Transfer of the Liposome Encapsulating Dexamethasone Production Method to a CO_2 PGSS Supercritical Process

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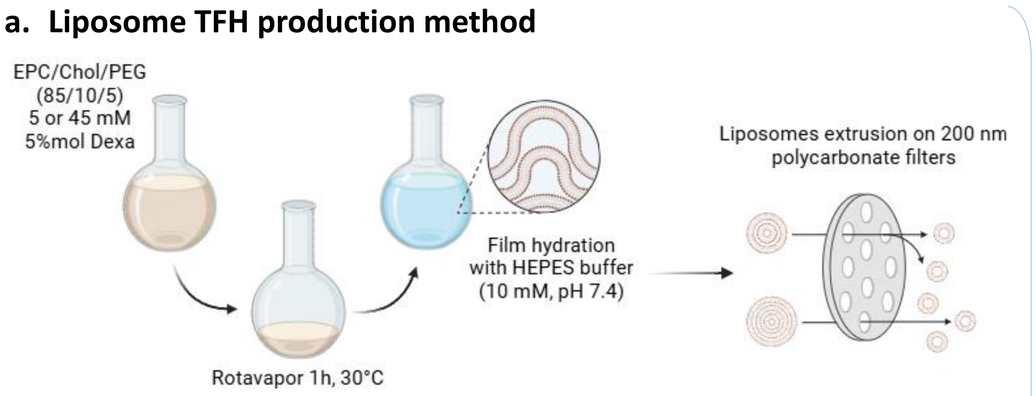
1. Introduction

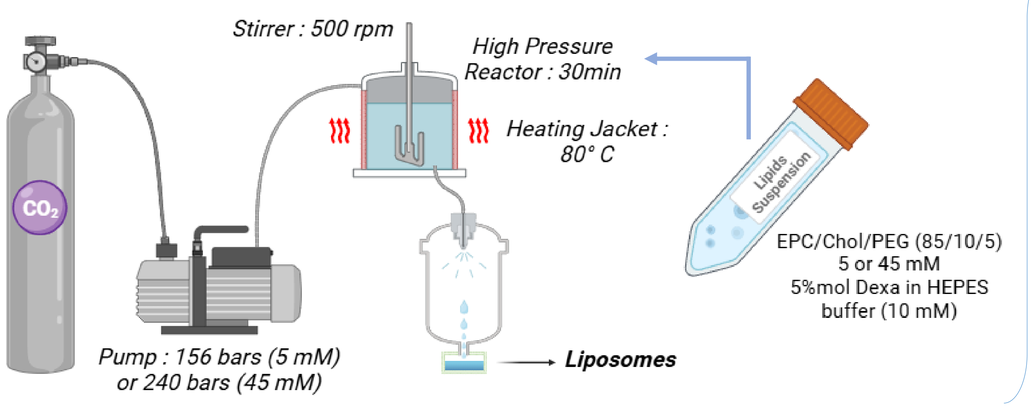
Several techniques exist to produce liposomes, such as thin film hydration (TFH) or rapid mixing. However, TFH only enables the production of small batches of liposomes, which are only suitable for laboratory-scale use. The aim of this work is therefore to explore a novel drug delivery system that encapsulates dexamethasone (Dexa) within liposomes produced using an innovative PGSS (Particles from Gas Saturated Solutions) method based on supercritical CO_2 ($scCO_2$) previously developed in our lab [1]. This process avoids any use of organic solvents, involves very few steps, uses a low-cost recyclable gas, and enables liposome production on a much larger scale. Liposomes produced using both methods will thus be compared based on their encapsulation efficiency (EE), size, polydispersity index (PdI) and zeta potential, to validate the transposability of the production process. A short-term stability study at 4°C will also be conducted to compare the liposomes produced at 45 mM via both TFH and $scCO_2$ methods.

2. Materials & Methods

a. Liposome TFH production method



b. Liposome PGSS production method using $scCO_2$



c. Liposome purification

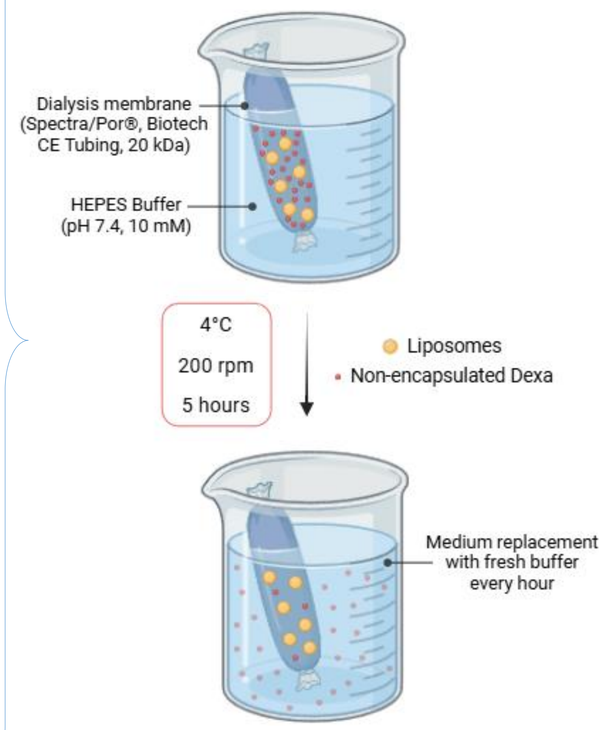


Figure 1. Liposome production using both TFH and $scCO_2$ methods, followed by their purification through dialysis [2].

3. Results & Discussion

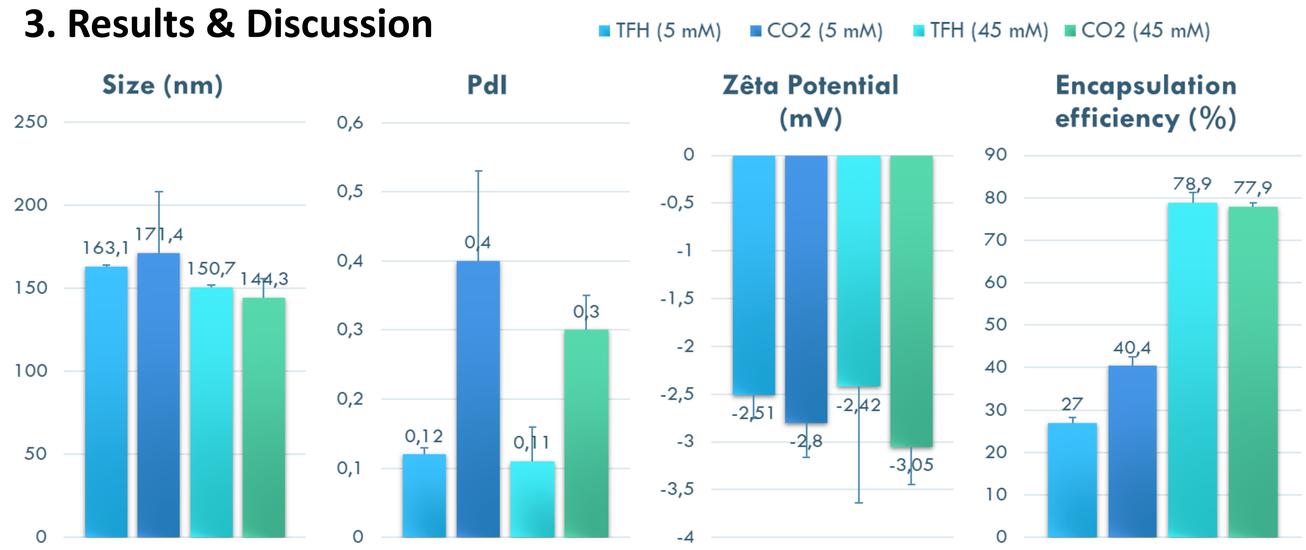


Figure 2. Comparison of liposome formulations produced at 5 and 45 mM using TFH and $scCO_2$ methods based on their size, PdI, zeta potential, and encapsulation efficiency, to validate the transition to a single-step and larger-scale production protocol.

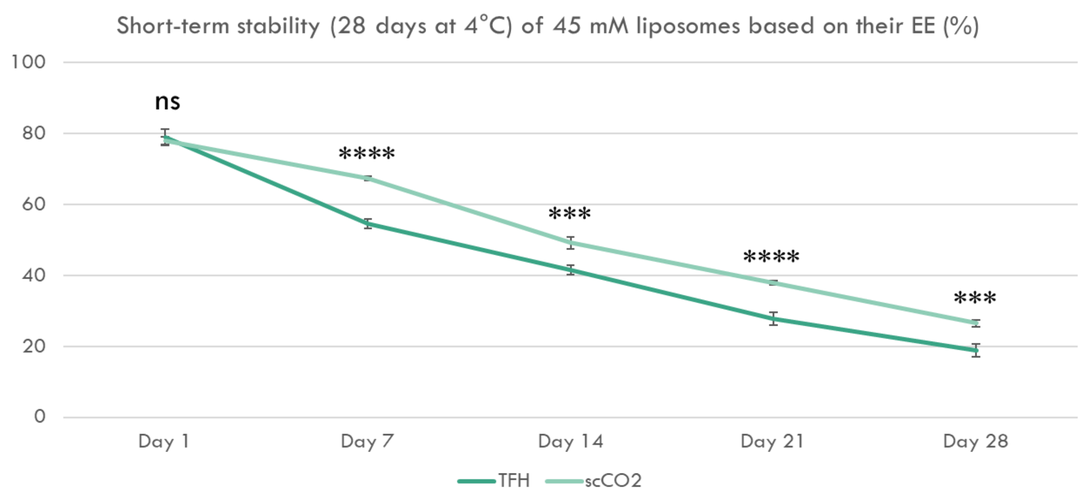


Figure 3. Comparison of the short-term stability (28 days at 4°C) of 45 mM liposomes produced by TFH and $scCO_2$ based on their encapsulation efficiency (EE - %).

The sizes of liposomes obtained by $scCO_2$ at 5 mM (171.4 ± 36.6 nm) and 45 mM (144.3 ± 11.6 nm) are comparable to those obtained by TFH at 5 mM (163.1 ± 1.0 nm) and 45 mM (150.7 ± 1.2 nm), which confirms the successful method transfer regarding this aspect (Figure 2). TFH implies a lower PdI of the particles (0.11 ± 0.05) thanks to the extrusion step, but $scCO_2$ still gives acceptable results at 45 mM (0.30 ± 0.05). All particles, whether produced by TFH or $scCO_2$, exhibit a zeta potential close to neutrality. Thus, the production method did not affect the charge. At 5 mM, the encapsulation efficiency of Dexa was higher with $scCO_2$ (40.4 ± 2.3 %) compared to TFH (27.0 ± 1.3 %). However, there was no encapsulation difference between $scCO_2$ (77.9 ± 1.0%) and TFH (78.9 ± 2.3%) methods at 45 mM, confirming the feasibility of transferring the production protocol from TFH to the $scCO_2$ method without impacting the liposome's delivery capacity. Also, when produced by $scCO_2$, 45 mM liposomes seem to show better stability after 7 days at 4° C (67.2 ± 0.6%) compared to those produced by TFH (54.5 ± 1.4%). This trend is consistent throughout the study. All of these results thus appear to validate the transfer of the method to an environmentally friendly, single-step production protocol based on $scCO_2$, adaptable to an industrial scale.

4. Conclusion & Perspectives

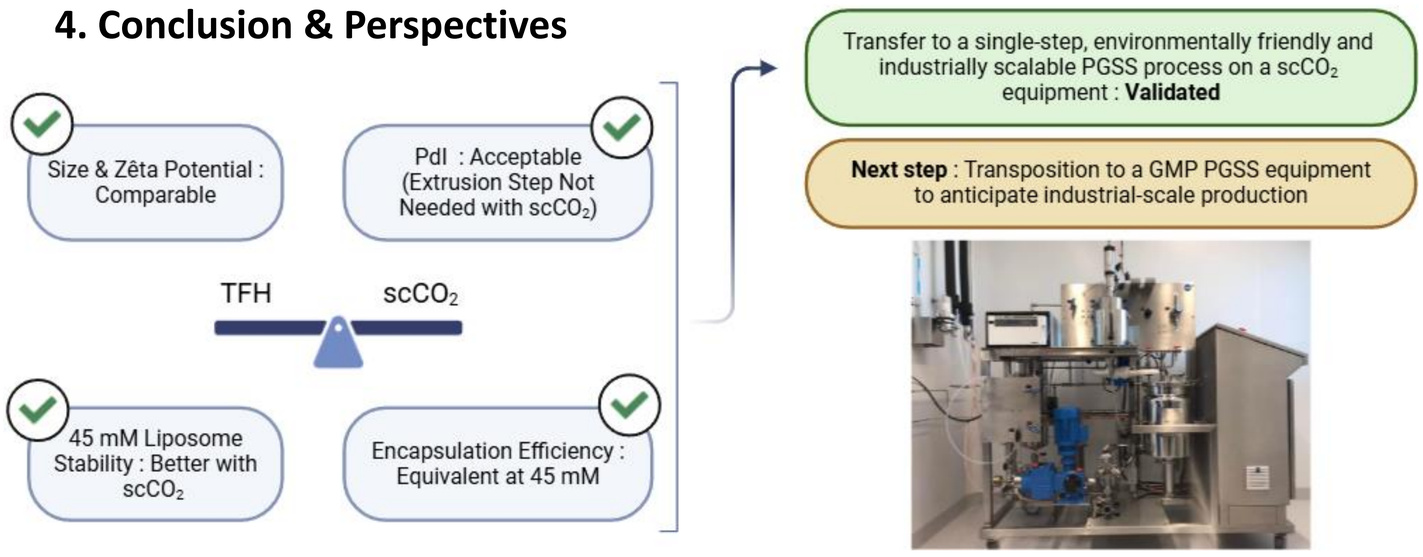


Figure 4. Validation of the transfer of a TFH production protocol to a PGSS equipment using $scCO_2$ [2].

5. References

[1] Penoy, N.; Delma, K.L.; Homkar, N.; Karim Sakira, A.; Egrek,S.; Sacheli, R.; Sacré, P.-Y.; Grignard, B.; Hayette, M.-P.; Somé, T.I.; Semdé, R.; Evrard, B. and Piel, G. Development and optimization of a one step process for the production and sterilization of liposomes using supercritical CO_2, Int. J. Pharm. 651, 123769 (2024).

[2] Created with BioRender.

6. Acknowledgements

Authors thank Wallonia and Dendrogenix for the financial support.

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