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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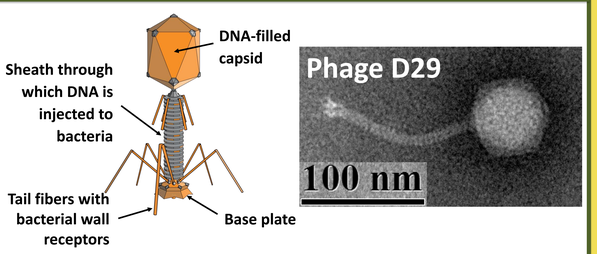
Introduction

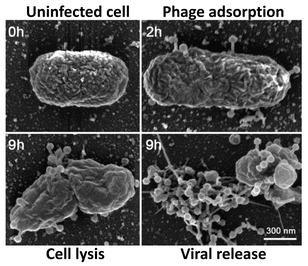
- Tuberculosis increasingly **antibiotic-resistant**
- **Phage therapy** is an alternative [1,2]
- Need many phage in lungs relative to bacteria [3]
- Processing must not **inactivate** the phage
- Alternatives to **long, expensive** lyophilization

Materials and Methods

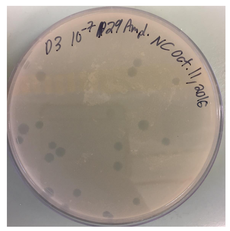


Morphological components of a tailed phage [4]

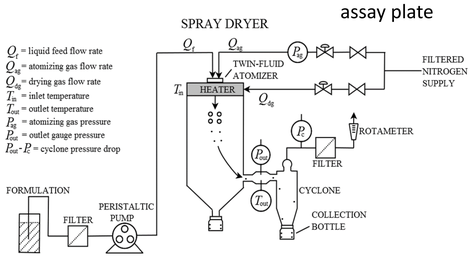
TEM of phage D29 [5], which lyses *M. tuberculosis*



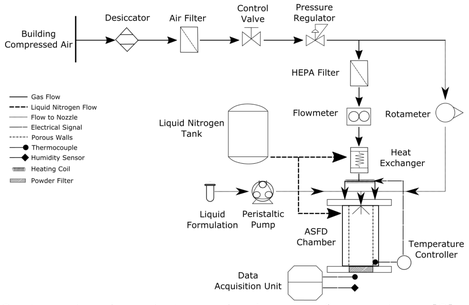
Lytic cycle [6]



M. smegmatis plaque assay plate



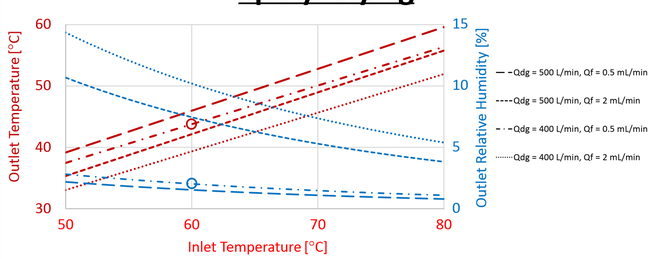
Modified Büchi B-191 spray dryer [7]



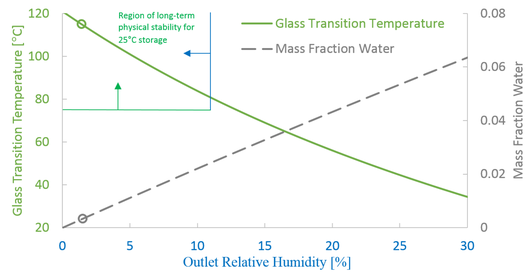
Custom developed atmospheric spray freeze dryer [8]

Results

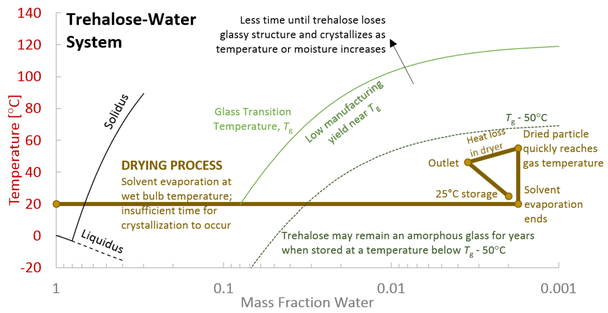
Spray Drying



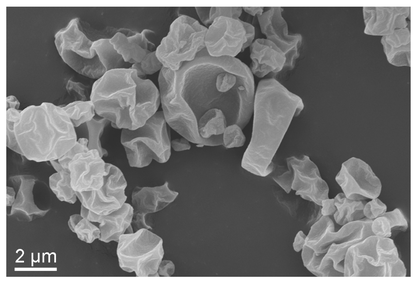
Processing modelling allows for quick choice of suitable processing conditions



Powder has suitable moisture content and glass transition temperature for long-term physical stability



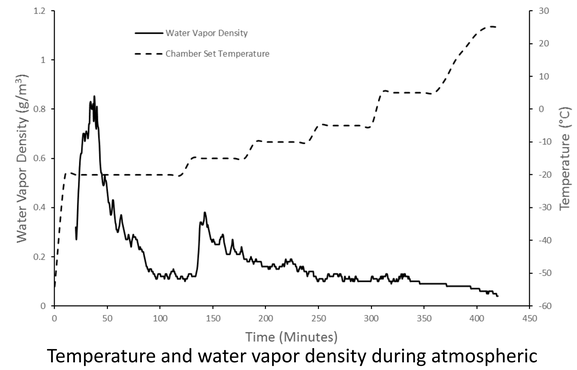
Supplemented phase diagram useful for predicting physical stability and potentially biological stability



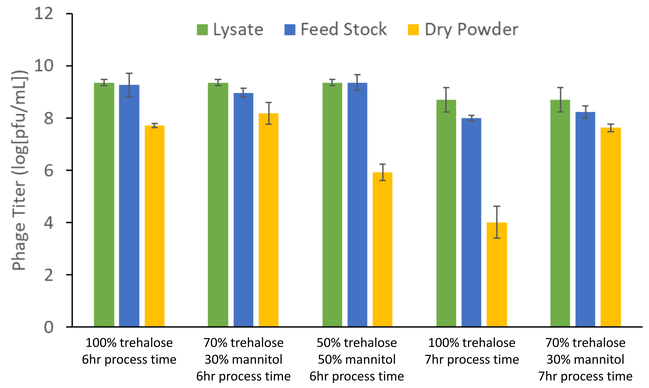
Spray dried 10% trileucine 90% trehalose particles contain active phage D29

Indicator	Value
Overall inactivation	1.2 ± 0.1 log(pfu/mL)
Process time	30 minutes
Yield	57%
MMAD	~3 µm
Morphology	Wrinkled

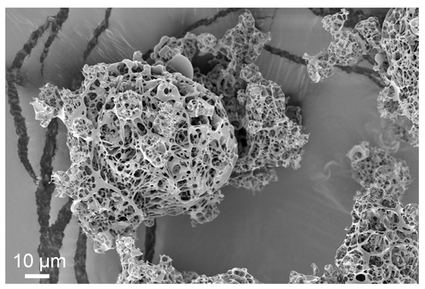
Atmospheric Spray Freeze Drying



Temperature and water vapor density during atmospheric spray freeze drying process monitored [8]



Least overall phage inactivation for 70% trehalose 30% mannitol [8]



Atmospheric spray freeze dried trehalose particles containing active phage D29

Indicator	Value [8]
Inactivation due to formulation	0.4 ± 0.1 log(pfu/mL)
Inactivation due to drying	0.7 ± 0.1 log(pfu/mL)
Overall inactivation	1.1 ± 0.2 log(pfu/mL)
Process time	6-7 hours
Morphology	Large porous

Conclusions

- Spray drying and atmospheric spray freeze drying both have **shorter processing times** than a traditional tray lyophilization cycle which lasts 3-5 days
- Processing methods are **promising for dry powder phage production**, exhibiting ~1 log(pfu/mL) titer reduction for unoptimized formulations and drying conditions
- Particles appear **suitable for inhalation**
- Exploring the use of spray drying and atmospheric spray freeze drying for **preserving other biologics** is of interest
- Powder glass transition temperature is predicted to be high enough to be **room temperature stable**, potentially allowing for distribution to developing countries without requiring cold-chain infrastructure
- Inhalation of high titer anti-tuberculosis phage D29 powder may be useful for providing **protection against active tuberculosis**

References

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3. Semler *et al.* 2014. Aerosol phage therapy efficacy in *Burkholderia cepacia* complex respiratory infections. *Antimicrob Agents Chemother* 58:4005-4013.
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5. Carrigy *et al.* 2017. Anti-tuberculosis bacteriophage D29 delivery with a vibrating mesh nebulizer, jet nebulizer, and soft mist inhaler. *Pharm Res* 34:2084-2096.
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8. Ly *et al.* 2019. Atmospheric spray freeze drying of sugar solution with phage D29. *Front Microbiol* 10:488.

Acknowledgements

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