence of immersion time in SBF solution, in the apparent porosity and density of the samples.

X-ray diffraction (XRD) patterns show that the immersion time in SBF solution has influence on the products. The peaks pointed in the diffractogram of Fig. 2, correspond to the diffraction pattern data sheets number 00-029-0359 (α-TCP) and 00-009-0432 (Hydroxyapatite) of the Joint Committee on Powder Standards Diffraction - JCPDS.

It is possible to observe the presence of phase α-TCP and Hydroxyapatite for the powder and for all samples, however in different amounts. The presence of β-TCP as phase undesirable, in the process of synthesis of α-TCP has been reported [18]. Currently,

there are no known data on literature about pure α-TCP for use as cement, independently of the synthesis method used [19]. The rate of transformation of β-TCP in α-TCP is reported in literature as low and reversible. For obtaining α-TCP of high purity, the use of high holding times, at temperatures higher than 1200 °C, and quenching is necessary to preserve the α-TCP phase at room temperature. In this work, it was not use high holding times and not uses quenching for obtaining the α-TCP, however the hydroxyapatite is present in powder synthesized.

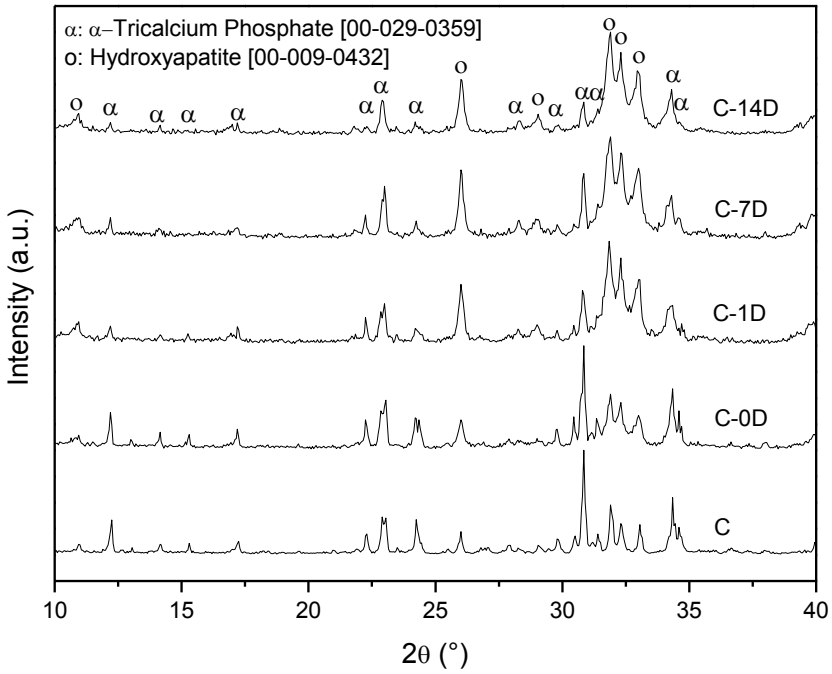


Figure 2: X-ray diffraction patterns of the samples.

By the diffractograms it is noted that the formation of hydroxyapatite gradually increased with the immersion time in SBF solution, but apparently remained unchanged for 1 and 7 days. However not all α-TCP was converted into hydroxyapatite, after 14 days of immersion.

The results obtained by compression strength of the samples, are shown in Table 1.

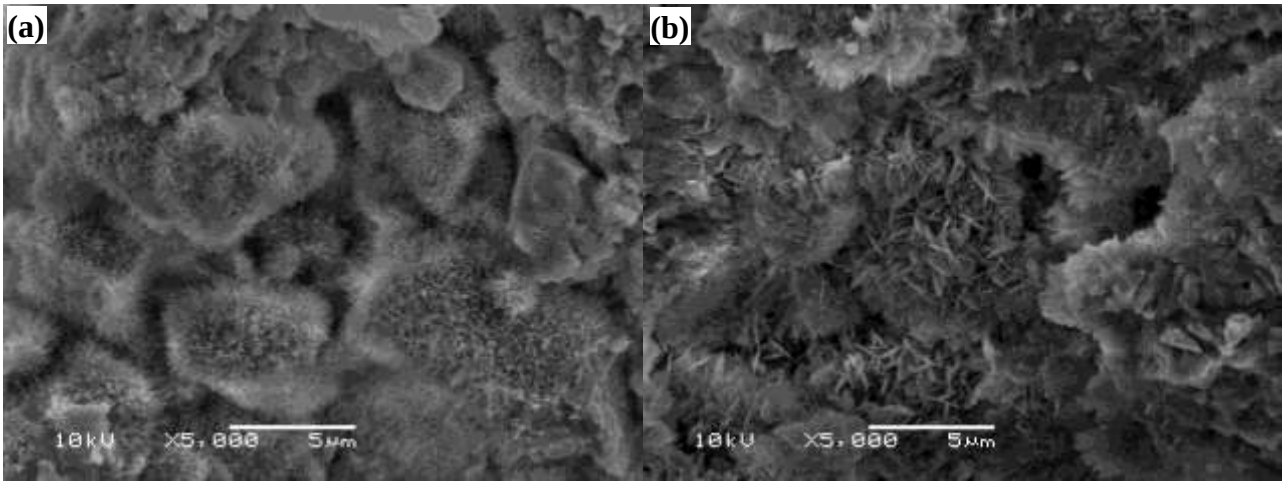
Table 1. Changes in compressive strength as a function of immersion time

Sample	*C-0D*	*C-1D*	*C-7D*	*C-14D*
Compression strength [MPa]	19.30 ± 3.00	30.90 ± 4.50	28.80 ± 3.30	34,30 ± 3.00

From the values shown in Table 1, it is found that the compressive strength is influenced by apparent porosity and by hydroxyapatite formation. As well as in the apparent porosity, for C-1D sample, the apatite formation was faster than the degradation of the sample, resulting in slightly greater compression strength. After this, the porosity of the samples decreased, but the compression strength was practically the same. At 14 days of immersion in SBF solution, the porosity was a slight increase as well as the compressive strength, possibly due to greater formation of hydroxyapatite. With longer times of immersion, it is expected that the compression strength achieves even higher values, due to higher formation and crosslinking of the hydroxyapatite needles.

The increase of mechanical strength of calcium phosphate cements, is attributed to hydration of α-TCP, resulting in hydroxyapatite. Hydroxyapatite crystallites are nucleated on the surface of α-TCP particles by supersaturated solution of Ca and P that surrounds the particles after the initial dissolution of the particles. Subsequent dissolution of α-TCP particles and growth of hydroxyapatite crystals are controlled by the diffusion process, by the layer of hydroxyapatite crystallites. The characteristics of hardening of the cement depend on the formation of a network of interlocking crystals, through this process of diffusion. Thus, the increased mechanical strength of some samples may be associated with greater interlacing of hydroxyapatite crystals [20].

The Fig. 3 shows the fracture surface micrographs of samples immersed in SBF solution, for different times.



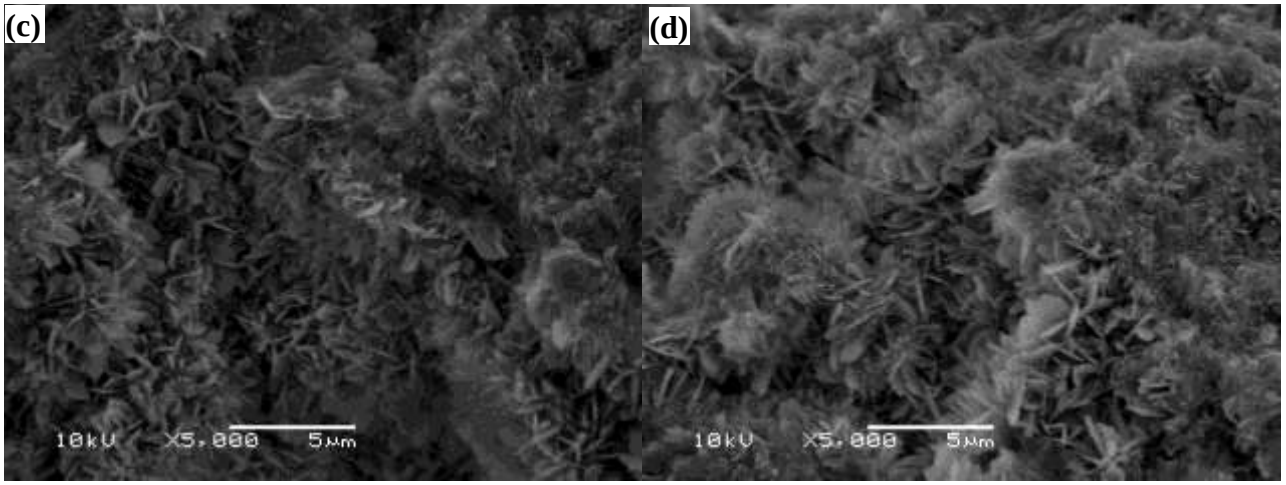


Figure 3. Fracture surface micrographs of (a) C-0D, (b) C-1D, (c) C-7D and (d) C-14D.

In micrographs can be observe hydroxyapatite needles formation in all the samples, however in different amounts and sizes. The C-0D sample shows a small hydroxyapatite needles. In the C-1D and C-7D samples, the hydroxyapatite needles are very similar. However in the C-14D the hydroxyapatite needles are greater than other samples.

The increase in mechanical strength observed in the C-14D sample, occurs as a result of the formation of the intersection of hydroxyapatite needles precipitate after solubilization of α-TCP [21]. In general, the compressive strength increases with increasing immersion time in SBF solution due to the growth and interlocking of these needles or crystals.

Thus possibly, the presence of hydroxyapatite needles increases the mechanical strength. The mechanical strength observed for the specimens developed can be considered low if it is taken into account the requirements related to trabecular bone, in which the main request is in compression. However, it should be noted that the behavior observed *in vitro* may not correspond to the behavior *in vivo*, once the bone has little irrigation and that should occur osteoconduction for the cement implant site. These factors can result an increase in mechanical strength at the implant site.

CONCLUSIONS

By wet precipitation method, was obtained powder with α-tricalcium phosphate as major phase and hydroxyapatite as undesirable phase. By immersion of specimens at different times in SBF solution, was possible verified the changes in the compression strength and morphology of the samples. With the increase of immersion time, can be observed the increase in compression strength of the samples and more hydroxyapatite needles in the morphology. The synthesis method was an efficient process to the

development α-tricalcium phosphate. However, the synthesis route to be improved in order to obtain powders of high purity, ie without the presence of undesirable phases. Thus it will be possible obtain greater compression strength. With the increase in immersion time also expected that there is an increase in the compression strength, possibly occur because the crosslink of the hydroxyapatite needles.

The results obtained for compressive strength is not yet satisfactory, but with this work, it is possible to establish parameter settings that will contribute to obtaining high quality materials and with great application in medical and dental area.

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