Modular oscillatory flow reactor as a new technology for the continuous production of customized Calcium Phosphates for tissue engineering

*s-anveiga@ucp.pt

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

[1]Laboratory for Process Engineering, Environment, Biotechnology & Energy, Dep. of Chemical Engineering, Faculty of Engineering of Porto, R. Dr. Roberto Frias, 4200-465 Porto, Portugal

[2]ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

[3]Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

MOTIVATION AND OBJECTIVES

Several technologies and synthesis routes have been implemented to produce calcium phosphates (CaPs) with distinct characteristics for tissue engineering (TE) [1]. However, produce CaPs in a controlled way still represents a challenge. Oscillatory flow reactors are a technology ready to deliver in terms of mixing intensification in multiphase systems. In particular, continuous processes improve control over the reaction conditions and can be implemented at an industrial scale [2].

The aim of the present work was to assess for the first time the influence of the oscillation amplitude (x_0: 4, 8 and 18 mm), frequency (f: 1.9, 4 and 6 Hz) and residence time (τ: 3.3, 6.6 min) on the final CaP physicochemical properties in a novel modular oscillatory flow plate reactor (MOFPR) (WO/2017/175207) (**Figure 1**). CaP synthesis was carried out by a simple precipitation route under near-physiological conditions described in previous work [3]. XRD, laser diffraction, SEM, FTIR and Ca/P ratio were performed to the different experimental conditions to obtain fully characterized CaP particles.

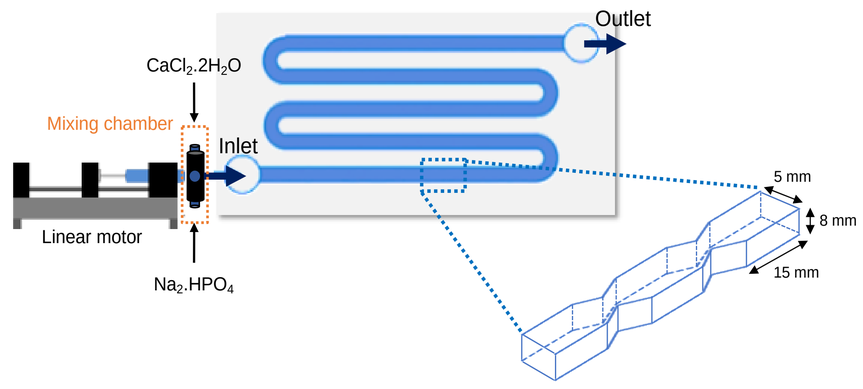

Figure 1. Modular Oscillatory Flow Plate Reactor (WO/2017/175207)

METHODOLOGY

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.

RESULTS AND DISCUSSION

For lower f (1.9 Hz), different x_0 (4, 8 and 18 mm) lead to the production of distinct nano-hydroxyapatite (HAp) particles with a sharper, elongated or even rod-like morphology. At higher f (4 and 6 Hz), the x_0 does not seem to have a significant influence on the particle's morphology. Plate-like and nano-HAp particles with traces of brushite were obtained at 4 Hz, while only HAp nanorods were identified at 6 Hz. τ affects the reaction yield but not the particle characteristics, which can result from the higher nucleation generated by more contact area between the reactant solutions at higher flow rates (**Figure 2**).

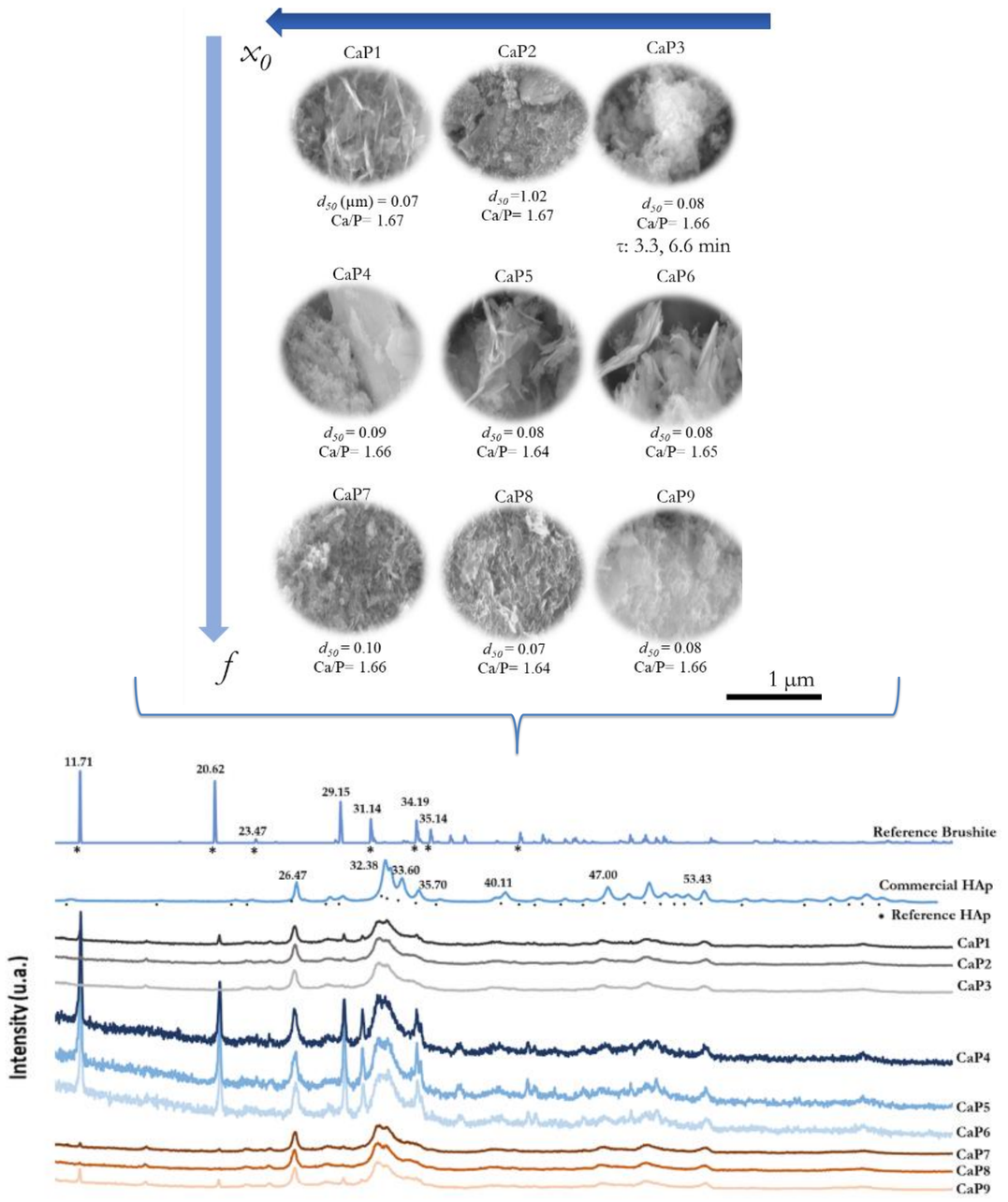

Figure 2. SEM, FTIR, mean size (d_{50}) and Ca/P molar ratio of the different CaP particles produced.

CONCLUSIONS

The unique properties of the synthetized CaPs using specific mixing conditions (f, x_0) provide a new window for the rational design of tailored CaP -nano and micro-carriers for specific biomedical applications. Hence, the MOFPR represents an attractive platform to meet the current industry demands for CaPs with different characteristics for biomaterials and TE.

References

[1] F. Castro, A. Ferreira, F. Rocha, A. Vicente, and J. António Teixeira, "Characterization of intermediate stages in the precipitation of hydroxyapatite at 37ºC," *Chem. Eng. Sci.*, vol. 77, no. 2, pp. 150–156, 2012.

[2] T. McGlone, N. E. B. Briggs, C. A. Clark, C. J. Brown, J. Sefcik, and A. J. Florence, "Oscillatory Flow eactors (OFRs) for Continuous Manufacturing and Crystallization," *Org. Process Res. Dev.*, vol. 19, no. 9, pp. 1186–1202, Sep. 2015.

[3] 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.

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.

For more information