Amorphous Pullulan Trehalose Microparticle Platform for Respiratory Delivery

AAAR 37th Annual Conference

Nicholas Carrigy

October 16, 2019

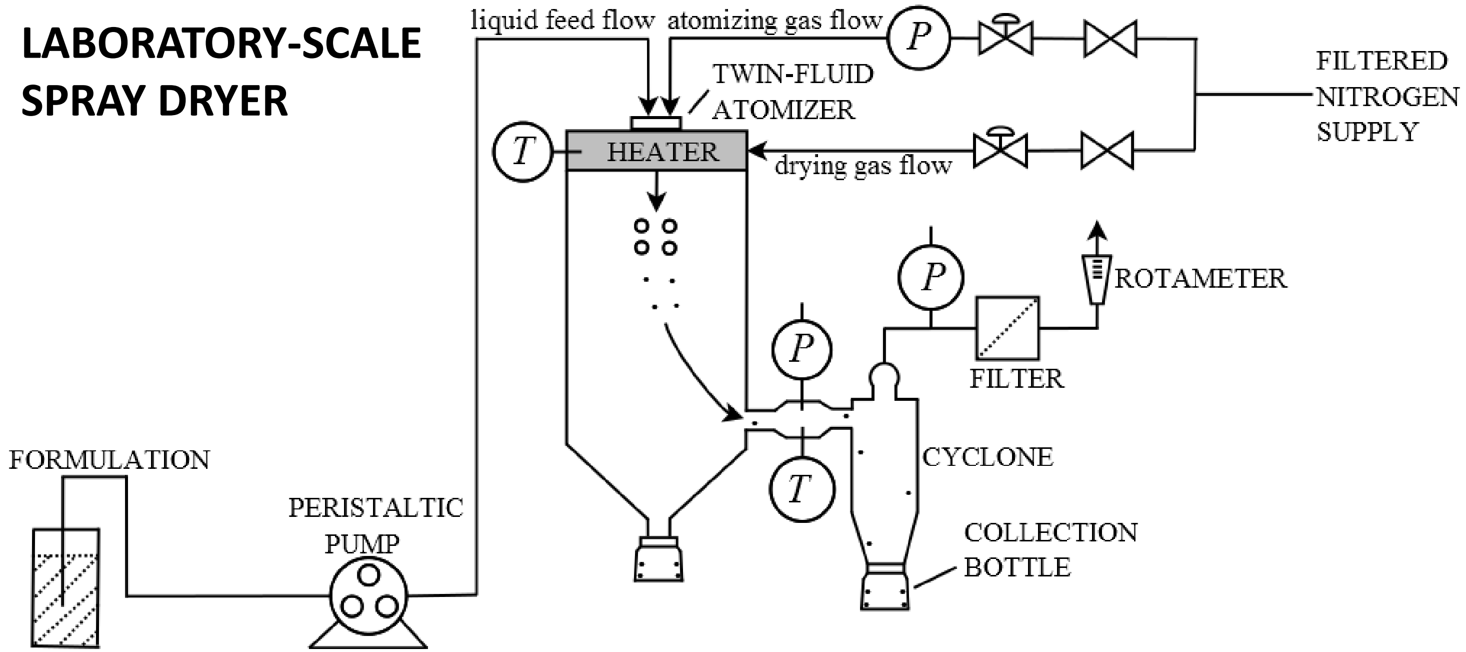
UNIVERSITY OF ALBERTA

MECHANICAL ENGINEERING

Production Techniques and Characterization of Pullulan Trehalose Microparticles

- Pullulan and trehalose are produced by microorganisms to protect against desiccation and have a high glass transition temperature that may be suitable for stabilizing biologics without requiring refrigeration
- Produce pullulan trehalose microparticles with a monodisperse droplet chain and a spray dryer and characterize to determine feasibility for inhalation
- Characterize: particle formation and morphology; glass stabilizing properties; manufacturability; aerosol performance; delivery device compatibility

LABORATORY-SCALE SPRAY DRYER



From: Carrigy & Vehring. Chapter 12. *Pharmaceutical Inhalation Aerosol Technology, Third Edition,* 2019.

MONODISPERSE DROPLET CHAIN GENERATOR

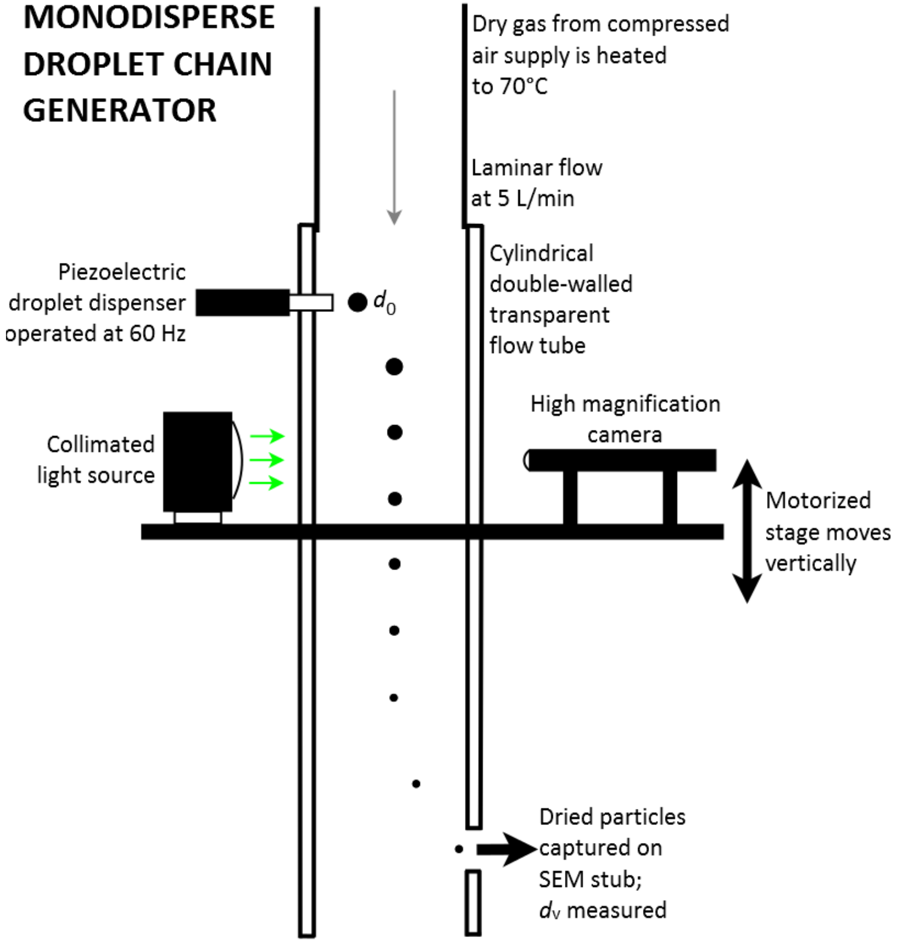
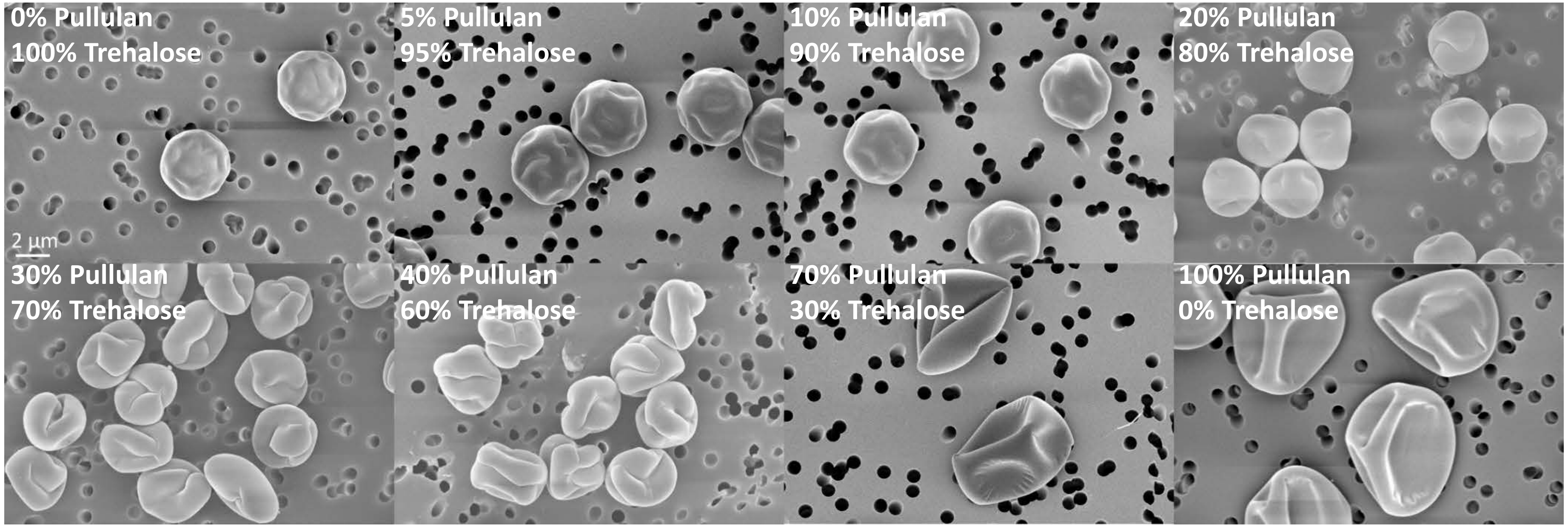
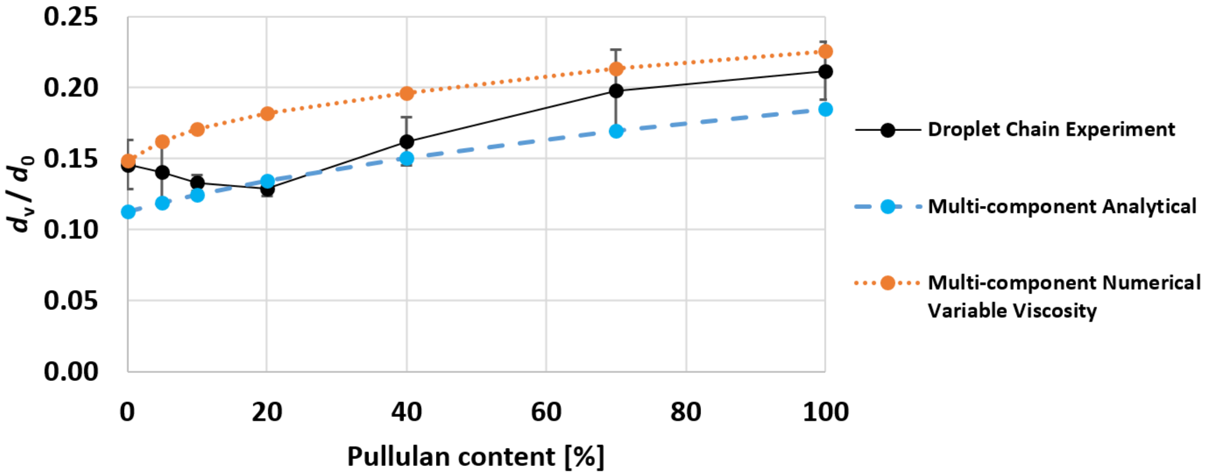


Figure adapted from: Gomez *et al. RDD 2018.*

Pullulan Makes Particles More Folded; New Particle Formation Model Predicts Diameter





Multi-component analytical model

Derived that: $t_{\mathrm{t,mix}} = \tau_{\mathrm{D}} \left[1 - \left(\sum_{\mathrm{i}} E_{\mathrm{i}} P_{\mathrm{i}} \right)^{\frac{2}{3}} \right]$

$d_{\mathrm{v}} = \sqrt{d_0^2 - \kappa t_{\mathrm{t,mix}}}$

where: $\tau_{\mathrm{D}} = \dfrac{d_0^2}{\kappa}$

$P_{\mathrm{i}} = \dfrac{C_{0,\mathrm{i}}}{\rho_{\mathrm{t,i}}}$

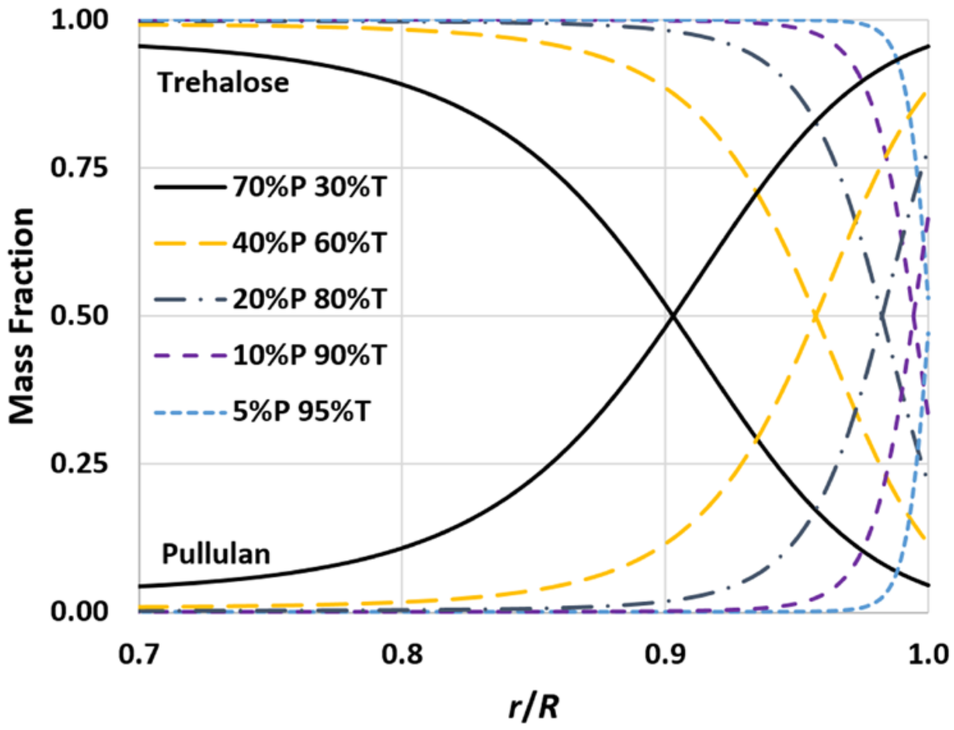
$Pe_{\mathrm{pullulan}} = 18$

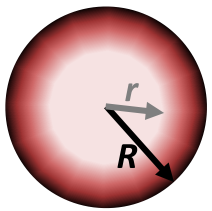
$Pe_{\mathrm{trehalose}} = 1.0$

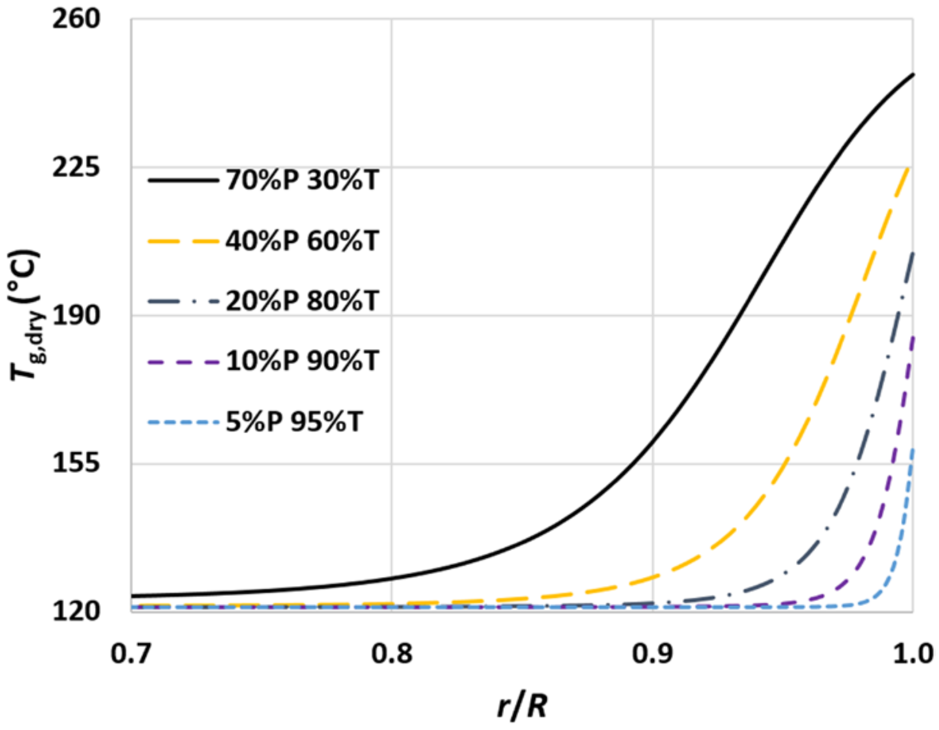
$E_{\mathrm{pullulan}} = 6.3$

$E_{\mathrm{trehalose}} = 1.2$

Pullulan Enriches on Surface and Increases Glass Transition Temperature Near Surface







Pullulan concentrates near surface due to high Péclet number (Pe_i) and surface enrichment (E_i)

$$Pe_i = \frac{\kappa}{8D_i}$$

$$E_i = 1 + \frac{Pe_i}{5} + \frac{Pe_i^2}{100} - \frac{Pe_i^3}{4000}$$

Pullulan concentration near surface increases glass transition temperature near surface

$$T_{g,dry}(r) = \frac{\omega_T(r) T_{g,T,dry} + k\omega_P(r) T_{g,P,dry}}{\omega_T(r) + k\omega_P(r)}$$

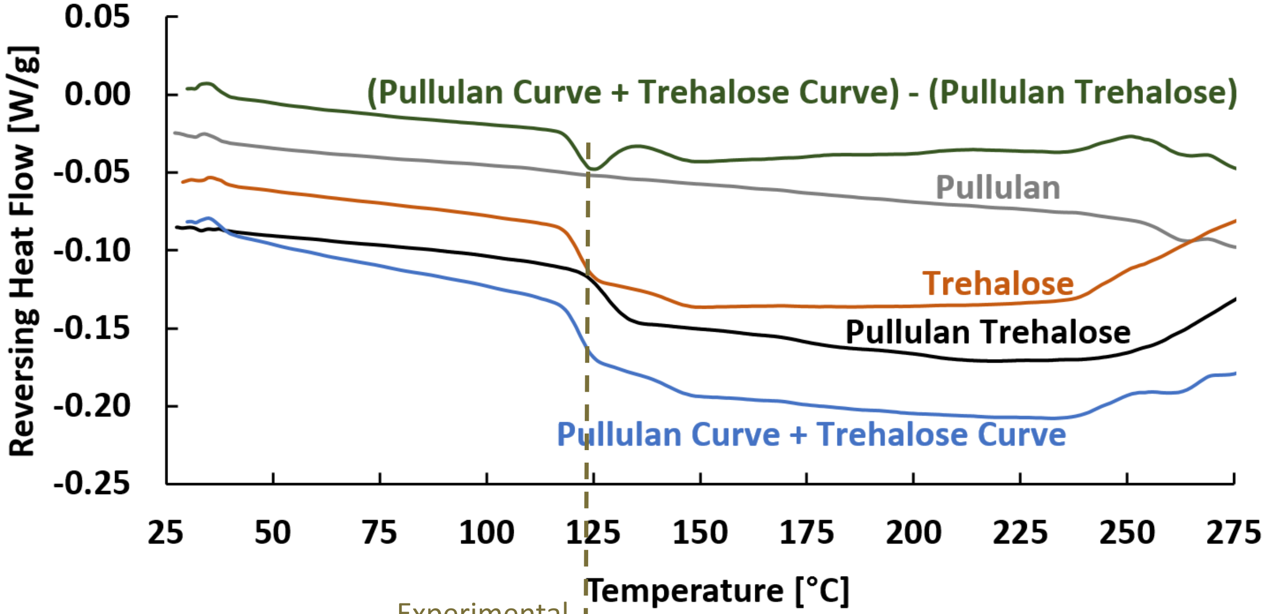
$k = 0.4163$

$T_{g,T,dry} = 394\ \mathrm{K}\ [121°\mathrm{C}]$

$T_{g,P,dry} = 534\ \mathrm{K}\ [261°\mathrm{C}]$

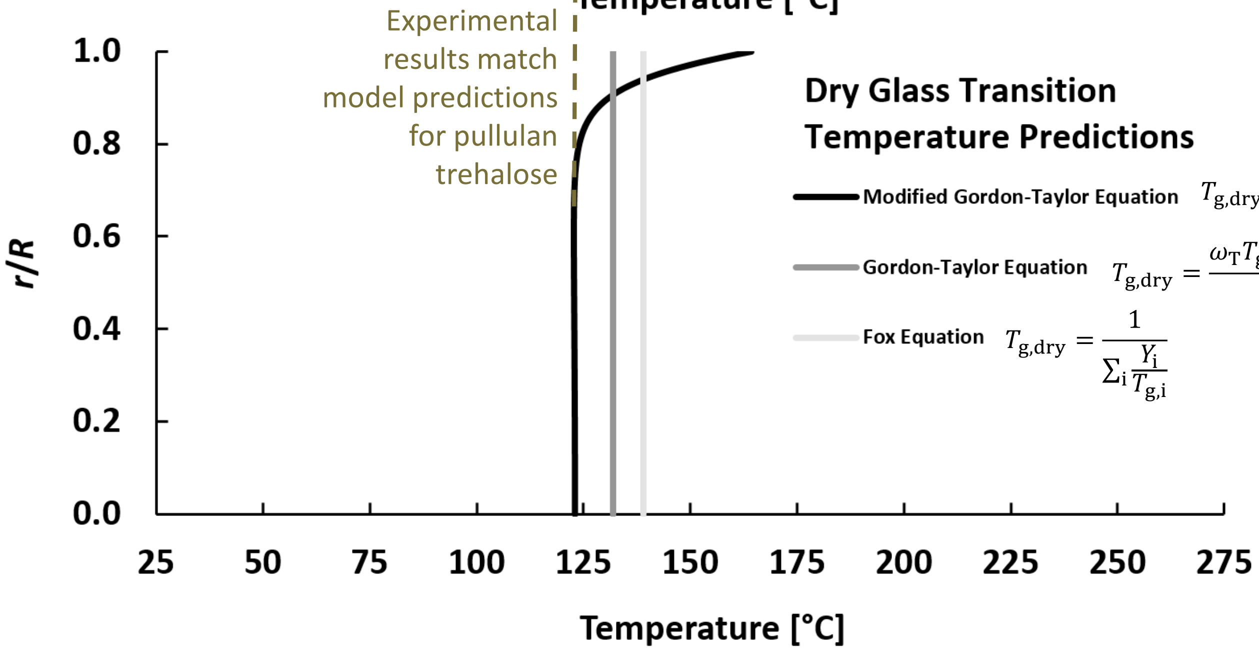
Parameters from: Teekamp *et al.* *Carbohydr Polym* 2017;176:374-380.

Modulated Differential Scanning Calorimetry Provides Evidence that Pullulan Increases Glass Transition Temperature and is Radially Distributed



Pullulan addition to formulation experimentally shown to increase powder glass transition temperature relative to trehalose alone

Evidence of well-mixed solid with associated co-solidification and radial distribution of glass transition temperature



Dry Glass Transition Temperature Predictions

Modified Gordon-Taylor Equation $T_{\mathrm{g,dry}}(r) = \dfrac{\omega_{\mathrm{T}}(r)T_{\mathrm{g,T,dry}} + k\omega_{\mathrm{P}}(r)T_{\mathrm{g,P,dry}}}{\omega_{\mathrm{T}}(r) + k\omega_{\mathrm{P}}(r)}$

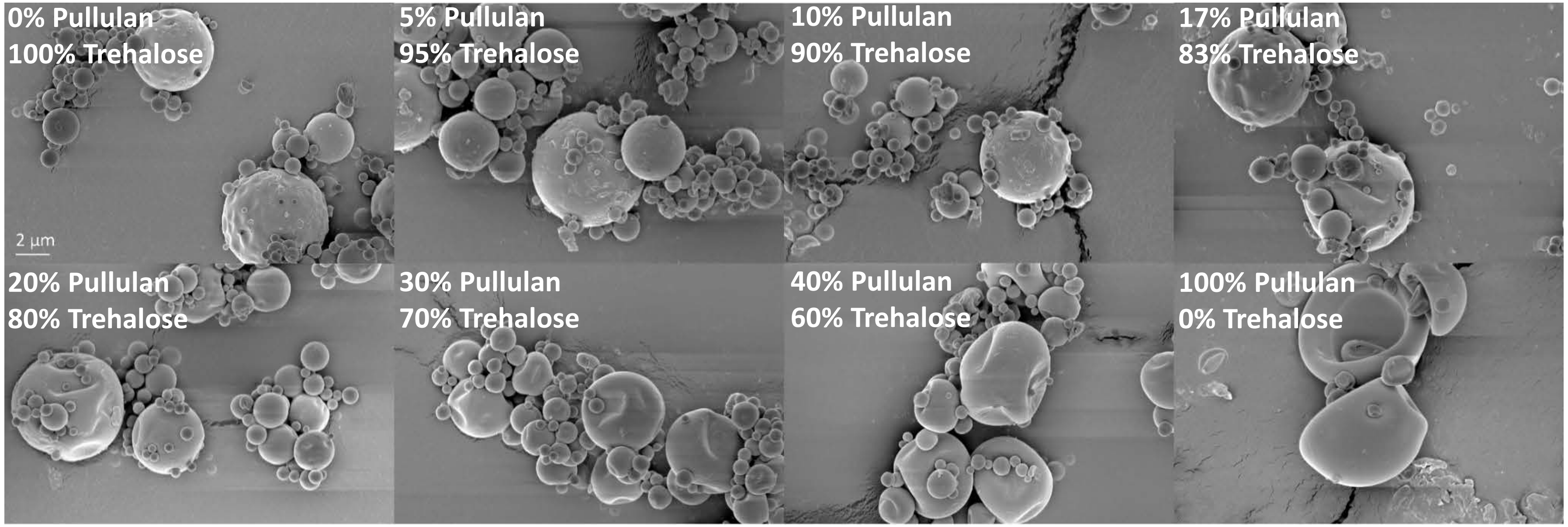
Gordon-Taylor Equation $T_{\mathrm{g,dry}} = \dfrac{\omega_{\mathrm{T}}T_{\mathrm{g,T,dry}} + k\omega_{\mathrm{P}}T_{\mathrm{g,P,dry}}}{\omega_{\mathrm{T}} + k\omega_{\mathrm{P}}}$

Fox Equation $T_{\mathrm{g,dry}} = \dfrac{1}{\sum_{\mathrm{i}} \frac{Y_{\mathrm{i}}}{T_{\mathrm{g,i}}}}$

Gordon & Taylor. *J Chem Technol Biotechnol.* 1952;2:493-500.

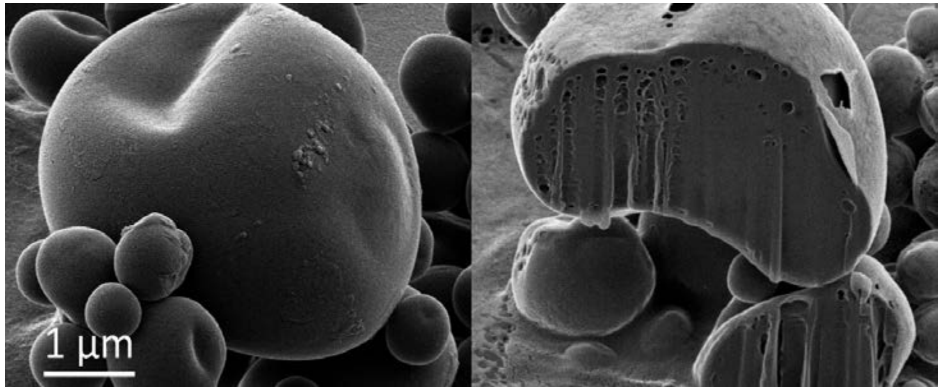
Fox. *Bull Am Phys Soc.* 1956;1:123-128.

Spray Dried Pullulan Trehalose Microparticles Produced and Appear to have Minimal Internal Void Space



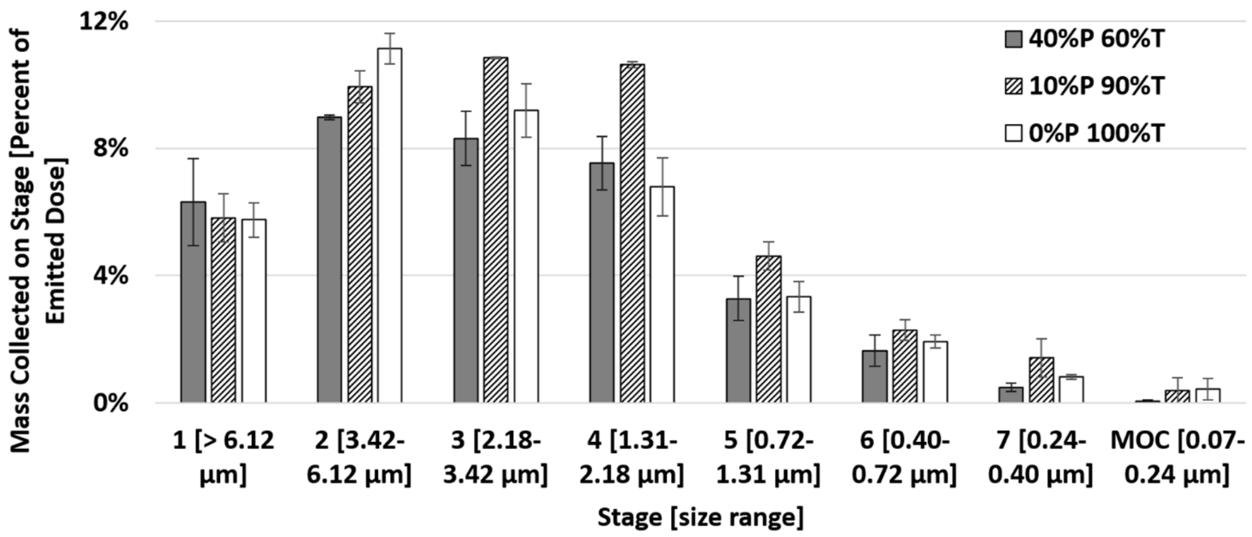
Yield 50-65%

Increased pullulan concentration increases folding in spray dried microparticles



Microparticles contract after solidification with resulting minimal internal void space

Pullulan Trehalose Microparticles have Suitable Aerosol Performance for Inhalation from a Dry Powder Inhaler



Aerosol performance of pullulan trehalose powder emitted from a Seebri® Breezhaler® dry powder inhaler through an Alberta Idealized Throat to a Next Generation Impactor. Emitted dose 93-94% of capsule dose.

Powder Composition	Total Lung Dose [%]	FPF (< 6.12µm) [%]	MMAD [µm]
0% Pullulan 100% Trehalose	39.3 ± 1.6	33.6 ± 2.1	2.45 ± 0.03
10% Pullulan 90% Trehalose	45.9 ± 3.2	40.1 ± 2.4	2.16 ± 0.09
40% Pullulan 60% Trehalose	36.5 ± 1.4	30.2 ± 2.8	2.38 ± 0.09

Commercial DPIs reported to have total lung doses of 5.5-40.5%, with a mean of 23%.[1]

Suitable MMAD for inhalation; GSD ~1.9

- Three actuations and inhalations per run to obtain sufficient powder mass for gravimetric measurement. Error bars represent s.d. from two runs.
- 100 L/min flow rate for 2.4 seconds provided 4 L inhalation volume.
- Pressure drop across DPI 3.4 kPa.
- Control measurements verified silicone spray used to coat plates did not evaporate and affect mass during run.

Fit to cumulative distribution function

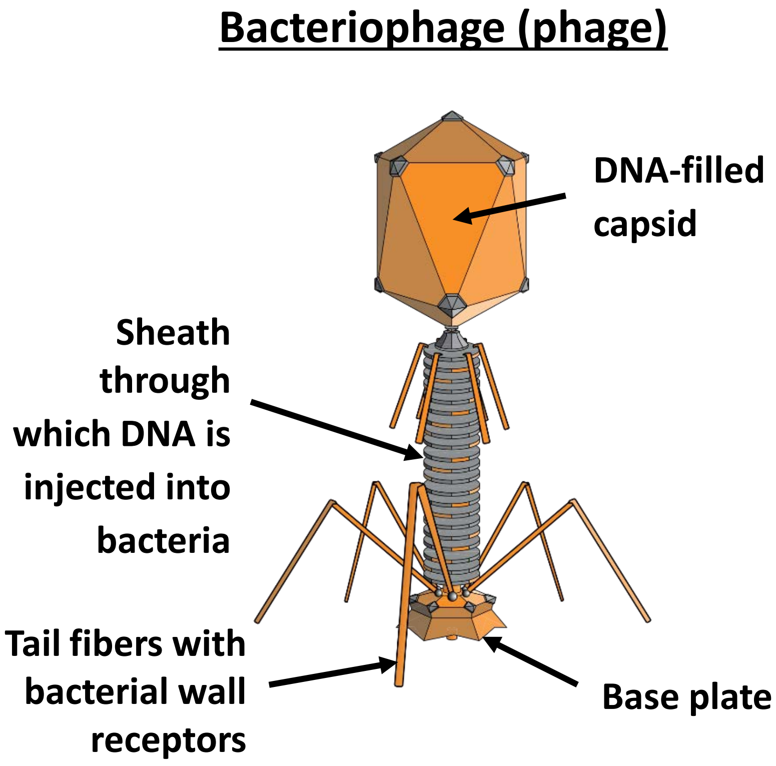
$$y = \frac{1}{2}\left[1 + \operatorname{erf}\left(\frac{\ln(x) - \ln(MMAD)}{\sqrt{2}\ln(GSD)}\right)\right]$$

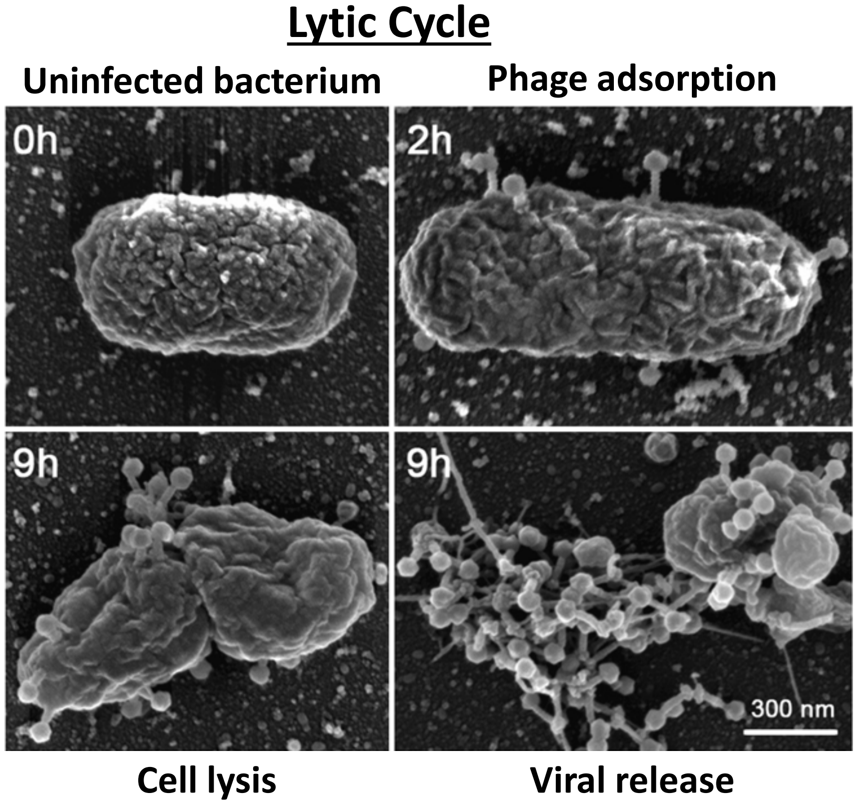
x = vector of upper size limits for stages 2-MOC

y = vector of corresponding cumulative mass data {0-1}

1. Matinkhoo *et al. J Pharm Sci.* 2011;100:5197-5205.

Bacteriophage – Virus that Infects Bacteria





- **Hypothesis**: Phage could be stabilized in a fully amorphous spray dried powder with a sufficiently high glass transition temperature to not require refrigeration

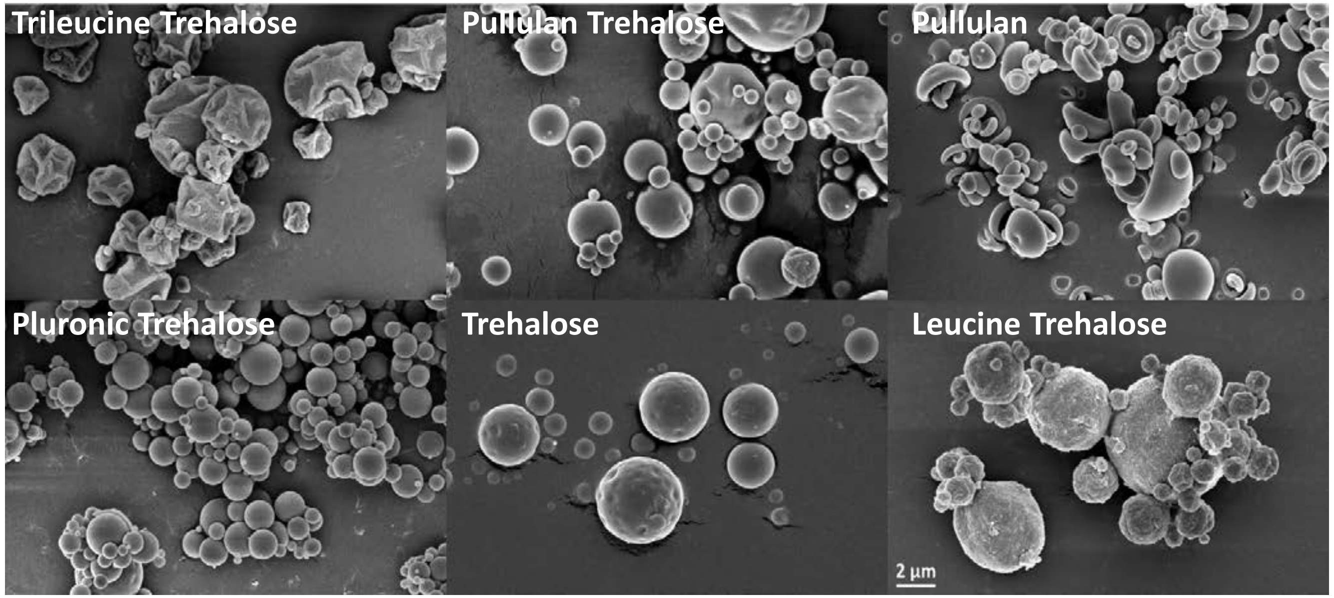
Image on left adapted from: Sabehi *et al. PNAS.* 2012;109:2037-2042
Image on right adapted from: https://en.wikipedia.org/wiki/Bacteriophage

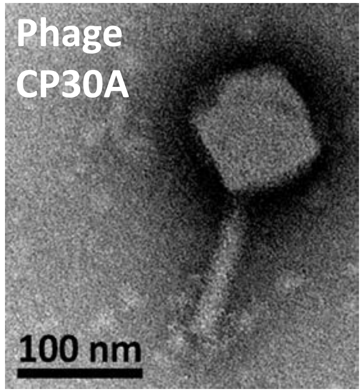
Pullulan Trehalose Microparticles Outperform Commonly Used Leucine Trehalose Microparticles for Stabilizing Phage CP30A

- **Pullulan trehalose microparticles stabilized phage CP30A better than the commonly used leucine trehalose formulation**

Formulation	Processing Titer Reduction [log(PFU/mL)]	Processing + 1 Month Storage Titer Reduction [log(PFU/mL)]
Trileucine 4 mg/mL, Trehalose 100 mg/mL	N.M.	0.6 ± 0.1 *
Pullulan 20 mg/mL, Trehalose 100 mg/mL	1.0 ± 0.1 * †	1.7 ± 0.1 ***
Leucine 20 mg/mL, Trehalose 100 mg/mL	1.7 ± 0.1 ** ††	1.9 ± 0.1 *
Pullulan 20 mg/mL	2.4 ± 0.2	N.M.
Pluronic F-68 4 mg/mL, Trehalose 100 mg/mL	2.4 ± 0.2	N.M.
Trehalose 100 mg/mL	2.4 ± 0.1 * †	3.9 ± 0.2
Trehalose 20 mg/mL	3.3 ± 0.0	N.M.

* significantly less ($p < 0.001$) titer reduction than below in same column
** significantly less ($p < 0.01$) titer reduction than below in same column
*** significantly less ($p < 0.05$) titer reduction than 20L 100T in same column
† significantly less titer reduction than with 1 month storage ($p < 0.0025$)
†† significantly less titer reduction than with 1 month storage ($p < 0.05$)





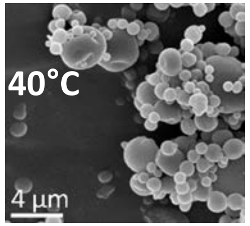
Pullulan and trehalose both have high glass transition temperatures[1] and protect against desiccation[2-3]

1. Teekamp *et al. Carbohydr Polym* 2017;176:374-380.
2. di Stefano. MSc Thesis, Politecnico di Milano, 2017.
3. Crowe *et al. Annu Rev Physiol* 1998;60:73-103.

Conclusions

- Spray dried pullulan trehalose microparticles are a promising non-reducing sugar-only, fully amorphous platform for respiratory delivery, for example of biologics, with suitable aerosol performance from a dry powder inhaler
  - Good manufacturability: reasonable spray drying yield and ability to easily fill the flowable powder into dry powder inhaler capsules, indicating it is not extremely cohesive

- Short-term physical stability (42 days): maintained amorphous phase during ambient temperature storage in a dry box and 40°C storage in pressurized metered-dose inhaler canisters containing commercial propellants HFA 134a and HFA 227; actuation from a pressurized metered-dose inhaler after storage did not affect the morphology or solid phase



- Particles theoretically predicted and experimentally supported to have a higher glass transition temperature near the surface, where biologics are expected to reside, than in the interior; this may provide more protection against fusing for temporary high temperature or high humidity excursions

$$T_{\mathrm{g,dry}}(r) = \frac{\omega_{\mathrm{T}}(r) T_{\mathrm{g,T,dry}} + k\omega_{\mathrm{P}}(r) T_{\mathrm{g,P,dry}}}{\omega_{\mathrm{T}}(r) + k\omega_{\mathrm{P}}(r)}$$

- Newly developed particle formation model for amorphous co-solidification from multi-component drying droplets demonstrated close match of diameter predictions to experimental measurements with the monodisperse droplet chain and may prove useful for accelerating the development process

$$t_{\mathrm{t,mix}} = \tau_{\mathrm{D}} \left[1 - \left(\sum_{\mathrm{i}} E_{\mathrm{i}} P_{\mathrm{i}} \right)^{\frac{2}{3}} \right]$$

THANK YOU FOR LISTENING!

Acknowledgements – Thank You!

University of Alberta
Alberta Innovates Technology Futures
Lauréats KILLAM Laureates
NSERC CRSNG
University of Nottingham UK | CHINA | MALAYSIA
BBSRC bioscience for the future