Bone mineral: Seeking a more efficient approach to produce nano-hydroxyapatite

Anabela Veiga[1,2,3*], Filipa Castro[2,3], Ana L. Oliveira[1] and Fernando Rocha[2,3]

[1]CBQF – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal;
[2]LEPABE - Faculty of Engineering of Porto, University of Porto, R. Dr. Roberto Frias, 4200-465 Porto, Portugal;
[3]ALiCE-Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal.

*s-anveiga@ucp.pt

Introduction/Resume

Calcium phosphate particles (CaPs) are one of the most investigated ceramic biomaterials, being chemically similar to the mineral phase of bone and teeth. Special attention has been given to nanometric-HAp (nano-HAp), because of its similarity in size, crystallinity and chemical composition with biological CaPs [1].

However, to produce nano-HAp in a controlled way still represents a challenge. Precipitation in stirred tank reactors (ST) is conventionally used [2]. Despite its simplicity, heterogeneous distribution of supersaturation in the reaction medium occurs as a consequence of the low micro mixing efficiency, in that regard, oscillatory flow mixing is presented as a "technology ready to deliver". The intensity of the mixture is controlled by the oscillation amplitude (*x0*) and frequency (*f*) [3]. Regarding the operation mode, continuous processes allow a more efficient use of the reagents and allow higher productivity to be achieved [4].

Objectives

In the present work, the development of fully characterized nano-HAp was achieved in a ST and meso-OFR (WO2015/056156A1) in batch and in a modular oscillator flow plate reactor (MOFPR) (WO/2017/175207) using a continuous process.

Methods

Experimental set-ups

As illustrated in **Figure 1**, the precipitation of HAp particles was carried out in batch in a ST that consists of a cylindrical vessel made of glass; a meso-OFR which is a glass jacketed tube with 2D smooth periodic constrictions (SPCs); and a MOFPR provided with SPCs that are present in two parallels faces of the rectangular cross section tube. The OFRs are connected to a mixing chamber that allow control of the oscillations of the fluid inside the reactors. All reactors are jacketed to allow temperature control and connected to a thermostatic bath. The experimental set-ups were also equipped with a pH electrode to measure the pH of the collected suspensions.

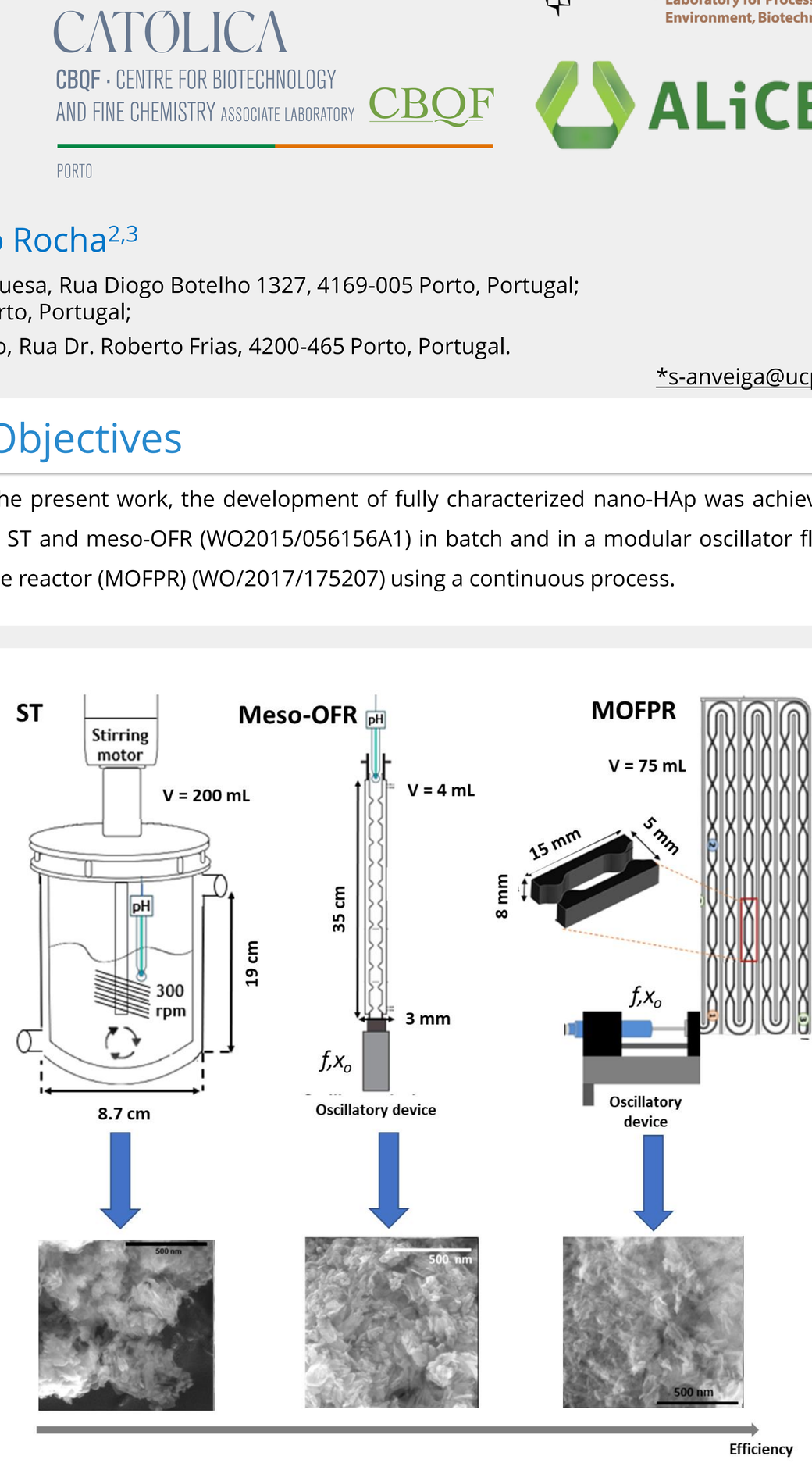

Figure 1. Experimental set-ups and produced nano-HAp particles.

Precipitation of nano-HAp

Nano-HAp materials were synthesized by mixing equal volumes of a solution $CaCl_2.2H_2O$ (Merck, 99.5%) (0,02 M) (pH ≈ 5.84) to a Na_2HPO_4 (Sigma-Aldrich, 99.0%) (0.012 M) (pH ≈ 8.52) solution (initial Ca/P molar ratio = 1.67). In order to main near-physiological conditions of pH and temperature (T), the T inside the reactors was maintained at 37 °C. pH profile was monitored over time for each experimental condition, since pH stabilization indicates the formation of the most stable CaP under the reaction conditions applied.

Physico-chemical characterization

The obtained powders were used for FTIR (Bruker Vertex 70), XRD (XPERT-PRO) analysis, SEM (FEI Quanta 400FEG ESEM/EDAX Genesis X4M) and to determine the final Ca/P molar ratio (Atomic Absorption and UV-Vis Spectrophotometry). For the particle size distribution (LS 230, Beckman Coulter), the suspensions were directly analyzed.

Conclusions

In all the reactors studied, single phase nano-HAp (d_{50} ≈ 80 μm) with low crystallinity and rod-like morphology were successfully synthetized. However, in the ST the formation of the most stable CaP took 4h, whereas in the meso-OFR and MOFPR HAp was produced only after 30 and ≈7min, respectively and without the formation of intermediate CaP phases. While the meso-OFR allows to reduce the reagent requirements and waste, which is ideal for initial testing at the laboratory scale, the MOFPR can be implemented at an industrial scale.

References

[1] T. Gong, J. Xie, J. Liao, T. Zhang, S. Lin, and Y. Lin, "Nanomaterials and bone regeneration," Bone Res., vol. 3, no. 8, p. 15029, 2015.
[2] A. Veiga, F. Castro, C. C. Reis, A. Sousa, A. L. Oliveira, and F. Rocha, "Hydroxyapatite/sericin composites: A simple synthesis route under near-physiological conditions of temperature and pH and preliminary study of the effect of sericin on the biomineralization process," Mater. Sci. Eng. C, vol. 108, p. 110400, Mar. 2020.
[3] F. Castro, A. Ferreira, F. Rocha, A. Vicente, and J. Teixeira, "Precipitation of Hydroxyapatite at 37o C in a Meso Oscillatory Flow Reactor Operated in Batch at Constant Power Density," AIChE J., vol. 7, no. Part 1, pp. 405–410, 2014.
[4] F. Castro, A. Ferreira, F. Rocha, A. Vicente, and J. A. Teixeira, "Continuous-Flow Precipitation of Hydroxyapatite at 37 ° C in a Meso Oscillatory Flow Reactor," AIChE J., vol. 59, no. 12, pp. 4483–4493, Dec. 2013.

Acknowledgements

This work was financially supported by: National Funds through FCT (Foundation for Science and Technology) under the project UIDB/50016/2020 of the Centre for Biotechnology and Fine Chemistry - CBQF; and by LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC). A. Veiga gratefully acknowledges doctoral scholarship [2020.08683.BD] from FCT.