Expedited Biologic Formulation and Spray Drying Process Development via Mechanistic Modeling

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INTRODUCTION

Spray drying is known to be a fast and cost-effective technique widely used in the manufacture of pharmaceutical dosage forms [1]. Spray drying biologics such as bacteriophages and protein vaccines into a dry powder can increase their thermal stability and shelf-life beyond those of traditional liquid formulations and thereby potentially reduce the associated cost for global distribution [2]. Compared to empirical and statistical design of experiments, predictive models based on an underlying mechanistic understanding can significantly reduce the cost, time and effort required to develop a suitable biologic formulation. The use of mechanistic models to expedite the biologic formulation development process is demonstrated in this study for a bacteriophage and a vaccine.

MATERIALS AND METHODS

Trehalose (177613, Fisher Sci., ON, Canada) and trileucine (BCBP2254V, Sigma-Aldrich, MO, USA) were selected as the excipients to stabilize the biologics of interest in the spray-dried formulations. Bacteriophage CP30A is a *Myoviridae* known to be present in the gut of chickens that is capable of infecting *Campylobacter jejuni*. The phage generation and assay have been described in detail in a previous study [3]. Stock phage lysates were first purified and then diluted into an aqueous solution consisting of trileucine

(4 mg/mL) and trehalose (100 mg/mL). The tuberculosis (TB) vaccine used in this study is a recombinant protein ID93 comprised of *Mycobacterium tuberculosis* (*Mtb*) antigens Rv3619, Rv1813, Rv3620, and Rv2608 and stabilized in the form of a nano-emulsion (~ 90 nm) consisting of squalene oil, DMPC (dimyristoyl-sn-glycero-3-phosphocholine), Tris 7.5, and GLA (glucopyranosyl lipid A). The vaccine feedstock for spray drying was prepared by diluting the nano-emulsion into an aqueous solution consisting of trileucine (1.3 mg/mL) and trehalose (33.3 mg/mL).

Two different laboratory-scale spray dryers, a modified Büchi B-191 and a custom-built PETRA dryer, were used to spray dry the phage and vaccine formulations respectively. Microparticle engineering models [4] were used to select component concentrations in the feedstock to achieve desired component distributions within the dried particles and resulting dried particle morphology. Heat loss experiments combined with spray dryer process modeling [5] were used to quantify how the temperature and relative humidity at the outlet of the two dryers varied under different operational conditions; the results were then used to predict appropriate spray drying parameters for the different biologics. This methodology ensured that the dried powder would meet pre-determined standards. A supplemented phase diagram [6] was used in combination with the models to predict the long-term physical stability (retention of the amorphous solid phase) of the spray-dried products for the chosen processing and storage conditions.

Table 1. Settings used for spray drying of biologic formulations

Active Biologics	**Bacteriophage CP30A**	**Tuberculosis Vaccine ID93**
Formulation Targets	Dry powder phage formulation with low titer reduction and long-term storage stability.	Dry powder vaccine formulation with long-term storage stability and high respirable delivery efficiency.

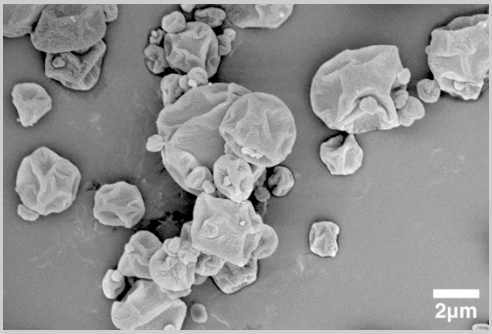
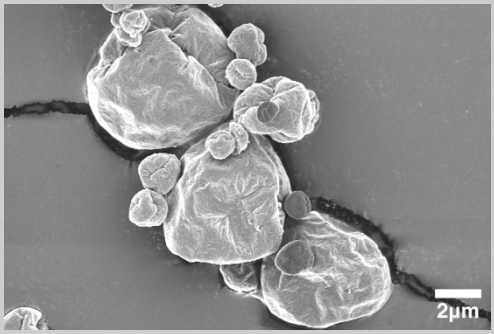
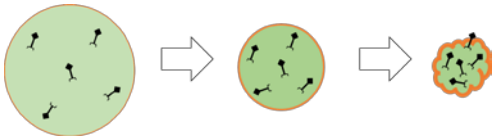
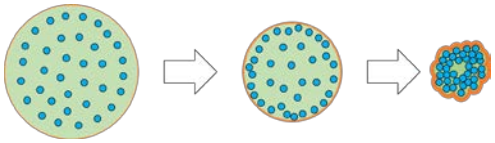
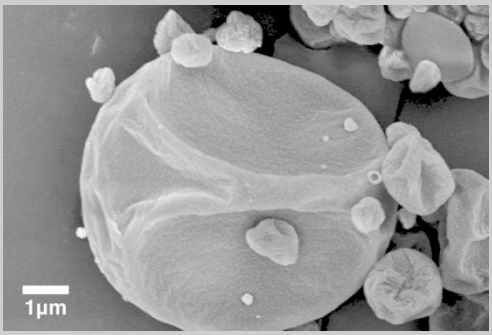
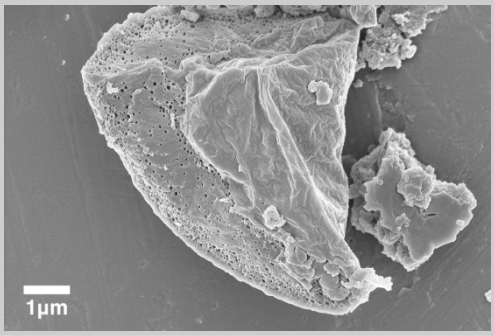
Spray Dryer	Modified Büchi B-191	Custom-built PETRA
Total Solids Concentration	~ 104 mg/mL	42 mg/mL
Formulation	Trileucine (4 mg/mL) Trehalose (100 mg/mL)	Trileucine (1.3 mg/mL) Trehalose (33.4 mg/mL)
Air-Liquid-Ratio	9	8
Predicted Droplet MMAD	~ 9 µm	~ 8 µm
Dryer Inlet Temperature	70 °C	65 °C
Predicted Outlet Temperature	49 °C	36 °C
Predicted Dryer Outlet Relative Humidity	3%	7%
Predicted Particle Median Volume Equivalent Diameter	3.9 µm	3.2 µm

RESULTS AND DISCUSSION

Two formulations containing anti-*Campylobacter* bacteriophage CP30A and tuberculosis vaccine ID93 were successfully formulated and spray dried with the help of various mechanistic models. The formulations, spray drying conditions, and product stability were well-predicted by the models as presented in Table 2. In both formulations, the excipient trehalose works as a glass stabilizer that solidifies into an amorphous solid phase during the droplet drying process and provides protection to the embedded biologics. For the phage formulation, a close-to-saturation concentration of trileucine was used. At this concentration, the trileucine was predicted to form a very thin amorphous shell at the surface early in the drying process that would prevent the phages from reaching the surface and being exposed to desiccation stress, thus leading to a much lower phage inactivation rate than for a pure-trehalose formulation [6]. Meanwhile, for both

formulations, trileucine, despite its low concentration (< 5%), also acted as an effective particle surface modifier that improved the dispersibility of the dried particles [7], increasing the production yield during the spray drying processes and significantly improving aerosol performance.

Table 2. Spray dried phage and vaccine formulations

Biologic	Phage Formulation	Vaccine Formulation
Spray Dried Particle Morphology		
Particle Formation Schematic		
Particle Surface Structure		
Spray Dryer Condition	Outlet temperatures matched predictions within 2°C during the spray drying processes of both formulations.	
Particle Size	• Consistent with predicted volume equivalent diameter	• Matched particle size prediction • Particles within inhalable range (1-5 µm)
Dry Powder Formulation Performance	• Dispersible powder • High production rate and yield (>53%) • Overall phage titer reduction after formulation, spray drying,	• Dispersible powder • High production rate and yield (>70%) • After reconstitution of dried powder

	1-month dry room temperature storage, and reconstitution for plaque assay < 1.0 log_{10}(pfu/mL) [6]	o Insignificant squalene and GLA content loss (<5%); o Insignificant nano-emulsion droplet size change (<5%); o Retention of vaccine antigen ID93 • Promising applicability for respiratory drug delivery (> 95% emitted dose, > 30% lung dose, >10% FPF tested in vitro by Next Generation Impactor)
Storage Stability	• Demonstrated physical stability at room temperature (amorphous > 4 months)	• Physical stability at different temperatures after 1 months (5°C, 25°C, 40°C, 50°C)

CONCLUSIONS

In this study, biologic formulations containing anti-Campylobacter bacteriophage CP30A and tuberculosis vaccine ID93+GLA-SE were successfully formulated and spray dried. Mechanistic models proved useful for informed selection of appropriate excipients, formulations, and process conditions, expediting the CMC (chemistry, manufacturing, and control) development process, as fewer experiments were required than would be for an empirical or statistical design approach. Performance of the spray-dried biologic formulations met the formulation design targets using the processing conditions and formulation compositions achieved by a significantly reduced number of experimental iterations.

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