Comparison of Spray Drying and Atmospheric Spray Freeze Drying for the Production of Active Anti-tuberculosis Bacteriophage D29 Dry Powder for Inhalation

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INTRODUCTION

Bacteriophage D29 is a parasitic virus capable of infecting various mycobacteria, including *Mycobacterium tuberculosis.* The cycle of infection consists of attachment to the cell wall, injection of double-stranded DNA, subsequent reprogramming of the cell to replicate bacteriophage progeny within it, cell lysis, and attachment of the released bacteriophage progeny to other bacteria [1]. In order for successful infection to occur the bacteriophage must remain active; in other words, it must not be irreparably damaged by the manufacturing, storage, or delivery process. Previously, it has been demonstrated that bacteriophage D29 maintains its activity after delivery with a vibrating mesh nebulizer or a soft mist inhaler, but it is substantially inactivated by a jet nebulizer [2]. However, a potential disadvantage of these aerosolization techniques is that liquid bacteriophage solution is required, and bacteriophage D29 has limited stability in solution at room temperature. Potentially, manufacturing into a dry powder could improve thermal stability. This is important since some developing countries have high

rates of drug-resistant tuberculosis yet do not have adequate cold-chain infrastructure for liquid transportation. In this study, we explore the inactivation of bacteriophage D29 associated with spray drying and compare the results to another dry powder manufacturing technique, atmospheric spray freeze drying.

MATERIALS AND METHODS

Spray drying was performed using a modified Büchi B-191 (Büchi Labortechnik AG, Flawil, Switzerland) with a custom twin-fluid atomizer. The spray drying equipment and process is described in detail elsewhere [3]. The inlet temperature was 60°C, the drying gas flow rate was ~500 L/min, and the air-to-liquid ratio was 15, which according to Hoe *et al.* [4] results in atomized droplets of an initial mass median diameter of 7 µm. Using an existing process model based on a mass and energy balance [3], the outlet temperature was predicted to be 46°C and the outlet relative humidity 1.5%.

Formulation for spray drying consisted of dissolving 140 µL of sterile-filtered bacteriophage D29 lysate at a titer of 11.7 ± 0.1 log(pfu/mL) into 14 mL aqueous solution containing 5 mg/mL trileucine (Product no. L0879; Sigma-Aldrich, MO, USA) and 45 mg/mL trehalose (Cat no. BP2687; Fisher BioReagents, NH, USA). The powder batch size was 700 mg. By theoretical considerations described elsewhere [3], the predicted mass median aerodynamic diameter of the produced particles was 2.7 µm. The dry glass transition temperature of the powder was predicted using the Fox Equation to be ~119°C, which is suitable for physical stability of the amorphous powder under room temperature storage conditions [3].

A field emission scanning electron microscope (Zeiss Sigma; Carl Zeiss Microscopy GmbH, Oberkochen, Germany) was used with an in-lens (immersion lens) detector, spot size of 20 µm, working distance of 6.1 mm, and accelerating voltage of 10 kV to image the spray dried powder coated with ~12 nm of gold with a sputter deposition system (Denton Desk II; Denton Vacuum LLC, NJ, USA).

Materials and methods for atmospheric spray freeze drying of bacteriophage D29 are described elsewhere [5]. Briefly, the process consisted of a spray-freezing step, followed by atmospheric drying. For spray-freezing, the chamber was pre-cooled to -130°C with liquid nitrogen in dry air. Liquid nitrogen was then shut off and the bacteriophage formulation was pumped through a twin-fluid nozzle, with a liquid flow rate of 20 mL/min and a compressed air flow rate of 10 L/min. At the end of spraying the chamber had warmed to -80°C. Atmospheric drying then began by gradually increasing the temperature of the chamber from -80°C to -20°C. The chamber temperature was kept at -20°C for two hours, and then held constant for one hour each at incrementally higher temperatures. The temperature was eventually brought up to 25°C at the end of the drying process.

Amplification of bacteriophage D29 and plaque assays to determine activity level were performed according to existing protocols [6]. Plaque assay was performed in triplicate at multiple dilution levels. Results are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

The outlet temperature of the spray dryer was within 1°C of the predicted value. The amount of powder collected was 57% of the batch size, which is typical for a laboratory-scale spray dryer with a small batch size, and would increase substantially upon scale-up. Generating the powder required approximately 30 minutes, which is much shorter than the 6-7 hours for atmospheric spray freeze drying, as spray drying requires no atmospheric drying step to remove ice. Nevertheless, atmospheric spray freeze drying is a faster process than traditional tray lyophilization, which typically takes 3-5 days [5].

The spray dried bacteriophage D29 powder had a titer of 6.7 ± 0.1 log(pfu/mg). The corresponding overall titer reduction due to formulation and spray drying was 1.2 ± 0.1 log(pfu/mL). This is a marginally acceptable manufacturing loss and is comparable to the total (formulation + drying) titer reduction of ~1.1 log(pfu/mL) for bacteriophage D29 atmospheric spray freeze dried with the lead formulation of 30 mg/mL mannitol and 70 mg/mL trehalose [5]. Ly *et al.* [5] found that about half of the total titer reduction occurred in the formulation step prior to the atmospheric spray freeze drying process. Further assessment would be required to determine the titer reduction occurring during the formulation step for spray drying.

A scanning electron micrograph of the spray dried powder is shown in Figure 1. The spray dried particles have a wrinkled morphology and a low particle density, which results in a lower aerodynamic diameter than geometric diameter. It is known that wrinkled spray dried particles containing trileucine are highly dispersible and suitable for inhalation with dry powder inhalers [7]. In comparison, atmospheric spray freeze dried trehalose particles are highly porous and appear to have a very low particle density [5].

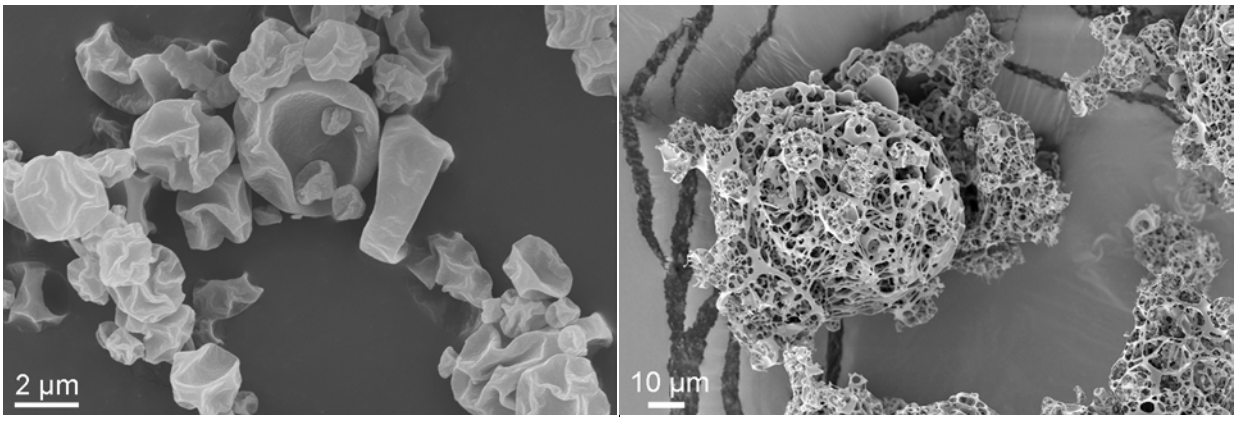

Figure 1: Scanning electron micrograph of spray dried trileucine trehalose powder containing active bacteriophage D29 (left) and atmospheric spray freeze dried trehalose powder containing active bacteriophage D29 (right).

CONCLUSIONS

Trileucine trehalose particles containing active bacteriophage D29 that appear suitable for inhalation can be efficiently produced by spray drying. The inactivation of bacteriophage D29 due to spray drying was marginally acceptable, and similar to that reported by Ly *et al.* [5] for atmospheric spray freeze drying of D29. Thermally-stable high titer bacteriophage D29 powder may be useful for transportation, storage, and respiratory delivery in developing countries without cold-chain infrastructure. Specifically, the inhalation of this spray dried anti-tuberculosis powder could potentially provide prophylactic protection to individuals at risk of inhaling tuberculosis bacteria or play a role in combination therapy with small molecule antibiotics.

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