Encapsulation of clofazimine in mesoporous silica as a potential dry powder formulation for treating tuberculosis

Jesús Enrique Campos Pacheco*[1,2], Azra Riaz[1,2], Peter Falkman[1,2], Adam Feiler[3,4,5], **Mikael Ekström[6]**, Georgia Pilkington[3,4] & Sabrina Valetti[1,2]

[1]Biomedical Science, Faculty of Health and Society, Malmö University, 205 06 Malmö, Sweden; [2]Biofilms – Research Center for Biointerfaces (BRCB), Malmö University, 205 06 Malmö, Sweden
[3]Nanologica AB (publ), Forskargatan 20G, 151 36 Södertälje, Sweden; [4]Surface and Corrosion Science, KTH Royal Institute of Technology, SE-100 44 Stockholm, Sweden; [5]Current affiliation Interrobang AB. Sweden; [6]Iconovo AB, Ideongatan 3A-B, 223 70 Lund, Sweden.

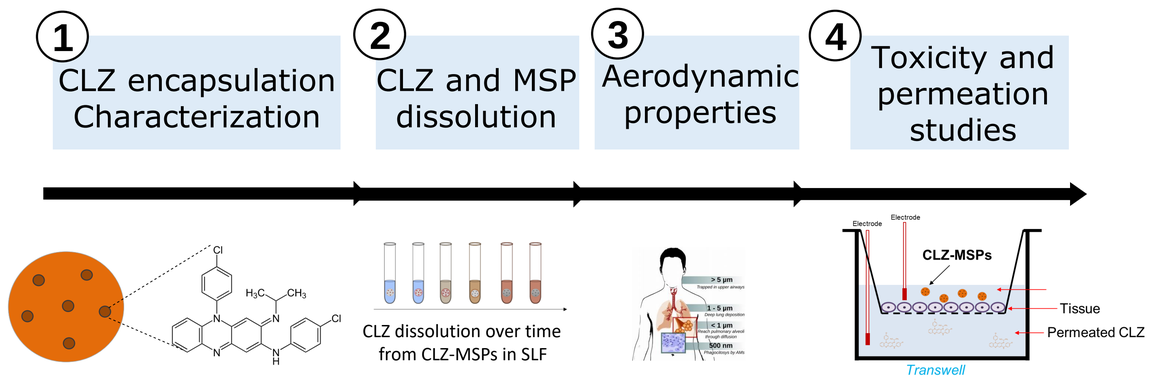
*jesus.campos-pacheco@mau.se

Introduction

Clofazimine (CLZ) has shown strong synergistic activity *in vitro* with numerous drugs used to treat mycobacterial infections. However, CLZ has limited solubility and, as consequence, limitations for oral administration. **Direct pulmonary delivery of CLZ** is an attractive strategy to tackle tuberculosis since it will target infected cells in the lungs, providing therapeutic drug concentrations directly at the main site of the disease [1]. One novel delivery route for delivering CLZ to the lungs is **mesoporous silica particles (MSPs). MSPs** are biocompatible carriers composed of amorphous silicon dioxide, which safety studies in rats have indicated is cleared and tolerated in the lungs [3]. MSPs are free flowing, easily aerosolisable, and have vast surface area, offering a high loading capacity (up to 50% w/w) [2].

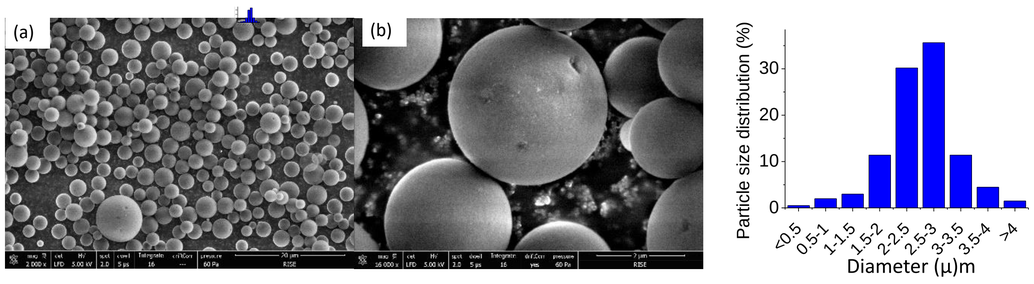
Aim

Investigate the feasibility of MSPs as microparticle carrier for dry powder formulation that could demonstrate good aerodynamic properties together with enhanced apparent solubility and permeation of CLZ.



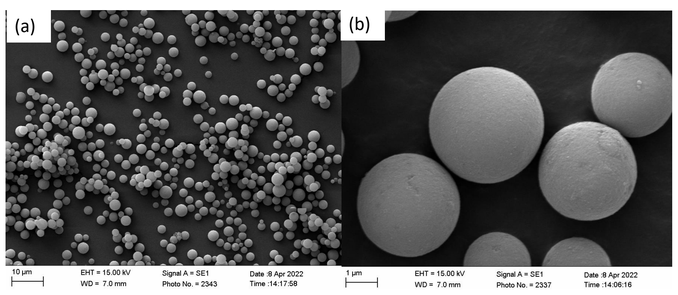
1. Dry powder formulation

- Characterization of mesoporous silica particles



SEM images of (unloaded) MSPs (2.4-2.6 µm) at a magnification of (a) 2000×, (b)16000× and (c) histogram of particle sizes.

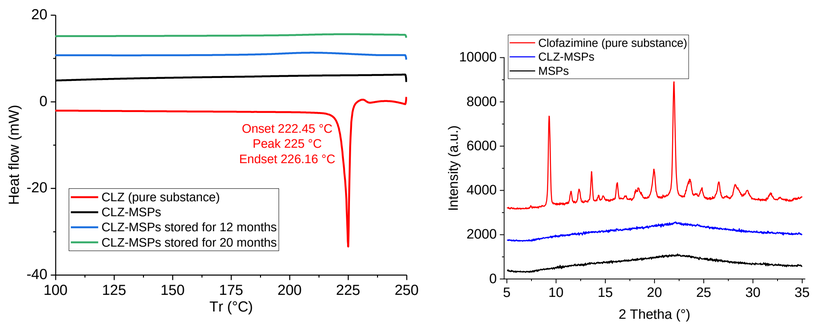
- Loaded particles with Clofazimine: CLZ-MSPs (loading method [2])



TGA confirmed 8-10 % of loaded amount

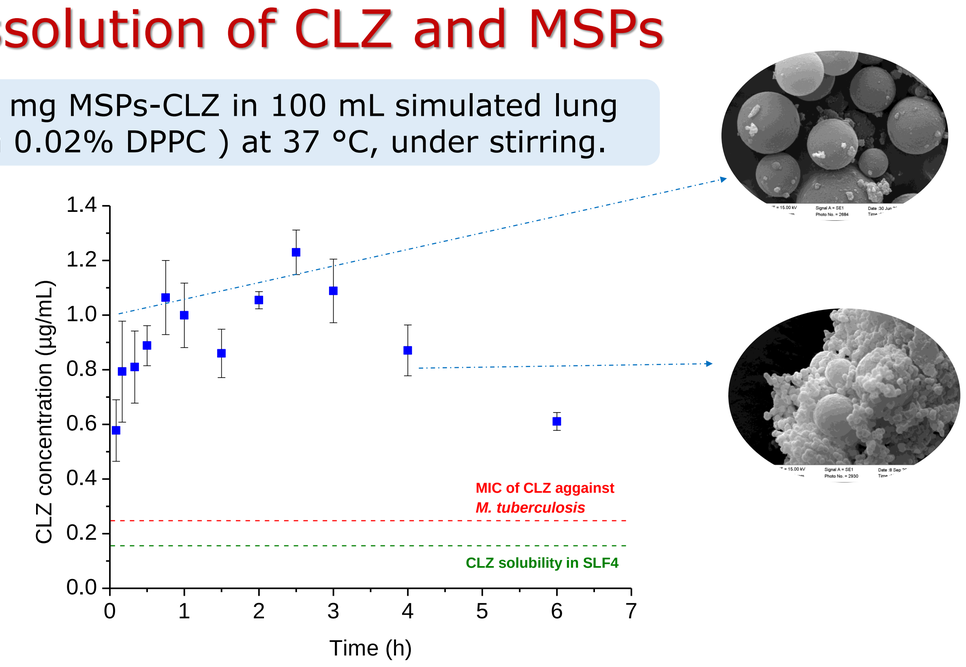
SEM images of CLZ-MSPs at (b) 2500×, (c) 25000×.

DSC and XRPD: Confirming amorphicity



2. Dissolution of CLZ and MSPs

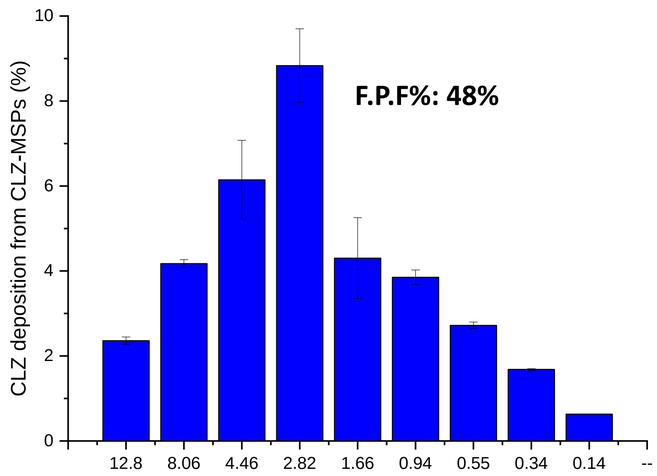
Dose: 3.1 mg MSPs-CLZ in 100 mL simulated lung fluid (with 0.02% DPPC) at 37 °C, under stirring.



Release profile of CLZ from CLZ-MSPs in SLF4 (mean ± standard deviation; *n*=3). Inserts show SEM images taken of solid fraction at indicated time points.

3. Aerodynamic properties

20 mg of CLZ-MSPs filling the ICOone® inhaler, flow rate of 60L/min, with a 4 kPa pressure drop.

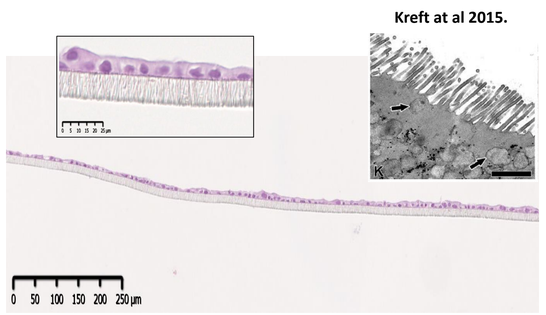


Aerodynamic distribution of CLZ-MSPs in the Next Generation Impactor (NGI). Calculated as averaged delivered dose ± standard deviation of CLZ.

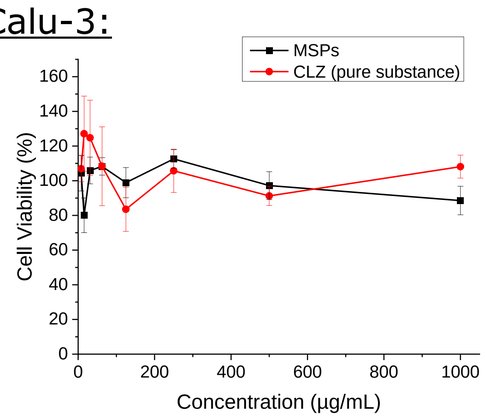
ICOone® inhaler

4. *In vitro* studies

- MTT cell toxicity analysis with Calu-3:

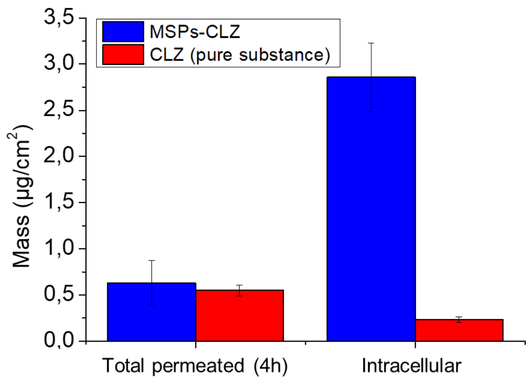


Calu-3 cells epithelial monolayer at air-liquid-interface



MTT test of MSPs and CLZ for 4 hours (mean ± standard deviation (*n*=8).

- Permeation studies:



Permeation of CLZ delivered from MSPs-CLZ and CLZ (crystals) through Calu-3 and intracellular deposition.

Conclusions

Micron-sized MSP showed a great potential as an inhalation carrier platform to encapsulate and release CLZ in simulated lung fluid with therapeutic concentration. The uniform size of the MSPs facilitated deposition of the formulation in the airways. *In vitro* studies demonstrated that MSPs and CLZ were not toxic on epithelial lung cells up to 1 mg/mL. CLZ released from MSPs showed enhanced intracellular concentration.

References

[1] Castro R, et al. *Development of inhaled formulation of modified clofazimine as an alternative to treatment of tuberculosis*, Journal of Drug Delivery Science and Technology, 2020.
[2] Valetti S, et al. *Clofazimine encapsulation in nanoporous silica particles for the oral treatment of antibiotic-resistant Mycobacterium tuberculosis infections*, Nanomedicine, 2017.
[3] Arts J, et al. *Five-day inhalation toxicity study of three types of synthetic amorphous silicas in Wistar rats and post-exposure evaluations for up to 3 months 45*, Food Chem Toxicol, 2017.

Acknowledgements

Biofilms-Research Center for Biointerfaces at Malmö University, Swedish Research Council (Vetenskapsrådet) grant 2019-05163, Knowledge foundation (KK-stiftelsen) grant 20170027. Nanologica A.B., Iconovo AB and the University of Copenhagen for the support with analysis and discussions.