

## Synthesis and Characterization of Novel Crystalline Mesoporous Beta-Tricalcium Phosphate Nanoparticles

Flávia Rodrigues Oliveira Silva<sup>a\*</sup>, Walter Kenji Yoshito<sup>b</sup>,  
Ivana Conte Cosentino<sup>c</sup>, Ana Helena Almeida Bressiani<sup>d</sup>  
and Nelson Batista Lima<sup>e</sup>

Centro de Ciência e Tecnologia de Materiais, Instituto de Pesquisas Energéticas e Nucleares,  
IPEN-CNEN/SP, Butantã, São Paulo - SP, Brazil

<sup>a</sup>\*frsilva@ipen.br, <sup>b</sup>wyoshito@ipen.br, <sup>c</sup>iconte@ipen.br, <sup>d</sup>abressia@ipen.br, <sup>e</sup>nblima@ipen.br

**Keywords:**  $\beta$ -tricalcium phosphate, magnesium, nanoparticles, mesoporous, aqueous precipitation synthesis.

**Abstract.** A nano-sized magnesium substituted beta-tricalcium phosphate (Mg: $\beta$ -TCP) was synthesized by an aqueous precipitation method, at room temperature, in one single step. In the present study, the novel and stable Mg: $\beta$ -TCP resulted in a crystalline and spherical nanoparticles (diameter of approximately 20 nm) with mesoporous structures and a high specific surface area (about 574 m<sup>2</sup>/g). These special characteristics make this novel crystalline mesoporous Mg:  $\beta$ -TCP nanoparticles ideal candidates for drug delivery system and a promising non-viral vector for gene therapy.

### Introduction

Synthetic calcium phosphates, especially hydroxyapatite (HA - Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub>) and beta-tricalcium phosphate ( $\beta$ -TCP - Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>), are of special interest in medicine because of their biocompatibility, bioactivity, non-cytotoxicity and their similarity with human bone and teeth [1]. Due to their excellent biological functions and responses in physiological environments, particular attention has been placed to  $\beta$ -tricalcium phosphate [2]. The bioresorbability and biodegradability features make the  $\beta$ -TCP being widely used in a number of biomedical applications such as orthopedics, dentistry, tissue engineering, and drug, gene and protein delivery.

$\beta$ -TCP is mainly prepared via the solid-state method above 1000°C [3] or wet-chemical method, wherein the calcium-deficient hydroxyapatite (CDHA) is obtained as a precursor and needs to be calcined above 700-800°C to transform into  $\beta$ -TCP phase [3, 4, 5],

Bow et al [2] have developed a route for synthesizing nano-sized  $\beta$ -TCP at room temperature, in methanol solvent, wherein an intermediate amorphous calcium phosphate, CaHPO<sub>4</sub> phase, is formed and the incorporation of carbonate during the synthesis process favors a  $\beta$ -TCP phase after 8h of aging stage.

Lee et al have synthesized [6]  $\beta$ -TCP using 50% magnesium ( $\beta$ -TCMP) substituted brushite as a precursor in a method of several digestion/boiling steps. They obtained ~80 nm sized platelets of  $\beta$ -TCMP, with some amount of Ca<sub>2</sub>(P<sub>2</sub>O<sub>7</sub>).

Substituting magnesium for calcium ions is known to stabilize  $\beta$ -TCP phase [3], Enderle et al [7] have found that Ca<sup>2+</sup> is preferably replaced by Mg<sup>2+</sup> on the six-fold coordinated Ca(5) site and on the nine-fold coordinated Ca(4) resulting in a change of lattice parameters c. Furthermore, Mg<sup>2+</sup> ion reduces the dissolution rate of Mg:  $\beta$ -TCP samples [8], decreases the particle size; improves the  $\beta$ -TCP phase thermal stability, the mechanical properties, biosolubility and biological responses *in vitro* and *in vivo* [9].

Until now, it was believed to be impossible synthesizing  $\beta$ -TCP structure directly from aqueous precipitation, at room temperature, even more in one single step. In this study, Mg<sup>2+</sup> substituted calcium phosphate crystalline nanoparticles have been prepared using the simple aqueous precipitation method. X-ray diffraction analysis, transmission electron microscopy and specific surface area analysis were used for samples characterization before and after thermal treatment.

## Materials and Methods

The Mg: $\beta$ -TCP powders were synthesized by co-precipitation method, wherein the phosphoric acid ( $\text{H}_3\text{PO}_4$ , Synth – Brazil) diluted in deionized water (0.3 M) was slowly added drop by drop (8 mL/min rate) into a suspension of calcium hydroxide ( $\text{Ca}(\text{OH})_2$ , Synth- Brazil) and magnesium hydroxide ( $\text{Mg}(\text{OH})_2$ , Synth Brazil) (0.5 M). The initial (Ca+Mg)/P ratio was fixed at 1.5. The pH value of one solution was adjusted below 6 during precipitation procedure by adding  $\text{HNO}_3$ . After the precipitation, the solutions were aged at room temperature and then the precipitates were filtered and washed several times with deionized water to remove free ions. Finally, they were dried at 60 °C for 24 h.

To evaluate the effect of thermal treatment, the dried powders were heat-treated at 950°C for 5 minutes or 1000°C for 30 seconds, using an adapted microwave oven [10]. Samples were characterized by X-ray diffraction (XRD) using a Multiflex Rigaku diffractometer using the Cu  $k\alpha$  radiation. The nanopowders were dispersed in absolute isopropanol and ultrasounds treated to avoid particles agglomeration, and then were dropped on a copper grid to be observed by transmission electronic microscopy (JEM 2010 - JEOL). Specific surface area was evaluated using the Brunauer Emmett-Teller method (BET).

## Results and Discussion

Fig. 1 shows the X-ray diffraction (XRD) patterns of the synthetic powders. All samples were identified as  $\beta$ -TCP according to JCPDS 09-0169 even those without thermal treatment (as-synthesized). The diffraction peaks are broadening for the as-synthesized samples and the X-ray diffraction pattern curves reveal subtle differences in the powders with and without pH adjustment.

Without pH adjustment during the synthesis - sample identified as  $\beta$ -TCP1 – the powders exhibit a broader profile due to nanoparticles with smaller crystallite size or a lower crystallinity. For the sample which one had the pH of solution modified to value below 6 (identified as  $\beta$ -TCP2), the diffraction pattern became narrower and defined. Differences in  $\beta$ -TCP1 and  $\beta$ -TCP2 crystallinity leads to distinguished dissolution rates.

In agreement with other studies [9], replacing  $\text{Ca}^{2+}$  for  $\text{Mg}^{2+}$  ions in calcium phosphate structure stabilizes the  $\beta$ -TCP phase and results in a shift in the X-ray diffraction peaks, due to a contraction in the unit cell dimension (attributed to the smaller ionic radius of  $\text{Mg}^{2+}$  (0.65Å) compared to  $\text{Ca}^{2+}$  (0.99Å).

The morphologies of the synthesized powders with and without pH adjustment (Fig. 2) show the influence of the pH in the size and shape of the particles. It is interesting to observe two different morphologies: without pH adjustment, the particles present no defined shape (Fig. 2.A), whereas the powders after the pH modification present a spherical and crystalline nanoparticle about 20 nm of diameter (Fig. 2.B).

After a rapid heating treatment (1000°C - 30 seconds) in an adapted microwave oven, the diffractogram remains similar of  $\beta$ -TCP2 as-synthesized, and for a longer period (950°C - 5 minutes) the peaks became narrower for both samples, confirming the  $\beta$ -TCP monophasic structure.

At high temperature, calcium phosphate absorbs microwaves efficiently leading to a complex ceramics sintering process [11]. The fast heating not only inhibits the growth of  $\beta$ -TCP2 (between 20 and 50 nm), but also promotes the mesoporous structure formation, as can be seen in Fig. 3.A, resulting in a significant increasing of the surface area valuated by BET analysis:  $\beta$ -TCP2 as-synthesized was  $116 \pm 2 \text{ (m}^2\cdot\text{g}^{-1}\text{)}$  and after the thermal treatment at 1000°C was almost 5-fold higher ( $574 \pm 7 \text{ (m}^2\cdot\text{g}^{-1}\text{)}$ ).

Adsorption is strongly enhanced since the sample has a high surface area [12], improving drug storage and release performance of the biomaterials. Their using is also promising as good adsorbents for the removal of dyes and heavy metal ions, in environmental applications [12].

The powders treated at 950°C for 5 minutes (Fig. 3.B) became somehow bigger (about 200 nm or more).

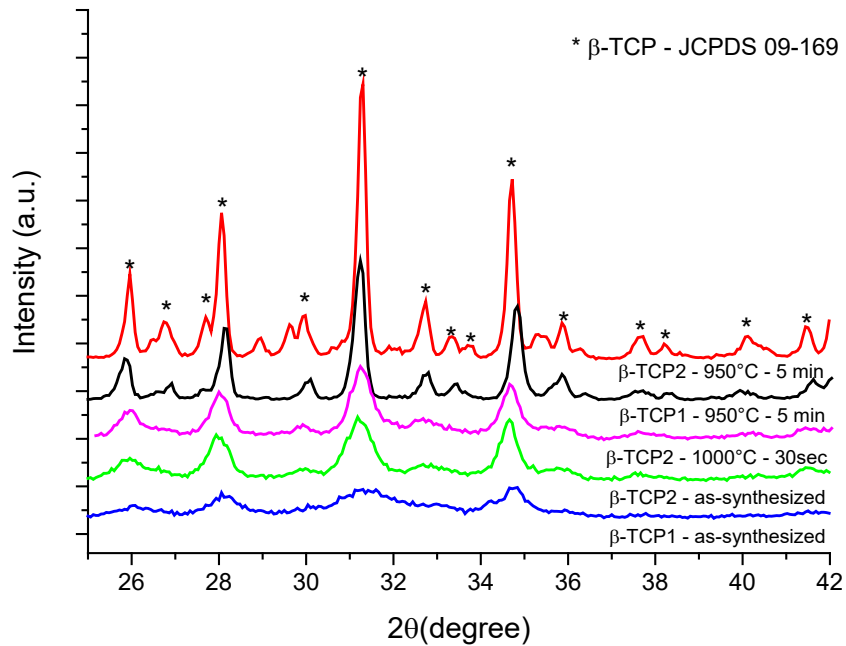


Fig. 1. X-Ray diffraction pattern of Mg:β-TCP1 (without pH adjustment), and Mg:β-TCP2 (pH adjusted below 6) as-synthesized and after thermal treatments, for 30 seconds, and for 5 minutes.

The shape, size, high crystallinity, mesoporous structures and high specific surface area of the novel beta-phosphate calcium synthesized at room temperature in aqueous solution, in one single step, should be advantageous for the aforementioned biological applications, especially as promising non-viral gene nano-vectors [13]. The small particle size and the positive cationic surface charge would increase the maximum amount of DNA condensation [13]. In addition, calcium dissolution rate, which one is important in endosomal escape and cytosolic stability, enhances nuclear uptake of DNA through nuclear pore complexes, improving the interaction with cell membrane and receptors as well as increasing transfection efficiency [13].

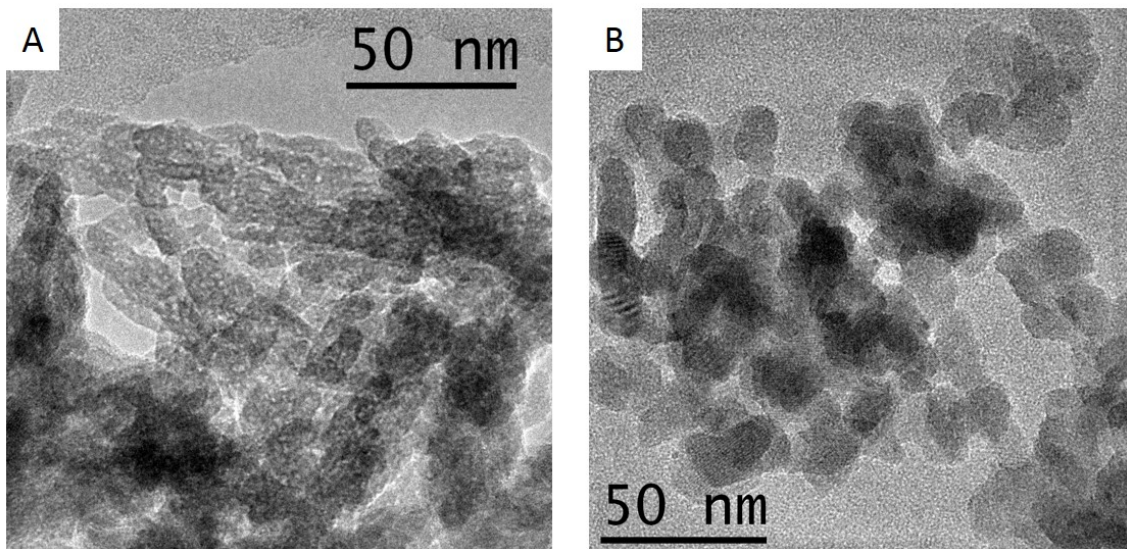


Fig. 2. TEM micrographs of (a) Mg:β-TCP1 (without pH adjustment) and (b) Mg:β-TCP2 (pH adjusted below 6), as-synthesized (no thermal treatment).

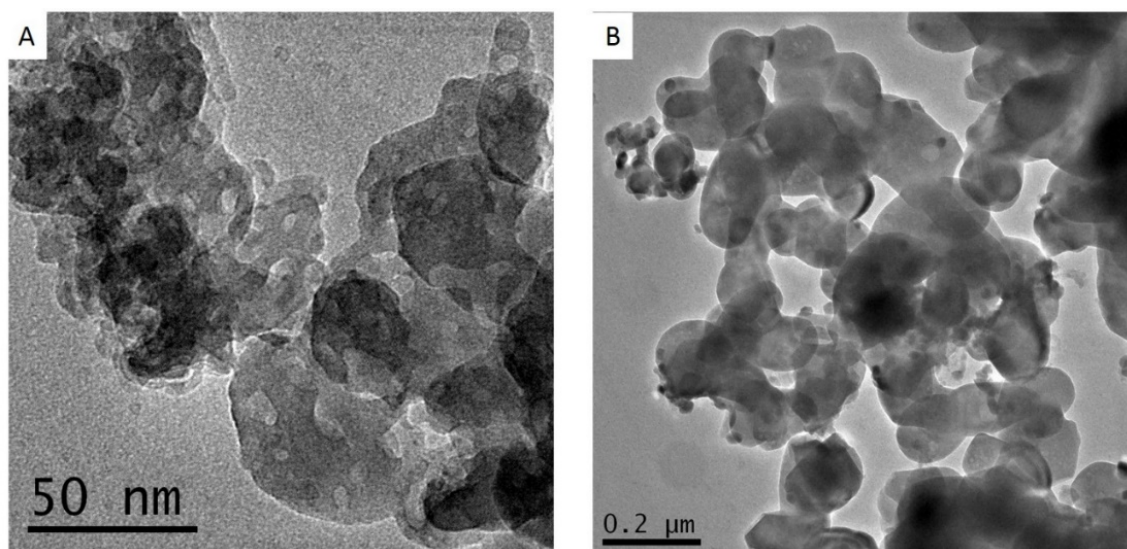


Fig. 3. TEM micrographs of Mg:β-TCP2 after thermal treatment: (a) 1000°C for 30 seconds and (b) 950°C for 5 minutes.

## Conclusion

Nano-sized β-TCP structure with ~20 nm in diameter has been well synthesized in water, at room temperature, in one single step. The results showed the ability to synthesize a stable magnesium doping beta-calcium phosphate presenting the ideal characteristics for biomedical applications: small particle size, spherical shape, mesoporous structures, high crystallinity, high specific surface area and high positive surface charge.

These special characteristics of the particular novel calcium phosphate structure would improve the widely biomedical and environmental applications, mainly the drug delivery and gene transfection efficiency as a non-viral vector for gene therapy, and as good adsorbents for water treatments.

## References

- [1] S.J. Kalita, A. Bhardwaj, H.A. Bhatt: *Mater. Sci. Eng. C* Vol. 27 (2007), p. 441.
- [2] J.S. Bow, S.C. Liou, S.Y. Chen: *Biomaterials* Vol. 25 (16) (2004), p. 3155.
- [3] S. Kannan, J. M. Ventura, J.M.F. Ferreira: *Ceramics International* Vol. 33 (4) (2007), p. 637.
- [4] W.I. Abdel Fattah, F.M. Reicha, T.A. Elkhooley: *Biomed. Mater.* Vol. 3 (3) (2008), p. 034121.
- [5] F.R.O. Silva, N.B. Lima, S.N. Guilhen, L.C. Courrol, A.H.A. Bressiani: *Journal of Luminescence* Vol. 180 (2016), p. 177.
- [6] D. Lee, C. Sfeir, P.N. Kumta: *Materials Science and Engineering C* Vol. 29 (2009), p. 69.
- [7] R. Enderle, F. Gotz-Neunhoeffler, M. Gobbels, F.A. Muller, P. Greil: *Biomaterials* Vol. 26 (2005), p. 3379.
- [8] C. Tardei, F. Gregore, I. Pasuk, S. Stoleriu: *J. Optoelect. Adv. Mater.* Vol. 8 (2) (2006), p. 568.
- [9] A. Hanifi, M.H. Fathi, H.M. M. Sadeghi, J. Varshosaz: *J Mater Sci. Mater. Med.* Vol. 21 (8) (2010), p. 2393.
- [10] F.R.O. Silva, N.B. Lima, W.K. Yoshito, A.H.A. Bressiani: *Journal of Luminescence* Vol. 187 (2017), p. 240.
- [11] Y. fang, D.K. Agrawal, D.M. Roy, R. Roy: *J. Mater. Res.* Vol. 9 (1) (1994), p. 180.
- [12] X. Li, W. Xiao, G. He, W. Zheng, N. Yu, M. Tan: *Colloids and Surfaces A: Physicochem. Eng. Aspects* Vol. 408 (2012), p. 79.
- [13] A. Maitra: *Expert Rev. Mol. Diagn* Vol. 5 (6) (2005), p. 893.