Atmospheric Spray Freeze Drying of Bacteriophage D29 with Sugars

Alvin Ly, Nicholas B. Carrigy, Hui Wang, Melissa Harrison, Dominic Sauvageau, Andrew R. Martin, Reinhard Vehring, Warren H. Finlay
University of Alberta, Edmonton, Canada

Introduction

- Bacteriophage (phage) are potential therapeutic agents for treating drug-resistant bacterial infections
- Phage D29 is a virus that lyses *Mycobacterium tuberculosis*
- Atmospheric spray freeze-drying (ASFD) is a unique drying process that has not previously been applied to phage processing
- Trehalose is useful as a preservation agent during drying

Materials & Methods

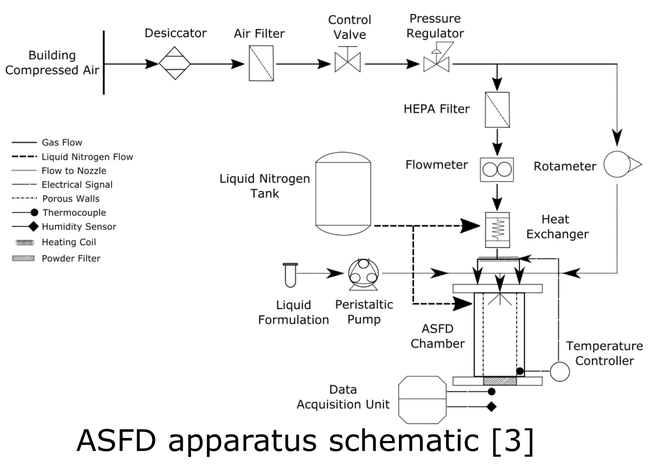
➢ Feedstock Formulation

- Phage D29 amplified on the host *Mycobacterium smegmatis* strain mc²155 with a solid media
- Phage buffer was poured onto plaques of host bacterium and incubated overnight at 4°C and filtered [1]
- Mannitol and trehalose was mixed with varying mass ratios and drying times with a total mass concentration of 100 mg/mL:

ASFD Feed Solution	Mass ratio (% w/w)		Volume (mL)	Process Time (min.)
	Trehalose	Mannitol		
A	100	0	10	360
B	70	30	10	360
C	50	50	10	360
A2	100	0	10	420
B2	70	30	10	420

➢ ASFD Process

- Atomize feedstock into cold chamber
- Liquid droplets instantly freeze and conveyed onto filter
- Cold drying gas passes through the powder and filter
- Low temperature and partial pressure at droplet surface sublimes the ice [2]



ASFD apparatus schematic [3]

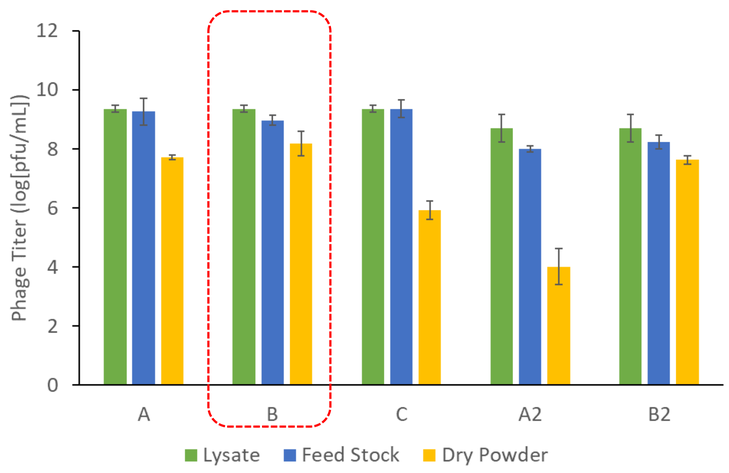
➢ Titer Measurement

- Plaque assays on the host *M. smegmatis* mc²155 were used to titer the phages, in plaque-forming units per mL (pfu/mL) [1]

Results & Discussion

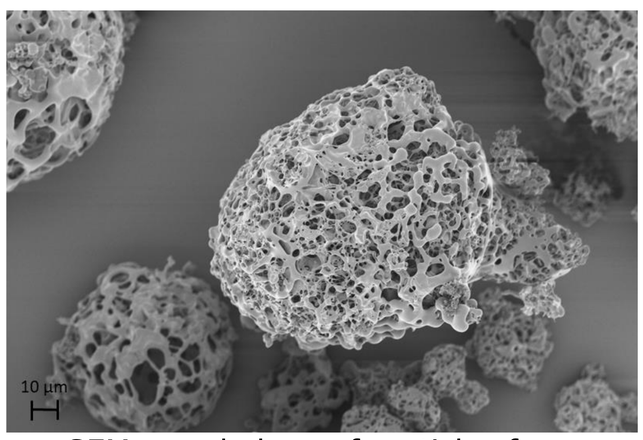
➢ Phage D29 Titer

- The trehalose powder had titer reductions greater than 1 log[pfu/mL]
- The sugar solution with equal mass ratios had titer reductions greater than 3 log[pfu/mL]
- Trehalose and mannitol powder at 7:3 mass ratio (powder B) had an acceptable titer reduction of only ~0.7 log[pfu/mL]

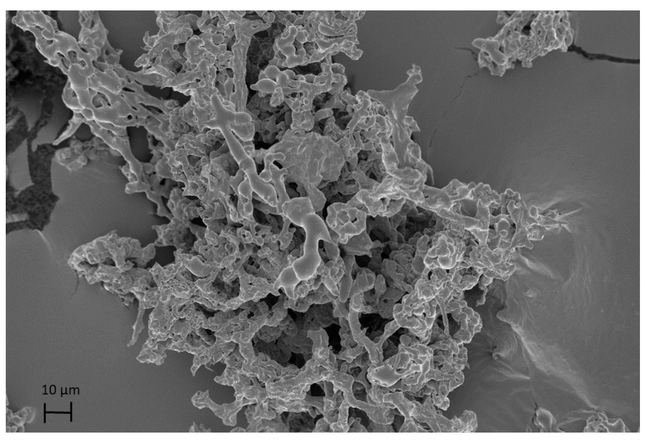


Phage titer measurement for each formulation [3]

➢ SEM Images [3]



SEM morphology of particles from formulation A shows individual spherical particles with typical porous structure



SEM morphology of particles from formulation B shows particles with porous structure but not spherical particle shapes

➢ Solid State Analysis

- Raman spectroscopy confirmed that mannitol was present in its crystalline forms with all three polymorphs present

Powder Sample	Trehalose	Mannitol		
		α	β	δ
A	amorphous	-	-	-
A2	amorphous	-	-	-
B	amorphous	15.4% ± 8.0%	15.4% ± 8.0%	69.2% ± 12.0%
B2	amorphous	19.1% ± 6.8%	10.6% ± 6.5%	70.2% ± 12.2%

- Trehalose remained amorphous in all cases

Conclusions

- ➢ ASFD is a promising technique for biological preservation, demonstrating titer reduction of less than 1 log
- ➢ ASFD has a shorter drying time (<7 hr) than traditional lyophilization (>24 hr)
- ➢ Trehalose remains amorphous, resulting in capacity for glass stabilization
- ➢ ASFD process produces porous particles with suitability for inhalation in future formulations
- ➢ Potential for controlled particle formation by changing formulation and operating conditions

References

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3. A. Ly, N. B. Carrigy, H. Wang, M. Harrison, D. Sauvageau, A. R. Martin, R. Vehring and W. H. Finlay, "Atmospheric spray freeze drying of sugar solution with phage D29," *Frontiers in Microbiology*, vol. 10, no. 488, pp. 1-11, 2019.

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