

Evaluating the *In Vitro* Regional Deposition Characteristics and Stability of a Spray Dried Tuberculosis Vaccine Candidate

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Summary

This work investigates the physical stability of an inhalable tuberculosis vaccine stored in hypromellose capsules for use in a capsule-based dry powder inhaler. The primary measure of stability was consistent regional deposition as predicted by an *in vitro* methodology; secondary measures included particle morphology assessed by scanning electron microscopy and solid phase analysis by Raman spectroscopy. The biological stability of the antigen-adjuvant system in powder form was demonstrated previously.

Regional deposition in the *in vitro* analogues of the upper respiratory tract, tracheobronchial, and alveolar regions remained consistent over nine months of storage at room temperature. Differences in regional deposition between the vaccine powder vs. a similarly manufactured vehicle powder were noted, highlighting the importance of device/formulation interactions. No morphological changes were observed over time indicating that storage of the vaccine powder within the hypromellose capsule did not compromise its previously demonstrated physical stability. This sustained performance supports the use of capsules filled with dry powder vaccines for global vaccination campaigns.

Key Message

Aerosol performance (measured via *in vitro* extrathoracic and regional lung deposition) of a spray-dried, inhalable vaccine was retained over nine months of storage in hypromellose capsules used in a capsule-based dry powder inhaler.

Introduction

Many vaccines rely on maintenance of a “cold chain” during transportation and storage. Equitable vaccine distribution could be improved globally by minimizing reliance on cold chains through the development of thermostable vaccines. Thermostable dry powder vaccines have the added benefit in that they may be formulated for delivery directly to the respiratory system with a suitable inhaler.

We have previously spray dried an experimental tuberculosis vaccine into an inhalable powder using a trehalose-trehalose excipient system [1]. Additional work has established that the vaccine exhibits excellent room temperature stability when stored as a bulk powder [2, 3]. Pre-filling dry powder inhaler (DPI) capsules prior to storage would take advantage of existing manufacturing capacity and allow for efficient vaccine rollout in the event of a pandemic. This work aims to evaluate the aerosol performance of the dry powder vaccine when stored within a hypromellose capsule compatible with existing DPIs. Various storage temperatures were evaluated for up to 9 months of storage.

Materials and Experimental Methods

Materials and formulation procedure: Trehalose dihydrate with a purity of 98% was used as the stabilizing excipient for spray drying. Tris(hydroxymethyl)aminomethane (tris) and hydrochloric acid (HCl) were used as a buffer system. ≥90% purity trehalose was added as a dispersibility enhancer. The antigen (ID93) and adjuvant (glucopyranosyl lipid adjuvant formulated in a stable emulsion [GLA-SE]) systems were provided by AAHI. To prepare the feedstock for the vehicle powder, trehalose, trehalose, and tris were dissolved in HPLC grade water on a stir plate at ~500 RPM overnight. The solution was pH-adjusted to pH 7.5 using 10% HCl, as monitored with a pH meter. The solution was then filtered with a 0.45 µm filter. The same procedure was repeated for the vaccine feedstock, with the addition of the antigen and adjuvant system after filtration. The GLA-SE was added and mixed, followed by the addition of ID93. The final solution was volume adjusted with HPLC water and mixed on a stir plate at ~500 RPM for about 30 minutes prior to spray drying. The concentrations of the components for the vaccine formulation are described in Gomez *et al.* [3]; the vehicle powder has identical composition except that the antigen and adjuvant system are omitted.

Spray drying: Spray drying was conducted using a custom research spray dryer with a twin fluid atomizer and a 0.3 mm nozzle. The vaccine and vehicle powders were dried at a drying gas flow rate of 400 SLPM, an atomizing gas pressure of 414 kPa, and a liquid feed rate of 1.0 mL/min. The inlet and outlet temperatures were 65 and 42°C, respectively, with a predicted outlet RH of 4.3%.

In vitro regional lung deposition: Combined powder-inhaler performance was characterized through measurements of powder deposition in *in vitro* analogues of the upper respiratory tract, tracheobronchial region, and alveolar region using a recently developed filter-based approach previously described by Tavernini et al. [4, 5]. A high resistance RS01 inhaler (Mod. 7 Size 3, Berry Global, Osnago, Italy) was selected as the single-dose DPI for this work as it is a low-cost option, is readily available, and there are no commercial restraints on using this device to deliver the vaccine. Air was drawn through the system using a breath simulator programmed to produce semi-realistic inhalation profiles, as proposed by Delvadia et al. [6]. The expected 50th percentile peak inspiratory flow rate (PIFR) for patients who have received instruction on inhaler use was used (76 L/min). The inhaled volume and time to PIFR were 2.7 L and 0.49 s, respectively. Three replicate measurements were performed for each timepoint and storage condition. The dose on each collection surface was determined by dissolving and collecting the powder in an 80% water, 20% methanol (v/v) solvent and then determining the trehalose concentration by liquid chromatography with tandem mass spectrometry (LC-MS/MS).

Stability study: Hypromellose capsules (Vcaps Plus Size 3, Capsugel, New Jersey, US) were manually filled with approximately 30 mg of powder (30.4 ± 1.3 mg, $n=89$); four to six capsules and a desiccant pouch were then placed within an aluminum bag which was heat sealed. To limit the chance of moisture ingress due to sealing failures, this bag was then placed within another aluminum bag with another desiccant pouch and heat sealed. Unique double-sealed packages were made for each storage condition and timepoint. The packaged product was then stored at 4°C, 25°C, or 40°C for up to six months. After six months, two sets of capsules which were subjected to two weeks of storage at 40°C and 50°C (after storage at 4°C) to evaluate robustness of aerosol performance. At the end of the planned stability study (6 months) all samples were stored at room temperature indefinitely.

Scanning electron microscopy: Micrographs of particles were taken using Field Emission Scanning Electron Microscopy (Zeiss Sigma FE-SEM; Carl Zeiss, Oberkochen, Germany). Samples were first sputtered with a coating of 80% gold/20% palladium (Leica ACE600 Carbon/Metal Coater; Concord, ON, Canada) to a thickness of 10 nm or with a gold coating (Denton Vacuum Desk II Sputter Coater; Denton, Moorestown, NJ, USA) to a thickness of ~16 nm.

Moisture content: Water acts as a plasticizer in trehalose-based formulations. Increase in water content may lead to reduced physicochemical stability of the powders. Water content of the powders over the course of the stability study was measured through Karl Fischer Titration (Karl Fischer Coulometric Titrator Model C30; Mettler Toledo; Mississauga, ON, Canada). Oven temperature was 110 °C, and results were given as a wet basis percentage based on the measured sample mass. Experiments were completed in triplicate.

Raman spectroscopy: Raman spectroscopy was used to verify the solid phase of the powder at the beginning and end of the stability study. Samples were measured at an RH of $\leq 5\%$ to minimize moisture uptake during the sampling process. Raman spectra analysis was completed on a liquid sample of squalene and spray-dried trehalose as a reference amorphous sample. The spectrum obtained after six months of storage at 25 °C was subtracted from the timepoint zero sample to determine if any changes could be detected in the difference spectrum. Powders where amorphous glass stabilizers are used should be monitored to ensure that crystallization does not occur over the intended shelf life of the product.

Results and Discussion

Spray drying the vaccine powder produced a yield of 81% compared to 87% when spray drying the vehicle powder. The two formulations produced particles with similar morphology, where inclusion of 1.3 mg/ml of trileucine in the feedstock led to a rugose surface (Figure 1a, 1b). However, the degree of rugosity was impacted by inclusion of vaccine components as the particles of the vaccine powder show less folding at the surface.

The vehicle and vaccine powders also showed differences in regional deposition when delivered with the RS01 DPI (Figure 1c). The vehicle powder showed significantly less deposition in the mouth-throat and higher deposition in the alveolar region as compared to the vaccine powder ($p<0.01$, Student's t-

test). The two powders were manufactured under identical conditions and were expected to have similar primary particle size distributions so were expected to have similar regional deposition profiles. The cause for this difference is not clear but highlights the importance of device-formulation interactions and the need to characterize expected regional deposition with the device one intends to use in clinical trials.

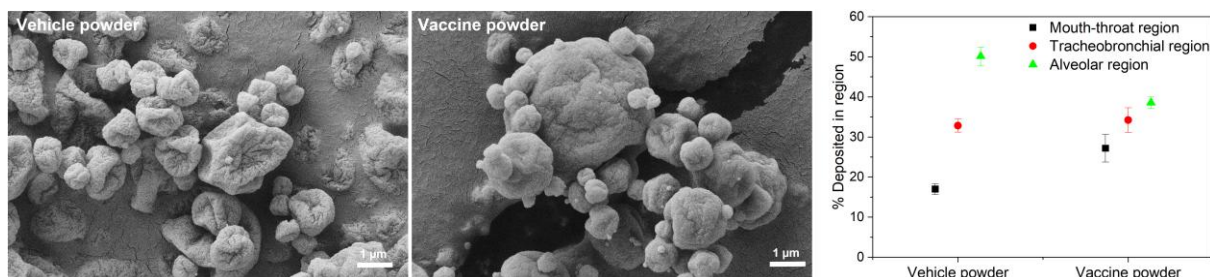


Figure 1: Comparison of particle morphology of the a) vehicle powder, b) vaccine powder and c) the regional deposition of the powders. Inclusion of vaccine components influences exterior morphology, with the vaccine powder exhibiting reduced rugosity and folding as compared to the vehicle powder. Scale bar, as given in figure, applies to both micrographs.

The outer morphology of the vaccine powder particles was maintained after both nine months of room temperature storage and six months of 40 °C followed by three months of room temperature storage within hypromellose capsules (Figure 2). Particles maintained their wrinkled appearance and there were no signs of particle fusing. The interior particle structure was also maintained over both storage conditions, with intentionally cracked particles showing the presence of voids left by the nanoemulsion droplets.

Similarly, Raman spectroscopy determined that the solid phase of the powder remained amorphous, i.e., did not change over storage. The moisture content of the powder by wet basis after manufacture was measured at $2.01 \pm 0.02\%$. This is very similar to previous batches (1.8-2.1% moisture content) [3]. After six months of storage at room temperature within the RS01 capsules, the moisture content of the powders fell to $0.33 \pm 0.07\%$, likely due to moisture scavenging by the desiccant packets.

Particle morphology and solid phase were not significantly affected by powder storage within RS01 capsules for the investigated conditions. The protective packaging method prevented moisture uptake, however, direct storage of the capsules next to desiccant led to drying out of the powder.

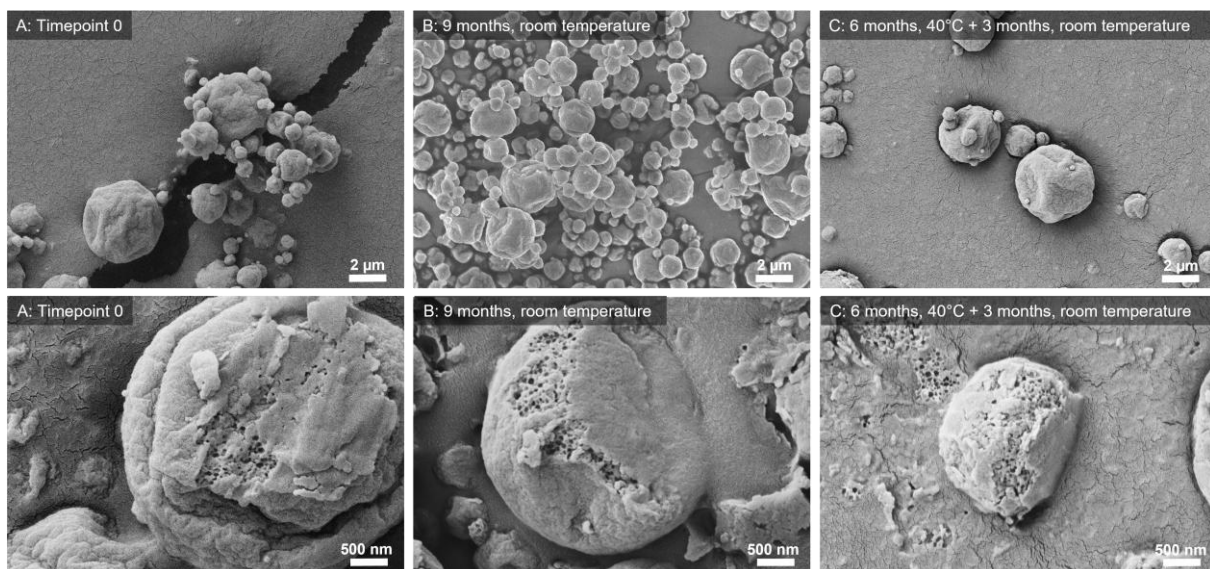


Figure 2: SEM images of the spray-dried vaccine at time point zero, after 9 months of storage at room temperature, and after 6 months of storage at 40°C plus 3 months of storage at room temperature. Powders were stored in RS01 inhaler capsules. Images depict the exterior (top) and interior (bottom) particle morphology. A: Timepoint 0; B: 9 months, room temperature; C: 6 months, 40°C + 3 months, room temperature.

All timepoints and storage conditions showed a consistent and relatively high deposition ($\geq 35\%$) in the alveolar region (Figure 3); similarly, a high lung dose ($>55\%$) was maintained. A departure from the 'standard' regional deposition pattern was noted at the six-month timepoint wherein mouth-throat deposition was increased and tracheobronchial deposition was concomitantly reduced. The regional deposition returned to the previously measured pattern in subsequent measurements, indicating the

departure was not due to instability in the formulation. During the 6-month timepoint, the relative humidity in the laboratory was notably low (6.1 to 9.1%). Such dry air conditions are known to reduce the ability of powders to dissipate static charge, which may have influenced powder dispersibility. While the ambient relative humidity was also low at the nine-month time point (11.6 to 12.2 %), the effect was not repeated, perhaps not surprisingly as electrostatic effects are notoriously variable.

The high alveolar and tracheobronchial doses were also retained after six months of refrigerated storage plus two weeks of storage at 40°C or 50°C indicating that the powder is robust enough to withstand extreme temperature excursions that may be encountered during the last leg of distribution in regions where the cold chain cannot be maintained.

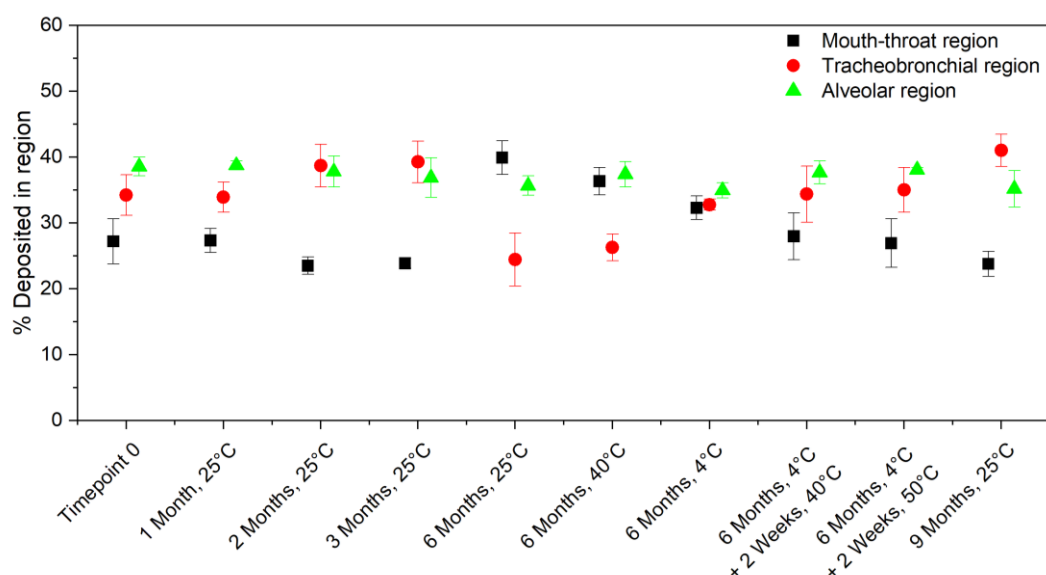


Figure 3: Regional deposition of vaccine powder delivered from the high resistance RS01 DPI after storage in hypromellose capsules over the course of the stability study

Conclusion

Particle engineering was used to prepare a dry powder tuberculosis vaccine that maintains robust aerosol performance when stored within capsules under variable temperatures. High lung deposition was demonstrated and maintained after nine months of storage at room temperature indicating this formulation may be well suited for global vaccination campaigns. The noted difference in regional deposition for the vaccine powder vs. a similarly manufactured vehicle powder highlights the need to consider device-formulation interactions early in the product development lifecycle.

Spray drying may be used as a method to create inhalable vaccines on the World Health Organization's list of vaccine priorities for the controlled temperature chain [7]. Furthermore, direct storage of the vaccine within inhaler capsules could allow for fast vaccine rollout with low storage-costs.

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