Long-term Room Temperature Stability of a Spray Dried Inhalable Tuberculosis Vaccine

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Introduction

- **Thermostability** can **improve global availability** of vaccines by **eliminating cold chain dependency**.
- There is evidence that **direct delivery to the lungs or nose improves protective immune response** for respiratory illness [1].
- **Two thermostable and inhalable tuberculosis (TB) adjuvanted subunit vaccines** were **spray dried** [2] and **evaluated for physical and chemical stability** over two years of storage at room temperature
  - A **control formulation** used **trehalose** as a **glass stabilizer for a GLA-SE** nano-emulsion **adjuvant** system and **ID93** recombinant fusion protein TB **antigen** [3]
  - A **lead formulation** in which **3% trileucine** was added as a **dispersibility and stability enhancer** [2]

Materials and Methods

Table 1. *Particle compositions of control and lead formulations* [2].

Component	Particle Composition (w/w)	
	Control	**Lead**
Trehalose	81%	78%
Tris (buffer)	2%	2%
Trileucine	**-**	**3%**
Squalene	14%	14%
DMPC	3%	3%
GLA	0.01%	0.01%
ID93	0.003%	0.003%

Table 2. *Low temperature spray drying parameters for inhalable TB vaccine powder* [2].

Parameter	Settings chosen for inhalable TB vaccine spray drying
Drying gas flowrate	200 SLPM
Inlet Temperature	65 °C
Liquid Feed Flowrate	0.6 ml/min
Outlet Temperature	36 °C
Predicted Outlet RH	7 %

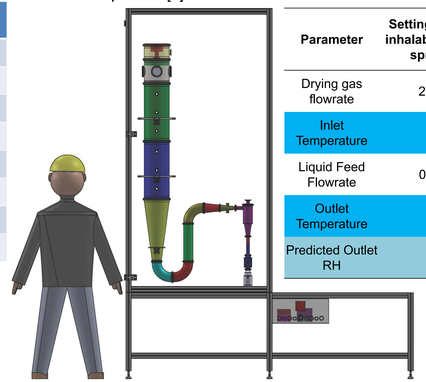

Figure 1. *Custom research dryer used for low temperature spray drying of inhalable TB vaccine powders.*

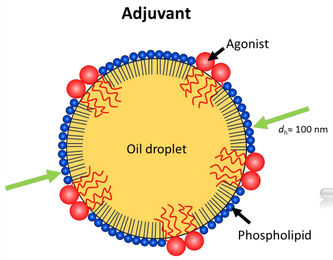

Figure 2. *GLA-SE Adjuvant System.*

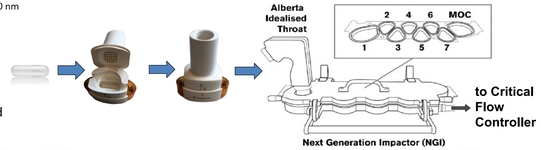

Figure 3. *Test setup for monitoring aerosol performance of inhalable TB vaccine powders.*

Physical stability was assessed by monitoring:
- **Particle morphology** via field emission scanning electron microscopy **(FESEM)**
- **Moisture content** via **Karl Fisher calorimetry**
- **Solid phase** via **Macro-Raman spectroscopy**
- **Aerosol performance** using a **commercial dry powder inhaler** (DPI, Seebri Breezhaler®, Novartis), **Alberta Idealized Throat (AIT), and Next Generation Impactor (NGI) setup** (Figure 3)

Chemical stability was assessed by monitoring:
- **Visual appearance**
- **pH**
- **Nanoemulsion droplet size distribution** via dynamic light scattering **(DLS)**
- **Squalene** and **GLA content** via reversed phase high-performance liquid chromatography **(HPLC)**
- **ID93 content** via densitometry analysis of reducing **SDS-PAGE** based on a standard curve

Results

- **Physical stability analysis** performed after 2 years' storage on:
  - **Lead** formulation stored at 25 and 40 °C
  - **Control** formulation stored at 25 °C
- **No change in particle morphology** observed for all samples after two years of storage at these temperatures
  - **Control** formulation (Figure 4A) consisted of **round, separate particles** with **no indication of fusing**
  - **Lead** formulation (Figures 4B and 4C) had **folded and rugose outer morphology** from inclusion of **trileucine**
    - Inclusion of **trileucine helped prevent particle fusing** (likely due to high glass transition temperature and contribution to rugose morphology [5]) – fusing was observed in control sample after one year's storage at 40 °C
  - **Interior voids** indicating **encapsulation of the nanoemulsion droplets** [2] were **maintained in all samples**
- **Powder moisture content** remained **consistently low** over two years of storage for **all samples**
  - **Packaging method** provided **excellent protection** against **moisture exposure**
- **Raman spectroscopy** indicated **no detectable phase change** in any of the three samples after two years of storage
  - Flat residual obtained for all three samples
- **Aerosol performance** for **all three samples remained consistent** throughout stability study
- **Lead formulation** showed **better aerosol performance** over the control formulation from **inclusion of trileucine** [2]
  - **Lead formulation** had **total lung dose of 37±2%** after 2 years of storage at 25 °C
  - **Control formulation** had total **lung dose of 17±4%** after 2 years of storage at 25 °C
- **Chemical stability analysis** performed after 2 years of storage on:
  - **Lead formulation** stored at -20, 4, 25, 40, and 50 °C
  - **Control formulation** stored at 4, 25, and 40 °C
- Consistent nanoemulsion droplet size for samples:
  - Below 4 °C in lead formulation
  - Below 40 °C in control formulation
- **GLA content was maintained** for all samples stored at **25 °C and below** (Figure 7A)
- **Squalene content was maintained** for all samples stored at **25 °C and below** (Figure 7B)
- **ID93 content** preservation was enhanced by **spray drying** and further aided by the **inclusion of trileucine**
  - Liquid presentation lost all ID93 content after 1 month at 37 °C [3]
  - **Control formulation** retained 38% of ID93 content after 3 months of storage at 40 °C [2]
  - **Lead formulation retained ~45% ID93 content** after **12 months of storage at 50 °C** [2]

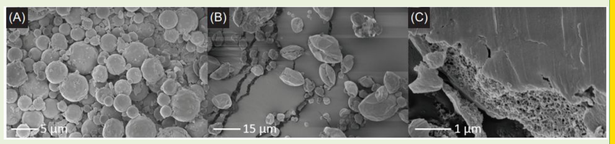

Figure 4. *Morphology of (A) control sample stored at 25 °C for two years; (B) lead candidate stored at 40 °C for two years; (C) interior structure of lead candidate stored at 40 °C for two years.*

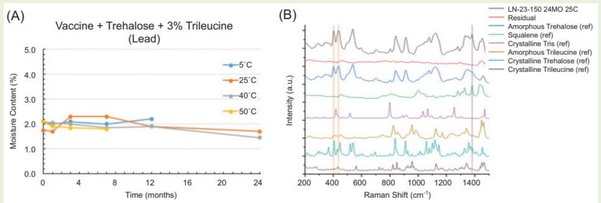

Figure 5. *(A) Moisture content for lead candidate over two years of storage and (B) Raman spectra for lead candidate stored at 25 °C for two years.*

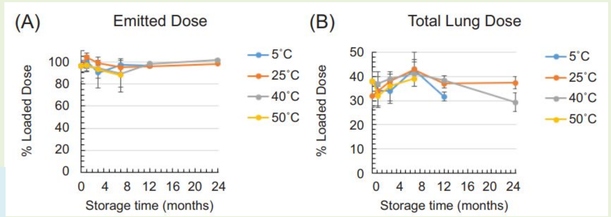

Figure 6. *(A) Emitted dose and (B) total lung dose for lead candidate over two years of storage.*

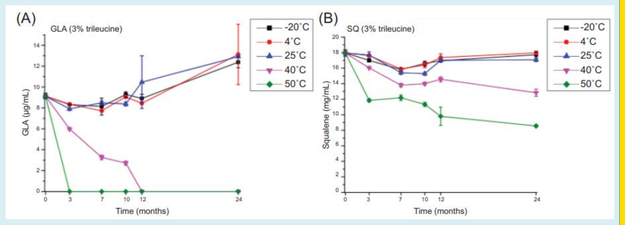

Figure 7. *(A) GLA content and (B) squalene content for lead candidate over two years of storage.*

Conclusions

- **Long-term physical and chemical storage stability** of **adjuvanted subunit vaccines** can be achieved by **low-temperature spray drying** with **glass stabilizers as excipients**
- Addition of **trileucine** to **increase the dispersibility** of the respirable vaccine particles contributed to **improved stability of the antigen**
- **Storage stability** under moisture protection was much improved relative to liquid presentation
- **Powder is resilient to higher temperatures** that might be encountered on transport or administration

References

[1] Aguilo N, Alvarez-Arguedas S, Uranga S, Marinova D, Monzón M, Badiola J *et al.*: Pulmonary but not subcutaneous delivery of BCG vaccine confers protection to tuberculosis-susceptible mice by an interleukin 17-dependent mechanism. *The Journal of Infectious Diseases* 2016,213(5): 831-839.
[2] Gomez M, McCollum J, Wang H, Bachchhav S, Tetreau I, Gerhardt A, Press C, Kramer RM, Fox CB, Vehring R: Evaluation of the stability of a spray-dried tuberculosis vaccine candidate designed for dry powder respiratory delivery. *Vaccine* 2021, 39(35): 5025-5236.
[3] Gomez M, Archer M, Barona D, Wang H, Ordoubadi M, Bin Karim S, Carrigy NB, Wang Z, McCollum J, Press C, Gerhardt A, Fox CB, Kramer RM, Verhing R: Microparticle encapsulation of a tuberculosis subunit vaccine candidate containing a nanoemulsion adjuvant via spray drying. *European Journal of Pharmaceutics and Biopharmaceutics* 2021,162: 23-37.
[4] United States Pharmacopeia. In <601> Aerosols, nasal sprays, metered-dose inhalers, and dry powder inhalers. Rockville, MD: United States Pharmacopeial Convention; 2017.
[5] Lechuga-Ballesteros D, Charan C, Stults CLM, Stevenson CL, Miller DP, Vehring R, Tep V, Kuo M. Trileucine improves aerosol performance and stability of spray-dried powders for inhalation. *Journal of Pharmaceutical Sciences* 2008, 97(1): 287-302.

Acknowledgements

This study was funded by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN272201400041C. The authors would also like to thank Conor Ruzycki for designing the custom mouthpiece adaptor used in aerosol performance testing.

Respiratory Drug Delivery 2022, Florida, USA