Application Note

Assessment of Nebulizer Performance for Aerosolization of Bacteriophages

Overview

Aerosolized bacteriophage therapy is gaining significant attention as a potential approach for managing a wide variety of respiratory infections, particularly those involving antibiotic-resistant bacteria. Delivering phages directly to the respiratory tract offers the advantage of localized antimicrobial activity, yet the nebulization process can expose phage particles to mechanical stress, thermal fluctuations, and air–liquid interfaces that may compromise their stability. Because of these challenges, it is essential to determine whether specific nebulizer technologies are compatible with phage formulations intended for inhalation. Vibrating mesh nebulizers are frequently considered suitable for biological aerosols due to their gentle aerosol generation mechanism, which minimizes shear forces. This application note describes an evaluation of a vibrating mesh nebulizer to determine its ability to produce respirable aerosols while maintaining the viability of bacteriophages across different formulations and experimental conditions.

Example: Evaluation of different nebulizers on the viability of bacteriophage

For this assessment, the bacteriophage was prepared at an initial concentration of approximately 10^8 PFU/mL and stored in buffered saline at 4 °C. Infectivity was quantified using plaque assays on the corresponding host bacterium.

Figure 1: Separation of aerosol particles based on aerodynamic size inside NGI.

Nebulization was performed using a vibrating mesh device with a reservoir capacity of 2–6 mL, operated according to manufacturer-specified instructions. The aerosol particle size distribution and gravimetric delivery were analyzed using cooled Next Generation Impactor (NGI) and laser diffraction, with the collected fraction samples plated to determine the phage titer. Experiments were conducted in a controlled environment maintained at 22 °C and 30–50% relative humidity.

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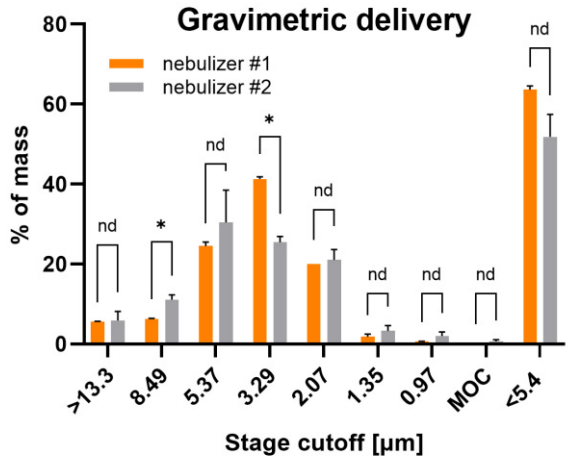

Figure 2: Distribution of separated mass in various NGI stages with various cutoff values. Shown are values from 3 repetitions with values presented as mean ± SD. *p < 0.05; nd = not different.

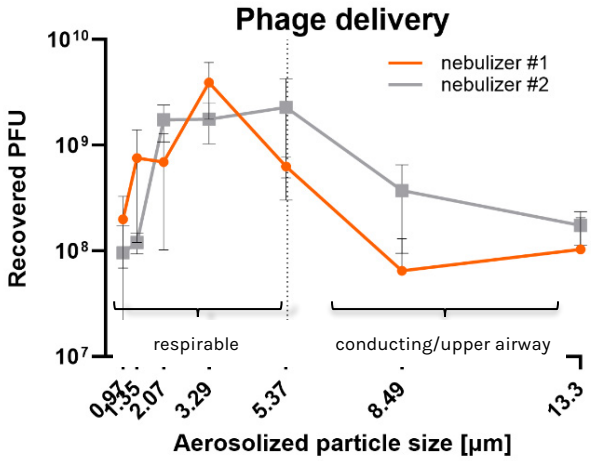

Figure 3: Phages were aerosolized with either nebulizer #1 or nebulizer #2 into a refrigerated NGI and nebulized particles of various sizes were assayed for viable phages. Shown are values from 3 experiments with values presented as mean ± SD of total PFU values.

Comparing the aerosol mass distribution from both devices, it is evident that nebulizer #1 is slightly more efficient in delivering particles <5.4 µm (based on gravimetric delivery), which can reach the lower lung airways, where most respiratory medications need to act.

Phage viability decreased during nebulization, with losses ranging from 0.5 to 2 log PFU depending on the specific nebulizer used. Despite this reduction, both nebulizers consistently preserved a meaningful proportion of infective particles, with around 45-60 % of phages presented in fraction <5.4 µm.

These findings align with previously published studies showing that mesh nebulizers are less damaging to phages than jet nebulizers, likely due to their lower shear stress and absence of compressed air. The aerosol produced exhibited a mass median aerodynamic diameter (MMAD) of approximately 4.2 µm, placing it well within the respirable range for pulmonary delivery. The device generated aerosol at a rate of roughly 0.4–0.6 mL/min and left less than 0.8 mL of residual volume, indicating efficient output. Phage formulations prepared in tested buffers remained stable throughout the process, with no visible aggregation or degradation.

Taken together, these observations indicate that both nebulizers are well suited for the aerosolization of bacteriophages intended for inhalation. Although some loss of viability is unavoidable, the magnitude of reduction observed here remains within a range that is likely acceptable for therapeutic applications. The performance of the device suggests that it can reliably produce respirable aerosols while maintaining clinically relevant levels of phage infectivity. These findings support the use of both nebulizers in research as well as clinical development of inhaled phage therapies and highlight their advantages over more aggressive aerosol-generation technologies.