11th International Conference on Advanced Materials
Rio de Janeiro Brazil
September 20 - 25
ICAM2009

# HRTEM analysis of the crystallization process of calcium phosphate nanostructures

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**Abstract** – Calcium phosphates nanostructures were precipitated without and in the presence of bovine serum albumin (BSA) to evaluate its first stages of the crystallization process by high-resolution transmission electron microscopy (HRTEM) and conventional characterization techniques. Results showed that at basic conditions (without BSA) the nanoparticles pass through an amorphous calcium phosphate phase, transforming into a carbonated hydroxyapatite structure. At lower pH it was obtained brushite and the inhibitory effect of the protein on this phase. The coexistence of amorphous and crystalline phases and different crystallographic configurations was observed by high-resolved images for both synthesis routes.

Hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$, is one of the most important biomaterials used for medical applications, better known by its osteoconduction and biocompatibility properties with the calcified tissues [1]. Another characteristic of its structure is the ability to allocate a large number of ionic substitutions maintaining its hexagonal arrangement. Carbonate ions replace partially the phosphate and hydroxyl ions [2], known as carbonated hydroxyapatite (C-HA); reproducing more properly the structure and chemistry of the apatites founded in bones and teeth. Complementary, apatite biomineralization is controlled by different types of proteins, among them, serum proteins [3]. In the other hand, it is difficult to obtain ultrastructural features of apatite formation by conventional characterization methods due to the nanometric dimensions of the individual crystals. High-resolution transmission electron microscopy (HRTEM) has become a currently powerful technique in achieving crystallographic information of nanostructured systems, by its spatial, spectral and temporal resolution advantages. The aim of this study was to analyze by HRTEM carbonated calcium phosphate nanostructures in the first steps of the ageing time, as well as the influence of the BSA on its crystallization process.

Diammonium phosphate and ammonium carbonate solutions were added to a calcium nitrate tetrahydrate aqueous solution, at 4°C, in slowly magnetic stirring and pH 11 by the addition of potassium hydroxide. Samples were collected at different hours of the maturation process and characterized by X-ray Fluorescence (XRF), X-ray diffraction (XRD), Fourier transformed Infra-red spectroscopy (FTIR) and HRTEM. Another set were precipitated in the presence of BSA (in the calcium solution) at pH 9 by the addition of ammonium hydroxide and gathered during ageing course.

HRTEM analyses of high-resolved, Fast Fourier Transformed (FFT) and calculated images shown that at pH 11, an amorphous calcium phosphate (ACP) precursor [4] transforms into a crystalline apatitic C-HA, as well as the observation of core crystalline/amorphous shell structures [5] and the occurrence of additional crystallographic configurations. Otherwise, at pH 9 was obtained a dicalcium phosphate dehydrate (DCPD or brushite) phase. The presence of the BSA had a remarkable inhibitory effect on this phase. These analyses reinforced the importance of HRTEM to acquire new crystallographic information of this fundamental biomaterial.

## References

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