

## Integrated manufacture of liposomal dry powder formulations for pulmonary enzyme delivery: When pharmaceutical technology meets green chemistry

Costa C.<sup>1,2,3</sup>, Liu Z.<sup>2</sup>, Simões S.<sup>4</sup>, Correia A.<sup>2</sup>, Rahikkala A.<sup>2</sup>, Seitsonen J.<sup>5</sup>, Ruokolainen J.<sup>5</sup>, Casimiro T.<sup>1</sup>, Santos H.A.<sup>2,6</sup>, Corvo M.L.<sup>3</sup>, Aguiar-Ricardo A.<sup>1</sup>

<sup>1</sup>LAQV-REQUIMTE, Departamento de Química, NOVA School of Science and Technology, 2829-516 Caparica, Portugal

<sup>2</sup>Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Helsinki FI-00014, Finland

<sup>3</sup>Instituto de Investigação do Medicamento (iMed.Ulisboa), Faculdade de Farmácia, Universidade de Lisboa, Avenida Professor Gama Pinto, 1649-003 Lisboa, Portugal

<sup>4</sup>Nanostructured Systems for Overcoming Biological Barriers Group of iMed.Ulisboa, Research Institute for Medicines, Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal

<sup>5</sup>Nanomicroscopy center, Aalto University, Aalto 00076, Finland

<sup>6</sup>Helsinki Institute of Life Science (HiLIFE), University of Helsinki, Helsinki FI-00014, Finland

Chronic obstructive pulmonary diseases, like COPD, asthma and cystic fibrosis, represent the third leading cause of death globally [1]. The treatment of these diseases requires long-term systemic therapy with high doses of anti-inflammatory drugs resulting in serious side effects, leading to cardiovascular diseases [2]. These disadvantages can be overcome resorting to suitable and biocompatible drug delivery systems, as liposomal dry powder formulations. These formulations can be obtained by the integration of a microfluidic system, where liposomes are produced and then dried using supercritical CO<sub>2</sub>-assisted spray-drying (SASD). In this work, through the nanoprecipitation method, using a one-step glass-capillary microfluidic device, Cu, Zn-Superoxide dismutase (SOD) – an enzyme with anti-inflammatory properties – was encapsulated in PEGylated liposomes (SOD@Lip), with an efficiency of 59 ± 6 %. *In vivo* experiments showed, through an ear edema model, that SOD@Lip administered by the intravenous route enable an improvement on edema inhibition over 45 % comparing to SOD in its free form [3]. To improve the storage stability of SOD@Lip without resorting to the cold chain, the SASD was used to convert SOD@Lip in suspension form into SOD-loaded liposomal dry powder formulations (SOD\_Lip@DPFs). Preliminary studies have shown promising results on the production of SOD-liposomal dry powder formulations using scCO<sub>2</sub> and their suitability to be administered through the pulmonary route.

### References

[1] World Health Organization (<https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death>). Accessed 26<sup>th</sup> January 2021; [2] Suer, H. *et al.* Biomed. Biotechnol. Res. J. 1 (2017) 1–8; [3] Costa, C. *et al.* Colloids Surf., B. 199 (2021) 111556.

### Acknowledgments

The authors are grateful for the financial support of the Associate Laboratory for Green Chemistry-LAQV- which is financed by national funds from FCT/MCTES UIDB/50006/2020 and UIDP/50006/2020) and the financial support from Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal, which is supported in part by UID/DTP/04138/2020 from FCT/MCTES, Portugal. C. Costa also acknowledges the FCT/MCTES for the grant PD/BD/142880/2018 and for the travel grant. H.A. Santos acknowledges financial support from the HiLIFE Research Funds and the Sigrid Jusélius Foundation.