Protein acqueos solutions for nebulization: assessment of aerodynamic particle size distribution

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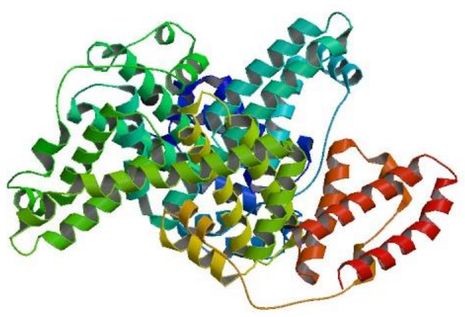
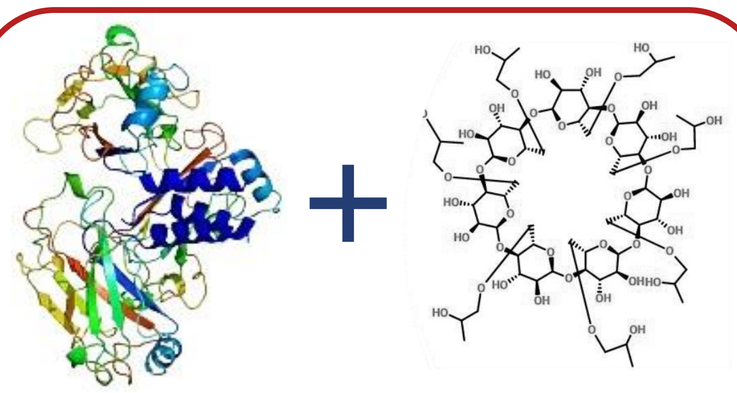
Introduction and Aim of the Work

The nebulization of therapeutic proteins presents several challenges, including protein unfolding, which can compromise their biological activity. To mitigate this issue, a promising strategy is the use of hydroxypropyl-beta-cyclodextrin (HPβCD) as complexing agent. HPβCD has been used for the formulation of a solution for inhalation containing the protein Plasminogen (PLG). One main aspect of inhalable solution is the aerodynamic particle size distribution (APSD) which can affect droplets deposition and the delivery to the lungs. In order to preserve the precious PLG based formulation, bovine serum albumin (BSA) was firstly investigated as model protein. This includes addressing challenges such as protein adsorption on filters and the required amount of drug for analytical setups, with the ultimate goal of improving the efficiency and reliability of biologics aerosolization. The deposition profile was assessed using a **Next Generation Impactor (NGI, Copley)** equipped with a **breathing simulator (BRS 300i, Copley)**. The method employs the Aerogen® Solo™ vibrating-mesh nebulizer, chosen for its ability to minimize physical and thermal stress, unlike traditional jet and ultrasonic nebulizers which may damage proteins[1].

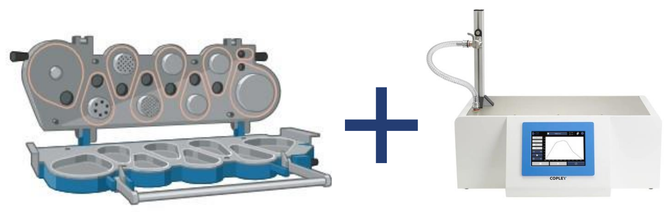


BSA standard protein, saline



PLG/HPβCD formulation, saline

Aerogen® Solo™ mesh nebulizer



NGI equipped with BRS

APSD of PLG/HPβCD and BSA protein formulations

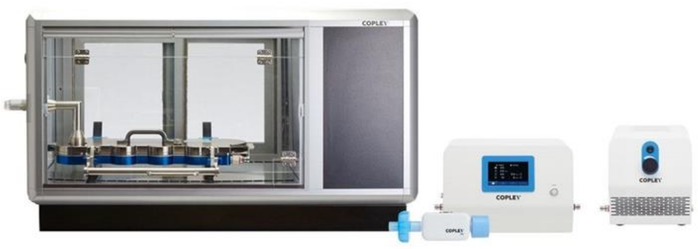
1. Determination of delivered dose (DD)

- Breathing simulator, BRS 300i (**healthy adult breathing profile setup**)
- DD was determined using low resistance filters connected to the nebulizer (according with the Eu.Ph)
- Nebulizer flow rate was checked > 0.4 mL/min
- **Loaded dose:** 3 mL of protein solution
- **Nebulization time:** 5 min (BSA) and 20 min (PLG/HPβCD)
- Filters pretreated with Tween 80 (to satisfy protein mass balance)
- PBS was used to collect the samples
- Bicinchoninic acid assay was applied for protein quantification

determination of DD

2. Aerodynamic assessment using NGI

- Pre-cool the impactor at 5°C for 90 min before the experiment
- Nebulizer flow rate was checked > 0.4 mL/min
- **Loaded dose:** 3 mL of protein solution
- **Nebulization time:** 5 min (BSA) and 20 min (PLG/HPβCD)
- Every stages was washed with a known volume of water
- Bicinchoninic acid assay was used for protein quantification

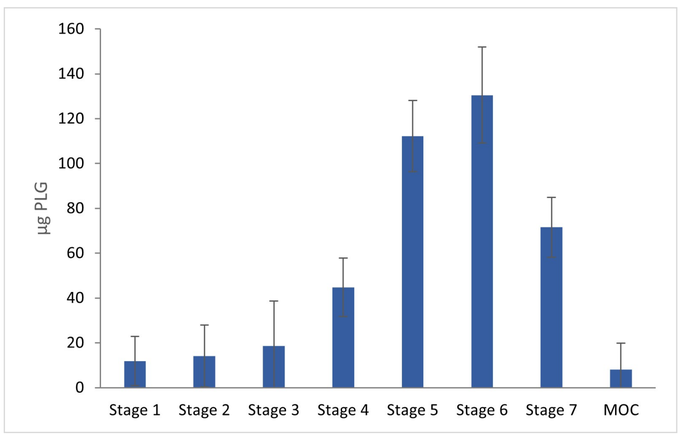


NGI inside the cooler and connected to the breath actuation controller and the low capacity vacuum pump, respectiveley

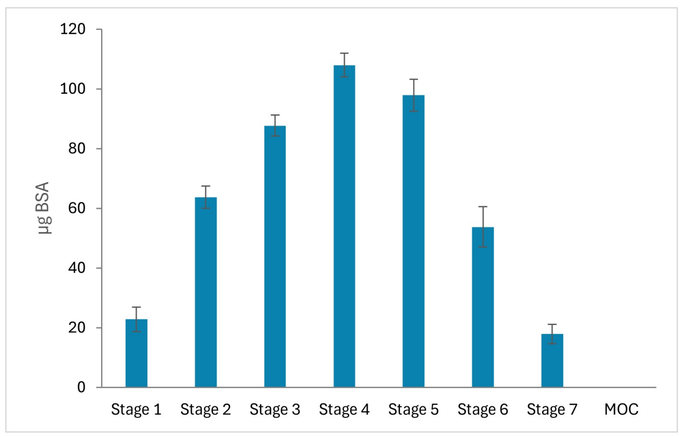
Results

	PLG/HPβCD (n=6)	BSA (n=5)
Delivered dose (µg)	403.9 ± 44.9	449.5 ± 20.3
FPF <5µm (%)	83.9%	52.8%
MMAD (µm)	2.033	4.58
GSD	1.555	2.107
R^2	0.995	1.0

APSD of PLG/HPβCD and BSA protein formulations, according to NGI deposition with Adult normal breathing profile (Eu.Ph). Delivered dose, Fine particle fraction (FPF; < 5 µm), Mass median aerodynamic diameter (MMAD), Geometric standard deviation (GSD) are shown



PLG distribution through NGI's different stages



BSA distribution through NGI's different stages

Despite the same breathing profile and mesh nebulizer were used, very different profiles were recorded for the two formulations

Conclusions

The aerodynamic particle size distribution for PLG/HPβCD formulation is promising: FPF > 50%, MMAD ideal for deep airway delivery, and GSD indicates a broad particle distribution. Refining the BSA profile after 20 minutes of nebulization is in progress. Challenges in assessing aerodynamic distribution include protein adsorption on filters and the required drug amount for analytical setup.

References

1. Vizzoni, L.; Migone, C.; Grassiri, B.; Zambito, Y.; Ferro, B.; Roncucci, P.; Mori, F.; Salvatore, A.; Ascione, E.; Crea, R.; et al. Biopharmaceutical Assessment of Mesh Aerosolised Plasminogen, a Step towards ARDS Treatment. Pharmaceutics 2023, 15, 1618.

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