Supercritical CO_2 applied on the production of liposomal dry powder formulations towards the lung inflammation treatment

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1. Introduction

Neutrophilic inflammatory lung diseases are the third leading cause of mortality in Europe, after cardiovascular and cancer diseases. The patients are commonly treated with high doses of anti-inflammatory drugs, like corticosteroids, resulting in adverse effects such as osteoporosis, adrenal suppression, diabetes, and cardiovascular diseases[1]. High and low molecular weight compounds such as biopharmaceuticals and flavonoids, respectively, with anti-inflammatory properties can overcome those drawbacks and provide treatment with higher patient compliance. PEGylated liposomes, owing to their physical and chemical features, can carry hydrophilic and hydrophobic drugs, like biopharmaceuticals and flavonoids, respectively, circulating in the bloodstream up to 24 h[2]. Yet, as the mRNA-based SARS-CoV-2 vaccines, the limited stability in suspension upon storage might limit their use. Therefore, it is advantageous to convert liposomal suspension into solid dosage forms. In this way, using a green technology like supercritical CO_2-assisted spray-drying (SASD), liposomal suspension is converted into dry powder formulations (Lip-DPFs) with capable of administration via different routes, including lungs.

2. Materials and Methods

Egg-phosphatidylcholine (E-PC) (> 99 %) and distearoylphosphatidylethanolamine-poly(ethyleneglycol)$_{2000}$ (DSPE-PEG$_{2000}$) (> 99 %) were obtained from Lipoid (Germany). Cholesterol (Chol) (> 99 %), monohydrate (> 99 %) and L-leucine (98 %) were purchased from Sigma-Aldrich (USA). Trehalose dehydrated (> 98 %) were acquired from Tokyo Chemical Industry (China), chloroform (> 99 %) was purchased from Carlo Erba (Spain) and ethanol absolute anhydrous (99.9%) was sourced from Scharlau (Spain). Air Liquide (Portugal) provided carbon dioxide (99.998 %). All components were used as received without further purification.

The hydrophilic[3] and hydrophobic compounds were separately encapsulated and incorporated, respectively, into PEGylated liposomes using the film hydration method, followed by extrusion. Free hydrophilic compound was separated from liposomes by ultracentrifugation, whereas the free hydrophobic one was removed by molecular exclusion. Loaded liposomes were mixed with a casting solution (4 %, v/v) and then dried into a laboratory scale SASD apparatus. Liposomes and Lip-DPFs were characterized in terms of size, polydispersity, lipid and molecules quantification. The aerodynamic behavior and storage stability of Lip-DPFs were also evaluated.

3. Results and discussion

The conversion of loaded PEGylated liposomes into Lip-DPFs was successfully achieved using scCO_2. The powders were then dissolved in water, to recover the liposomes. Resuspended liposomes showed a slightly size decrease, due to ethanol and leucine action. The encapsulation efficiency (*EE*) of the hydrophilic

compound remained above 95 %. Powders showed a mass median aerodynamic diameter of 1.75 µm and a fine particle fraction of 65 %, being suitable to be reach the terminal bronchi. The storage stability assays showed that the dry powder formulations were able to maintain liposome stability at relative humidity of 4 % and 50 % at 20 °C for 30 days.

Regarding to the Lip-DPFs incorporated with a hydrophobic compound, preliminary results have shown that resuspended liposomes are able to keep approximately of 40 % of incorporated molecule. The powders also have shown be suitable for inhalation, reaching the terminal bronchi, as well.

4. Conclusions

This work is a proof-of-concept that highlights the innovative potential of supercritical CO_2-assisted spray-drying for converting liposomes into liposomal dry powder formulations**.** Results showed that at determined concentrations of leucine and ethanol, liposomes kept their structure and the encapsulation efficiency the water-soluble dye above 95 %. Formulations incorporated with hydrophobic compounds kept the incorporation efficiency approximately 40 %. The challenges faced with regards to the storage stability of hydrophilic loaded liposomal formulations were surmounted through the production of solid form dosages using the supercritical fluid drying method with optimized ratios of excipients. The aerodynamic performance of the dry powders showed to be suitable for inhalation. Our findings point to promising synergies between pharmaceutical and chemical technologies in the search for effective alternative liposome storage techniques that forgo the need for cold chain storage, being able to target the lungs.

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