Sodium hyaluronate nanoparticles for lung delivery of amphotericin B in cystic fibrosis

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Background

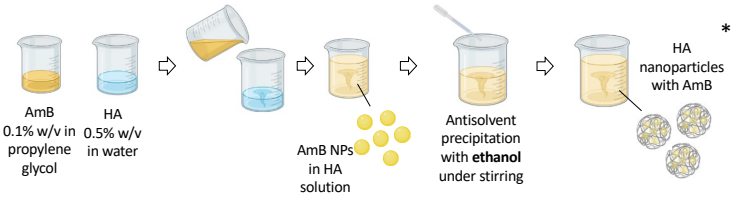
Amphotericin B (AmB) is an antimycotic drug with a high affinity for sterol lipids. If used below its MIC, in particular when the molar ratio of AmB:sterol is lower than one (i.e. [AmB] < 5 μM), AmB can self-assemble into nonspecific membrane-embedded ion channels, that are permeable both to cations and anions. This channels could be exploited in ion-channel related diseases, such as cystic fibrosis (CF), a genetic disease caused by mutations in cystic fibrosis transmembrane regulator (CFTR) gene, that results in compromise secretion of Cl^- and HCO_3^-. Evidences in literature show that AmB channels can restore HCO_3^- secretion and Cl^- current in *in vitro* models of CF epithelium[1,2].

Aim

Considering the pulmonary manifestations of CF, a targeted delivery of AmB to the lungs could constitute a useful strategy to help restoring ion traffic in CF patients, on a mutation- and genotype-independent basis. In this work, we propose the formulation of AmB in high molecular weight sodium hyaluronate (HA, 850 kDa) nanoparticles (NPs) to be administered as a dry powder to the lungs.

Methods

Preparation of NPs



The size of NPs was estimated by Dynamic light scattering using a NanoZS (Malvern Instruments Ltd).

Drying and characterization of resulting powders

Nozzle diameter: 0.7 mm
Feed rate: 2 mL/min
Aspiration: 35 m^3/h
Atomization air flow: 473 L/h
Inlet temperature: 80°C

The formulation as such or with the addition of bulking agents was converted into a powder by spray drying. The three bulking agents and their relative weight ratios to NPs were:

α- cyclodextrin (5:1), β-cyclodextrin (1:1) or leucine (1.5:1).

After drying, AmB content was estimated by HPLC and particle size distribution was measured using a Spraytec laser diffraction system (Malvern Instruments Ltd).
SEM analysis was performed with a FESEM SUPRA™ 40 (Carl Zeiss).

Aerodynamic Assessment

The formulations were loaded in size HPMC capsules and placed in a RS01® inhaler. Emitted fraction (EF%) and fine particle fraction (FPF%) were estimated by Fast Screening Impactor (FSI, Copley scientific).

RS01 HR (Plastiape)
Flow rate: 60 L/min
Aerosolization: 4 s

Results

The precipitation of HA on AmB lead to the formation of NPs having an average size of 60.9 ± 6.9 nm, that was not significantly increased by the addition of bulking agents. Encapsulation efficiency was 80%. Unexpectedly, despite the different solid concentration in the formulations before drying, the yields of spray drying were comparable, with or without excipients and independently on the type of bulking agent used (Table 1). The average sizes of resulting powders, that are all potentially adequate for inhalation, are reported as median diameter (Dv50) in Figure 1. Anyway, the content of AmB was different among formulations, and was about 65% of expected amount for all formulations tested.

Table 1: Size of NPs in suspension and characteristics of powders after spray-drying with different bulking agents

Bulking agent	Size (nm)	Yield (%)	AmB content (%w/w)	
			Theoretical	Actual
None	60.9 ± 6.9	73 ± 9	8.3	5.3
α-CD	80.8 ± 25	78 ± 7	1.4	0.91
β-CD	62.4 ± 8.1	67 ± 6	4.2	2.7
Leucine	66.7 ± 23.1	74 ± 1	3.3	2

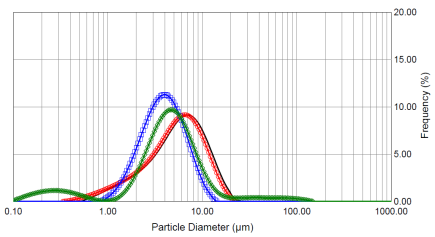


Figure 1: Particle size distribution of AmB nano-embedded HA microparticles SD without excipients (green), or in presence of α-CD (black), β-CD (red) and leucine (blue).

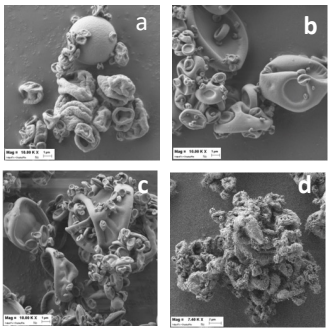
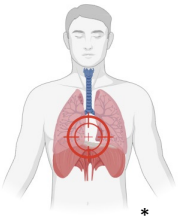


Figure 2: Figure 1. SEM images of dried powders (a) without bulking agent, (b) with α-CD, (c) with β-CD or (d) with leucine.

SEM analysis (Figure 2) shows a comparable morphology for particles prepared from NPs suspension as such or with addition of CDs, that appear composed of a mixture of smooth and deflated particles with larger ones acting as carrier for smaller ones. On the contrary, powders prepared with leucine show a rough appearence, with aggregates.

Table 2: Aerodynamic parameters of powders prepared with or without bulking agents determined by FSI

Bulking agent	EF%	FPF%
None	74.0 ± 16.8	57.9 ± 34.4
α-CD	68.8 ± 1.4	31.3 ± 0.3
β-CD	88.1 ± 4.5	36.2 ± 1.5
Leucine	91.4 ± 1.2	27.3 ± 1.1



Overall, excipients did not improve the performances of powders, since, despite a significant increase in emitted fraction in the presence of β-CD and leucine, the fine particle fraction was lower with respect to the formulation without bulking agents.

Conclusions

AmB was successfully encapsulated in HA NPs and transformed into inhalable powders by spray drying in the presence of different excipients. Bulking agents did not significantly improve the yield of drying nor the aerodynamic performance, with respect to the formulation without excipients, having an overall higher content of API. This formulation could allow the administration of an adequate dosage of AmB to the lungs to restore ion flux in CF patients.

1. Chorghade, R. S. *et al.* Amphotericin B induces epithelial voltage responses in people with cystic fibrosis. *Journal of Cystic Fibrosis* **20**, 540–550 (2021).
2. Muraglia, K. A. *et al.* Small-molecule ion channels increase host defences in cystic fibrosis airway epithelia. *Nature* **567**, 405–408 (2019).
3. Rayahin, J. E., Buhrman, J. S., Zhang, Y., Koh, T. J. & Gemeinhart, R. A. High and low molecular weight hyaluronic acid differentially influence macrophage activation. *ACS Biomater Sci Eng* **1**, 481–493 (2015).

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