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. Furthermore, there is evidence that direct delivery of vaccines to the lungs improves the protective immune response for respiratory illnesses [1]. Two thermostable and inhalable tuberculosis (TB) adjuvanted subunit vaccine candidates were spray dried to address these needs [2]. This study evaluated the physical and chemical stability over two years of storage at room temperature for these two spray-dried inhalable TB vaccine candidates: a control candidate using trehalose to achieve glass stabilization of a nano-emulsion adjuvant system (GLA-SE) composed of a synthetic Toll-like receptor 4 agonist called glucopyranosyl lipid A (GLA) and a squalene oil-in-water stable emulsion (SE) [3] and a recombinant fusion protein (ID93) comprised of four *Mycobacterium tuberculosis* antigens: Rv3619, Rv1813, Rv3620, and Rv2608 [3], and a lead candidate, with 3% trileucine added as a dispersibility and stability enhancer [2].

METHODS

Two inhalable TB vaccine candidate powders were designed and manufactured using a custom research spray dryer. Low-temperature spray drying was used to minimize processing losses. An inlet temperature of 65 °C, a drying gas flow rate of 200 SLPM, and a feedstock flow rate of 0.6 mL/min were used, producing a 36 °C outlet temperature and 7% outlet relative humidity (RH). The feed was prepared by dissolving trehalose, Tris, and trileucine in deionized water, adjusting the solution to a pH of 7.5 using hydrochloric acid, syringe-filtering the solutions through a 0.22 μm pore size sterile filter, adding GLA-SE and gently stirring, and finally adding ID93 [2]. These powders for stability study were prepared using an established packaging protocol, where the powders were aliquoted into low bind snap cap tubes and sealed in inner aluminum bags containing desiccant pouches equilibrated to the outlet relative humidity (RH) of the spray dryer. The inner aluminum bags were sealed inside of outer aluminum bags containing desiccant pouches equilibrated to 0% RH [3]. This maintained the initial powder moisture content during long-term storage at different temperatures at -20, 5, 25, 40, and 50 °C. The powders were assessed initially and at 1-month, 3-month, 7-month, 12-month, and 24-month timepoints [3].

Physical stability was assessed by monitoring particle morphology, moisture content, solid phase, and aerosol performance over time. Interior and exterior morphology was examined via field emission scanning electron microscopy. Particles were manually scraped against a substrate to crack open particles and view their interior structure. Moisture content was measured by Karl Fisher calorimetry. Solid phase was assessed via Macro-Raman spectroscopy using a custom instrument [3] and by deconvolution

using reference spectra obtained for Tris buffer, squalene oil, and amorphous and crystalline trehalose and trileucine [2]. As per the USP 601 standard [4], aerosol performance was characterized by actuating a commercial dry powder inhaler (DPI, Seebri Breezhaler®, Novartis) loaded with ~40 mg of powder into an Alberta Idealized Throat (AIT) connected to a Next Generation Impactor (NGI) at a simulated square inhalation profile at 100 L/min for 2.4 s [2].

Chemical stability was assessed by monitoring visual appearance, pH, nanoemulsion droplet size distribution, squalene content, GLA content, and ID93 content during storage. Nanoemulsion droplet size, including its mean hydrodynamic diameter and polydispersity index, was measured using dynamic light scattering (DLS) after the powder was reconstituted to its feedstock concentration. Squalene and GLA content were monitored using reversed phase high-performance liquid chromatography (HPLC), and antigen content was assessed using densitometry analysis of reducing SDS-PAGE based on a standard curve. For these assessments, the powders were reconstituted to threefold feedstock concentration for content assays, and to feedstock concentration for DLS and all other measurements.

RESULTS AND DISCUSSION

The lead formulation was assessed for physical stability after storage at 40 °C and 25 °C for 2 years, and the control formulation was assessed for physical stability after storage at 25 °C for 2 years. All three samples were physically stable after 2 years of storage at these temperatures. The particle morphology remained for all samples. Figure 1a shows the control sample stored at 25 °C for 2 years, which still consists of round, separate particles with no indication of fusing. The control sample also maintained its interior voids,

which indicate encapsulation of the nanoemulsion droplets [2]. Figures 1b and 1c show the lead sample stored at 40 °C for 2 years. Figure 1b demonstrates that the outer morphology is still folded and rugose, due to the inclusion of trileucine, and that the particles are still separate with no evidence of bridging. The inclusion of trileucine helped prevent high-temperature particle fusing, likely due to trileucine's high glass transition temperature and its contribution to rugose particle morphology and low cohesivity [5], as bridging occurred in small particles in the control sample held at 40 °C after 1 year [2]. Figure 1c shows that the interior voids were also maintained.

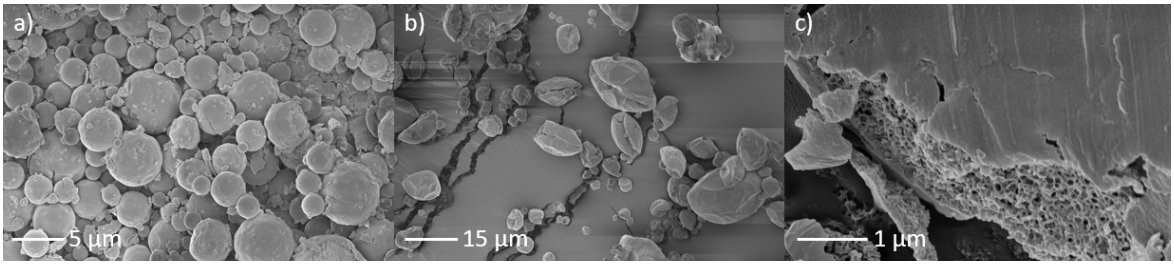

Figure 1. Morphology of a) control sample stored at 25 °C for 2 years, b) lead candidate stored at 40 °C for 2 years, c) interior structure of lead candidate stored at 40 °C for 2 years.

The powder moisture content remained consistently low over the course of the study for all samples (Figure 2a), indicating that the packaging method used provided excellent protection against moisture exposure. Raman spectroscopy indicated no detectable phase change in any of the three samples after 2 years of storage; a flat residual was obtained for all three samples. Fluorescence was detected in the lead candidate stored at 40 °C for 2 years, indicating some chemical degradation had occurred. The spectra used for the lead candidate stored at 25 °C are provided as an example in Figure 2b.

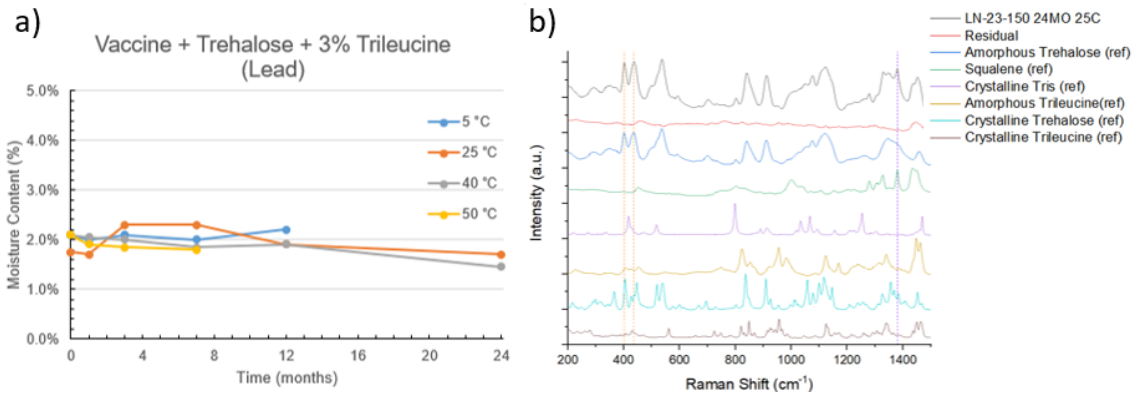

Figure 2. a) Moisture content for lead candidate over 2 years storage and b) raman spectra for lead candidate stored at 25 °C for 2 years.

The aerosol performance of all three samples remained consistent throughout the stability study. The lead formulation benefitted in terms of aerosol performance from the inclusion of trileucine over the control formulation [2]. After 2 years of storage at 25 °C, the lead formulation achieved a total lung dose of 37±2% compared to the control formulation's 17±4%. The emitted dose and total lung dose are shown for the lead formulation in Figures 3a and 3b respectively.

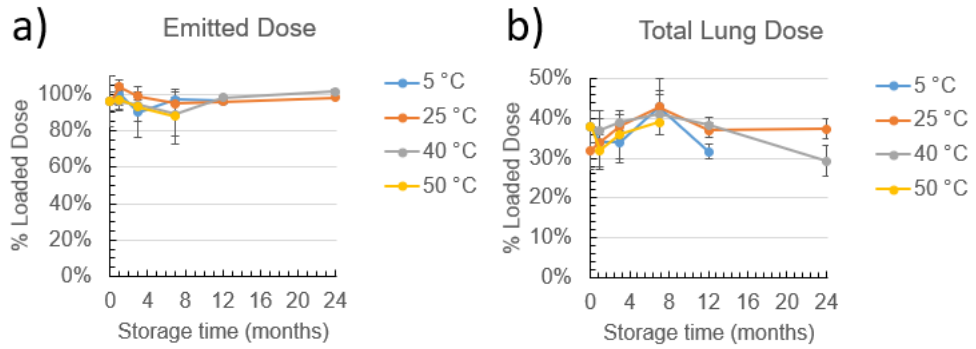

Figure 3. a) Emitted dose and b) total lung dose for lead candidate over 2 years storage

Chemical stability analysis was performed on the lead formulation after storage for 2 years at -20, 4, 25, 40, and 50 °C, and on the control formulation after storage for 2 years

at 4, 25, and 40 °C. Nanoemulsion droplet size remained consistent for samples held below 40 °C in the control formulation and for samples held below 4 °C in the lead formulation. GLA content, shown for the lead formulation in Figure 4a, was maintained for all samples stored at 25 °C and below. Squalene content, shown for the lead formulation in Figure 4b, was also maintained for samples held at 25 °C and below. While trehalose-only samples lost all ID93 content after two years storage at all tested temperature conditions, samples containing trileucine maintained substantial ID93 content even after storage at the higher temperatures. Approximately 45% of the initial ID93 content was present in this formulation after 12 months of storage at 50 °C [2], contrasting the complete loss of ID93 content in the liquid presentation after only one month of storage at 37 °C [3].

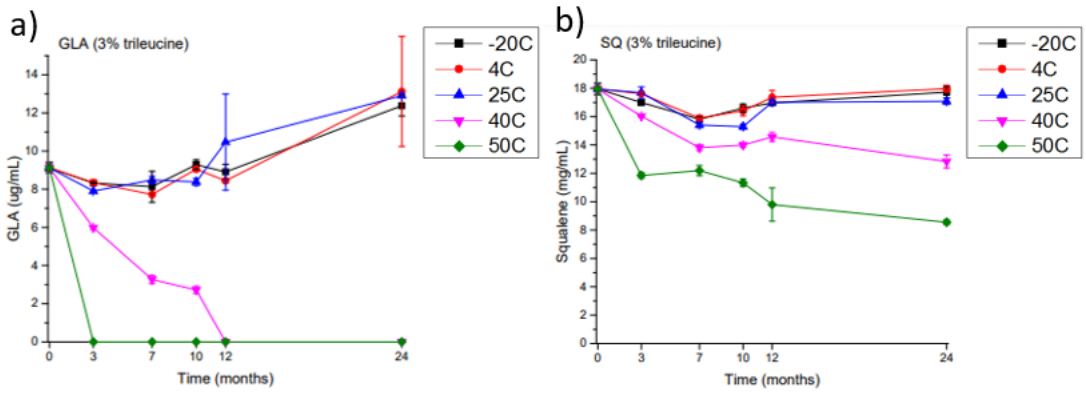

Figure 4. a) GLA content and b) squalene content for lead candidate over 2 years storage.

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 respirable vaccine particles contributed to

improved stability of the antigen. Storage under moisture protection at room temperature appears feasible and the powder is resilient to higher temperatures that might be. encountered on transport.

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