Liposome containing ciclesonide with/without indacaterol produced by supercritical carbon dioxide-based technologies

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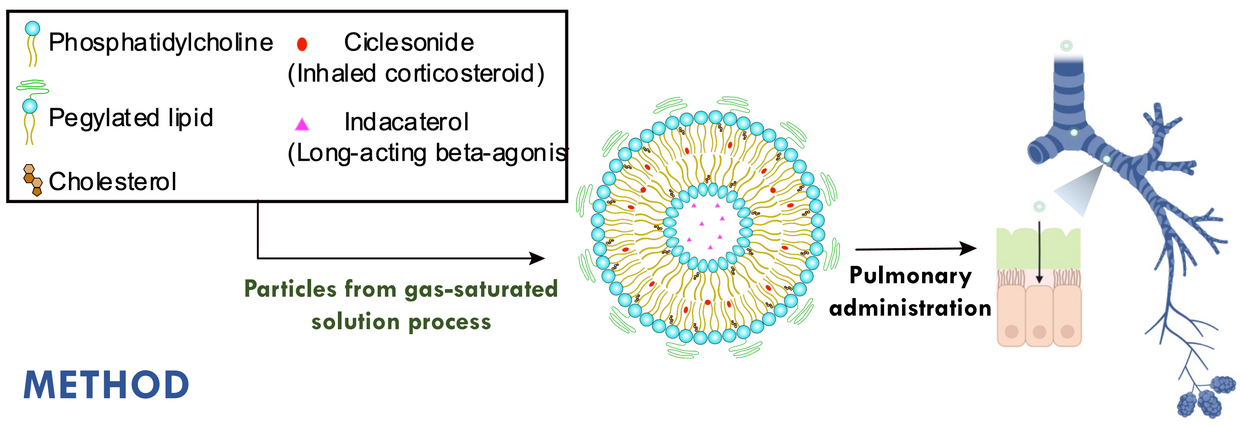
INTRODUCTION

This study is aimed to produce the **first liposomal** formulation loaded with an inhaled corticosteroid with an encapsulated or free long-acting beta agonist for the treatment of **Chronic obstructive pulmonary disease (COPD).** After deposition in bronchial tubes, PEGylated liposome could enhance penetration of these active pharmaceutical ingredients (APIs) through hypersecreted mucus layer, protect them from degradation by macrophages during the passage and control their liberation at target sites. In this study, the liposome is prepared by the **particles from gas-saturated process** in which supercritical carbon dioxide acts as a surfactant. This one step production method allow us to remove the use of organic solvent and improve the feasibility of fabricating the liposome at industrial scale. In summary, by optimizing the liposome production of by the supercritical carbon dioxide-based process, we expect to obtain nanoparticles exhibiting adequate properties for pulmonary delivery of prementioned therapeutic agents.

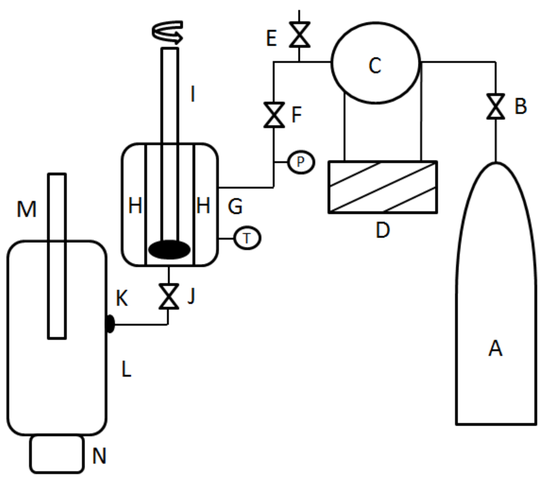


METHOD

1/ Expected properties for liposome administered by pulmonary route for COPD treatment

Property	Expected value	Objectives
Size	≤ 200 nm	Bypassing 'size' filtering by mucus network and uptake by macrophages
PDI	< 0.3	Ensuring particles homogeneity
Zeta potential	≤ 0 mV	Avoiding electrostatic interaction with mucus network
Polyethylene glycol-covered surface		Limiting hydrophobic interaction with mucus network and uptake by macrophages
Biocompatibility with lung epithelial cells		Avoiding APIs macrophages-mediated clearance and toxicity on lung epithelial cells
Controlled release profile of APIs		Reducing daily dose and APIs toxicity

2/ Optimizing of liposome production by Particles from gas-saturated solution process



Schematic representation of the PGSS equipment:

(A) CO_2 bottle
(B, E, and F) on/off valves
(C) Pump
(D) Refrigerant
(G) high pressure reactor
(H) heating jacket
(I) Stirrer
(J) pneumatic valve
(K) Nozzle
(L) expansion tank
(M) CO_2 outlet
(N) sample recovery vial
(P) pressure gauge, (T) thermostat

Parameter/Factor		Type/Investigated range
From lipid composition	Phosphatidylcholine	Soy phosphatidylcholine Hydrogenated soy phosphatidylcholine Dipalmitoylphosphatidylcholine
	PEGylated lipid	DSPE-PEG2000 DSG-PEG2000 DMG-PEG2000 C8-PEG2000-Ceramide
From production process	Lipids concentration	5-50 mM
	Volume of dispersion	10-30 mL
	Temperature	35-80°C
	Pressure	120-250 bar

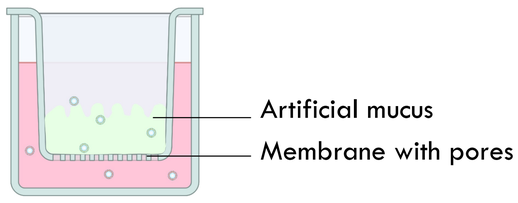
3/ Liposome characterization

<u>Dynamic light scattering:</u>

Measuring size, PDI and zeta potential of diluted liposome formulations.

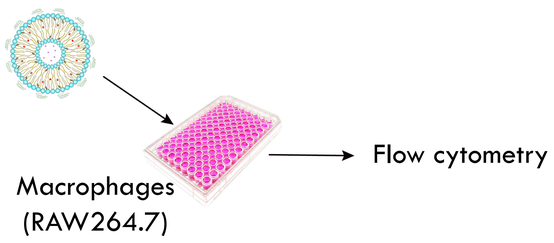
<u>Mucopenetration test:</u>

Tracking diffusion of fluorescently labeled liposomes through artificial mucus overtime in Transwell® model.



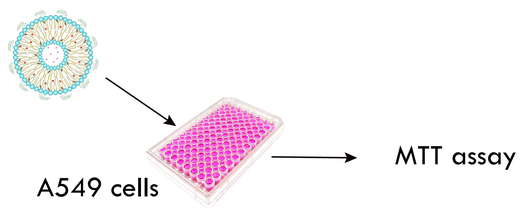
<u>Phagocytosis assay:</u>

Tracking fluorescent liposomes uptake by macrophages by flow cytometry.



<u>Cytotoxicity test:</u>

Evaluating toxicity of liposomes on lung epithelial cells



<u>Release profile test:</u>

Monitoring release of active molecules from liposomes