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Stabilizing formulations for inhalable powders of an adenovirus 35-vectored tuberculosis (TB) vaccine (AERAS-402)

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ABSTRACT

A powder vaccine intended for aerosol delivery was formulated by spray drying the Ad35-vectored tuberculosis (TB) AERAS-402 vaccine with mannitol-based stabilizers. Thermodynamic properties, water absorption, particle size distribution and morphology of the powders were evaluated. Virus survival during spray drying and storage was determined by medium Tissue Culture Infectious Dose ($TCID_{50}$). A mannitol-based powder (mannitol–cyclodextrin–trehalose–dextran, MCTD) had a narrow size distribution with a median volume diameter around 3.2–3.5 μm (suitable for pulmonary vaccination of humans) and good aerosolization characteristics. The spray dry powders generated from mannitol-based formulations were hydrophobic, which benefits virus survival during both production and storage at 4 °C, 25 °C and 37 °C as compared to the hygroscopic formulations (trehalose, sucrose, dextran, PVP, leucine). In conclusion, this study demonstrates that it is possible to produce in a one-step spray drying process a stable dry powder formulation of a TB vaccine suitable for mass vaccination.

1. Introduction

The Bacille Calmette-Guérin (BCG) vaccine, created in 1921, is the only approved vaccine against tuberculosis (TB) [1]. Unfortunately, it is only partially effective. It provides some protection against severe forms of pediatric TB, but is unreliable against pediatric and adult pulmonary TB, which accounts for most of the disease burden worldwide [2]. Treating TB is challenging, even in developed countries where there is a modern health care system and infrastructure. Current treatment regimens last 6–9 months, and often result in erratic or inconsistent treatment which breeds multidrug-resistant and even extensively drug-resistant TB, which means that this pandemic could become even more difficult to control throughout the world [3]. Although BCG is the most widely administered vaccine in the world, there have never been as many cases of TB as there are now. Globally, 9.2 million new cases and 1.7 million deaths from TB occurred in 2006, of which 0.7 million cases and 0.2 million deaths were in HIV-positive individuals [4]. There is therefore an urgent need for new, safe and effective vaccine that would prevent all forms of TB, including the drug-resistant strains, in all age groups and among people with HIV.

BCG vaccination, boosted with an adenoviral-delivered boost, represents a reasonable strategy to augment, broaden, and prolong immune protection against tuberculosis (TB) [5–10]. *r*Ad35 was chosen due to the prevalence of Ad5-specific immune responses in Africa [11–13], where the TB burden is high and novel vaccination strategies are needed. For example, the seroprevalence of adenovirus 5 (Ad5) in sub-Saharan Africa patients infected with HIV-1 is 90% compared to 20% for anti-Ad35 reactivity with less then 2% of individuals having titers >200 [12]. Recombinant adenovirus 35 (Ad35) AERAS-402 has been shown to induce high levels of $CD4^+$ and $CD8^+$ specific T cells in mice and in non-human primates following recombinant BCG priming as measured by intracellular staining (ICS) after in vitro short stimulation with peptides pools Ag85A/B (composed of Ag85A plus peptide regions of Ag85B that are not shared with Ag85A) and TB10.4 [9,10]. In humans that have been primed with BCG, AERAS-402 has also induced high levels of antigen specific polyfunctional CD4 T cells and INFγ secreting antigen CD8+ T cells [14].

Since *M. tuberculosis* infection occurs in the lung, a critical issue is whether vaccines can be administered in different ways to induce high frequencies of T cells in the lung that will prevent or ameliorate TB infection. One such approach is to deliver the vaccine directly to the lung. This was achieved with AERAS-402 by creating a 2–4 micro mist with a Pari nebulizer used in children with cystic fibrosis to deliver enzymes to the deep lung. Following aerosol administration of AERAS-402 in this manner to non-human primates high levels of Ag85A/B-specific $CD4^+$ and $CD8^+$ responses in the bronchial alveolar lavage (BAL) following immunization at weeks zero and eight were detected in the group receiving 10^{10} virus particles, as compared to the control group of unvaccinated

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animals, animals receiving BCG or animals receiving AERAS-402 parenterally. It was also shown that the pre-existing anti-vector immunity did not significantly affect the strong immune response inducted by the pulmonary-delivered vaccine (unpublished). These preliminary findings encouraged us to explore production of a dry powder of AERAS-402 for aerosol delivery which would be practical and economically feasible for use in the developing world.

The impelling interest in pharmaceutical dry powder aerosols in recent years is driven by the urge to find a suitable replacement for the chlorofluorocarbons (CFC) propellant used for liquid aerosols, as well as recognition of the lung's capacity to deliver drugs systemically [15]. Additionally, because of emerging technologies capable of permitting production of stable powders of respirable size, as well as devices competent to deliver flexible and accurate dosing, dry powder aerosols are no longer perceived as the "second choice" after the propellant driven liquid aerosols for inhaled drugs and vaccines [15–18].

To advance powder aerosol technologies, scientists and manufacturers have recognized the importance of understanding the determinants affecting powder dispersion. The influences of particle surface characteristics, environmental conditions, air flow rate, inhaler resistance, and excipients on aerosol generation are some of the fundamental areas that have been under continuous investigation [19–21].

Spray drying transforms a liquid into dry powder particles by nebulization of droplets in hot drying air. It has been recommended as an alternative to freeze drying for the preparation of inhalation products, as it represents an elegant one-step process for producing biopharmaceutical formulations with unique particle characteristics. Spray drying has the additional advantage of being a faster and more cost-effective dehydration process than freeze drying [22–25]. Over the past decade, numerous protein delivery technologies have emerged, of which several are powder-based methods – such as microspheres for long-acting delivery, fine powders for pulmonary delivery, and biopharmaceutical/vaccine powders for intradermal delivery [15,26–33]. With the advent of these technologies, efforts to identify appropriate powder formation methods are increasing. In this paper, the properties of AERAS-402 vaccine in dry powder forms were investigated and a spray-dried formulation was discovered with remarkable room temperature stability and the characteristics required for easy delivery to the deep lung parenchyma.

2. Materials and methods

2.1. Chemicals

Leucine was bought from Spectrum, Gardena, CA; mannitol, sucrose, histidine, trehalose were from J.T. Baker, Phillipsburg, NJ; dextran (MW 60,000–90,000) was from MP Biomedicals, Solon, OH; β-cyclodextrin was from TCI-GR, Kita-Ku, Tokyo, Japan; polyvinylpyrrolidone (PVP, MW 8000, K16-18) was from ACROS, NJ; and inositol was bought from EMD, Gibbstown, NJ.

2.2. CPS Disc centrifuge

The size of AERAS-402 vaccine virus was measured by CPS Disc centrifuge (CPS Instruments, Inc., Stuart, FL). Sucrose (8% and 24%) in sample buffer was used for gradient solutions. CPSV95 software was set up for data collection, analysis and process control. The maximum speed was selected at 24,000 rpm. The total injection volume for each analysis was 100 μL. A solution of PVC (20%, v/v) 0.377 Micro Calibration Standard was used for calibration.

2.3. Spray drying

The spray drying powders were generated by a Büchi Mini Spray Dryer B-290. Nitrogen was used as drying and atomizing gas. Ten different feed solutions were prepared: 15% mannitol (Man); 10% trehalose (Tre); 0.5% leucine (Leu); 15% mannitol mixed with 0.9% leucine (ManLeu) or 0.1% sucrose (ManSuc); 10% mannitol with 0.5% PVP (ManPVP) or inositol (ManIno); 10% mannitol in PBS buffer (ManPBS); 10% mannitol, 0.02% cyclodextrin, 5 mM histidine, 0.2% trehalose and 0.1% dextran (MCTD), and 10% mannitol, 0.2% trehalose, 1 mM histidine, 0.1% dextran and 0.01% tween 80 (MTDT). Formulations with the same concentrations without AERAS-402 were used for placebo tests. The inlet temperature was set at 65–125 °C and the drying gas flow rate at 439–538 L/h resulting in an outlet temperature of 34–50 °C. The aspirator rate was 35 m^3/h. The spray drying process and subsequent powder aliquoting were executed in a BioProtect II hood (The Baker Co.). To minimize both environmental microbial contamination to the powder and small powder particles released to environment, the spray dryer was assembled with a PTFE outlet filter and a 0.2-μm EMFLON Filter (Pall Life Sciences, USA) fitted to the compressed air line.Differential scanning calorimetry (DSC)

The thermodynamic behavior of the powders was determined on a DSC 823^e (METTLER TOLEDO, Switzerland). The cover of the crucible containing the powder sample was punched with a small hole before analysis. The sample (around 10 mg powder) was heated from 25 °C to 170 °C with a scanning rate of 10.0 °C/min. The sample cell was purged with a nitrogen gas of 10.0 mL/min. The glass transition temperature (T_g) was recognized on the reversing heat flow curve as an endothermic shift of the baseline and determined as the midpoint of this transition by a STAR^{e}SW9.01 software (METTLER).

2.5. Particle size distribution, polydispersity and morphology

Particle size distributions were measured by laser diffraction (Mastersizer 2000, Malvern, Worcs, UK). The polydispersity of the powder was expressed by the span. Span = $[D(v, 90) - D(v, 10)]/D(v, 50)$. The particle size of the primary powders was described by the volume median diameter (VMD), which is related to the mass median diameter (MMD) by the density of the particles (assuming a size independent density for the particles). A microscope (Axioskop 40, ZEISS) was used to examine particle morphology of spray-dried powders. The mannitol powders were re-suspended in anhydrous methanol at around 20 mg/mL. A drop of this suspension was placed on a clean microscope slide. After 2 min, the slide was examined with the oil immersion objective (100×) and a 10× ocular. Re-suspension of trehalose powder in methanol was found to be impractical because of high solubility. For trehalose, the method of Tracy et al. [49] was modified as follows: about 20 mg of powder was mixed with 1 mL of Halocarbon 0.8 oil, and a drop of the suspension was examined with a cover slip.

2.6. Adenovirus 35 and $TCID_{50}$ assay

Recombinant adenovirus 35 expressing TB antigens Ag85A, Ag85B and TB10.4 was manufactured by Crucell (Leiden, the Netherlands) using PER.C6 cell line. Ad35 virus titers in the original feed solutions and in the corresponding powders were determined by titration in $TCID_{50}$ tests. The TCID titer value (tissue culture infectious dose) was determined by the greatest dilution at which cytopathic effects (CPE) were observed on human embryonic retinoblast cells (HER: 911 cells) in a $TCID_{50}$ assay. The procedure was adopted from Crucell, in collaboration with Aeras Global TB Vaccine foundation, Rockville, MD. Briefly, the 911 cells were

cultured in a 75 cm^2 flask containing Dulbecco's modified Eagle medium supplemented with10% fetal bovine serum and antibiotics (penicillin and streptomycin). When cells were confluent, cells were detached using Trypsin-EDTA solution and the cell concentration was adjusted to 4×10^5 cells/mL. Cell suspension (100 μL) was seeded in one or two 96-well flat bottom tissue culture plates and incubated at 37 °C with 10% CO_2 for 4 h. After cells were attached the media was removed and 160 μL of medium was dispensed into all the wells. Then 40 μL each of pre-diluted virus was added to 8 wells in the first column and subsequently 5-fold serial dilution was performed in the plates for dilutions ranging from 10^{-1} to 10^{-11} or 10^{-7} to 10^{-17}, depending on the expected titer value, and the plates were incubated for 14 days. CPE was scored on day 14 and the virus titer was determined employing the Spearman–Karber formula as follows.

$$\frac{\log \mathrm{TCID}_{50}}{100\,\mathrm{mL}} = X_0 - \left(\frac{d}{2}\right) + \left(\frac{d}{n}\right)\sum_{Xi}$$

where 'X_0' is the $\log_{10}$ of the reciprocal of the highest dilution at which all testing columns are CPE positive, 'd' represent the $\log_{10}$ value of the dilution factor ($d = 0.699$ for 5-fold dilution factor) and 'n' is the number of wells for each dilution. '$\sum Xi$' is the sum of all wells that give CPE, from the dilution 'X_0', including CPE of dilution

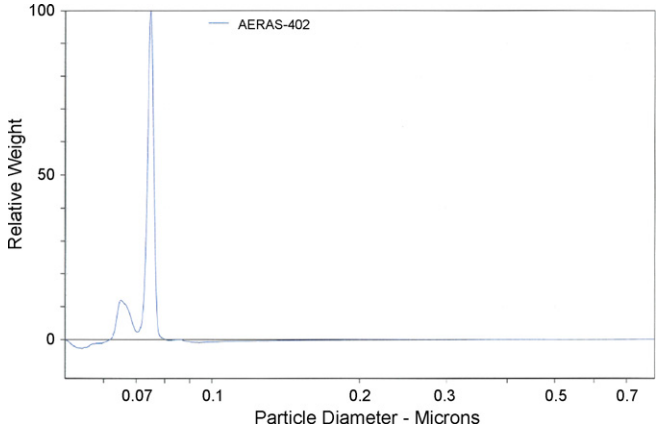

Fig. 1. The virus particle size distribution of AERAS-402. The size of AERAS-402 vaccine virus was measured by CPS Disc centrifuge. 8% and 24% sucrose in sample buffer were used for gradient solutions.

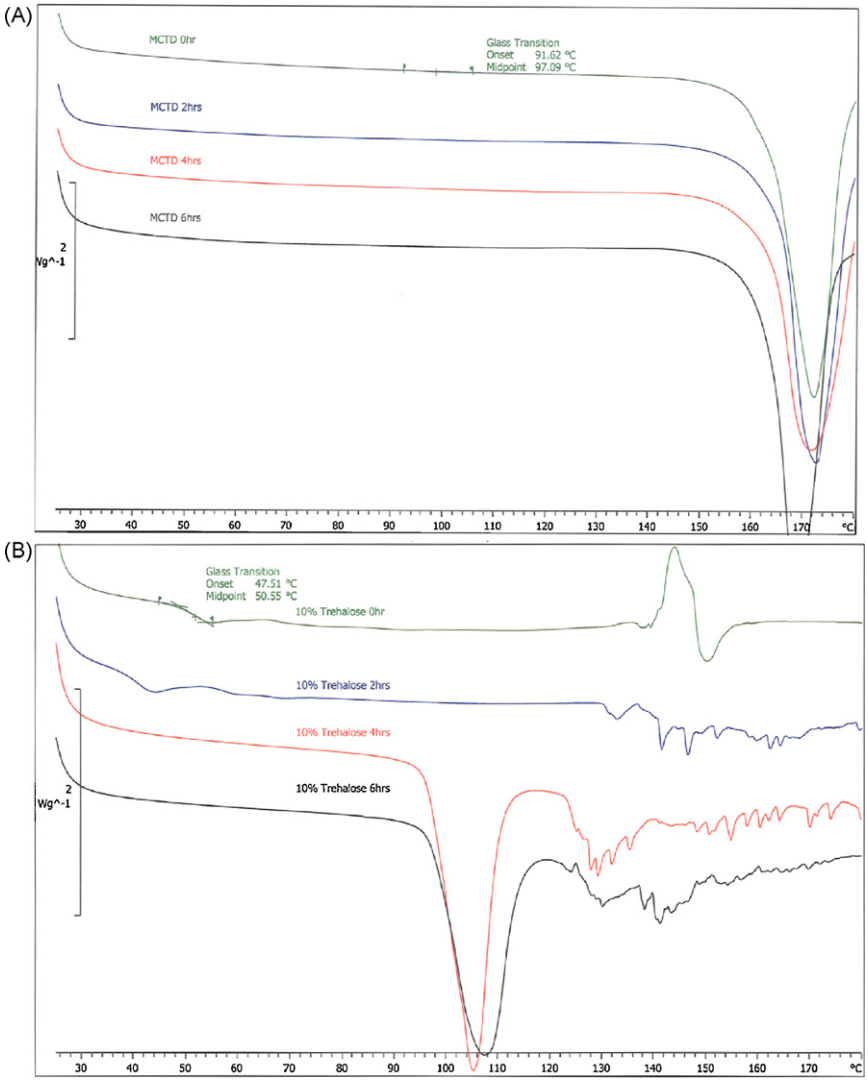

Fig. 2. (A) The glass transition temperature (T_g) of MCTD powder sample. (B) The glass transition temperature (T_g) of trehalose powder sample. The glass transition temperature (T_g) was determined by a DSC 823^e. The cover of the crucible was punched with a small hole before analysis. The sample (about 10 mg) was heated from 25 °C to 170 °C with a scanning rate of 10.0 °C/min. The sample cell was purged with nitrogen gas at 10.0 ml/min.

'X_0'. The resulting titer value was adjusted for initial dilutions and reported as $TCID_{50}$/mL.

2.7. Karl Fischer titration

The residual moisture content after spray drying and the water content after 2–6 h storage in a humidity box (70%, humidity detector from VWR, USA), were evaluated by a TIM550 Karl Fischer (Radiometer Analytical) in a dry box. The samples (±30 mg) were re-suspended in absolute, dry methanol (Phillipsburg, NJ). After background standby, the titration started automatically. During this titration, water molecules react stoichiometrically with the AQUA STAR® CombiTitrant 5 (EMD) reagent; subsequently, the volume of CombiTitrant 5 used to reach the endpoint of titration is used to calculate the percentage of water present in the sample (1 mL CombiTitrant 5 = 5 mg water). All titrations were performed in triplicate.

3. Results

3.1. AERAS-402 vaccine virus particle size

The main size distribution peak of AERAS-402 on the Disc centrifuge curve was around 77 nm (Fig. 1). There was a smaller peak of virus size of 65 nm. The two peaks did not shift even when the virus bulk material was concentrated 10 times (data not shown).

3.2. Thermodynamic properties

Glass transition temperatures of the drying powders were determined after production and storage at high humidity. The mannitol-based powder (mannitol–cyclodextrin–trehalose–dextran, MCTD) had the highest T_g value of 97.09 °C, with a melting point of 166.53 °C for the crystalline mannitol component (Fig. 2A). The T_g of trehalose powder after spray drying was 50.55 °C (Fig. 2B). Mannitol alone did not have a T_g, and mannitol with PVP powder had a T_g of 85.78 °C. The thermodynamic curves and glass transition temperatures of representative spray powders are shown in Table 1.

3.3. Particle size distribution and polydispersity

Spray drying the formulation of MCTD resulted in fine powder with an average particle size range of $D(v, 50) = 3.2 - 3.5$ μm.

Table 1
The characteristics of different spray drying powders.

Formulation	T_g (°C)	Yield[a] (%)	$d(0.5)$[b], VMD	M.C.[c] (%)
Mannitol	–	30.0	3.1	1.28
Mannitol with PVP	85.78	19.9	7.0	1.94
Trehalose	50.55	6.0	2.6	5.40
Leucine	–	4.3	–	1.64
MCTD	97.09	35.3	3.2	1.45

[a] Powder yield was calculated by the w/w of pre and post spray drying solid. The total amount of pre-spray drying solid was determined from lyophilization weight of pre-spray drying mixture.
[b] $d(0.5)$ is particle size at $D(v, 50)$, which is the equivalent volume diameter at 50% cumulative volume. The particle size of the powders was described by the volume median diameter (VMD).
[c] M.C., moisture content. The residual moisture contents were evaluated by a TIM550 Karl Fischer (radiometer analytical) in a dry box, the resulting water percents were expressed based on w/w.

Combined with $D(v, 0.1)$ and $D(v, 0.9)$, the span was around 1.5 μm [$D(v, 90)$, $D(v, 10)$ and $D(v, 50)$ are the equivalent volume diameters at 90, 10 and 50% cumulative volume, respectively]. The percent of inhalable particles (IP, 1 μm < IP < 5 μm) could reach to 72.6% of the total particles, and most powders are spherical (Figs. 3 and 4A). The spray-dried powder using the MCTD formation did not have either different distribution or thermodynamic properties in AREAS-402 and placebo tests (data not shown). The Mannitol with PVP formulation had a dry powder size of $D(v, 50) = 7.0$ μm and the trehalose product had a $D(v, 50) = 2.6$ μm (Table 1). Although the trehalose formulation had a smaller particle size after spray drying (Table 1 and Fig. 4B), the trehalose powder clumped easily after exposure to high humidity, and particle size could increase significantly which was not suitable for laser diffraction measurement. MCTD, however, did not have any detectable decrease in IP < 5 μm, even after storage at 37 °C for 28 days (results were the same as shown in Fig. 3).

3.4. Moisture content and shifting of glass transition temperature

To compare the moisture content variations under high humidity (70%) of different formulations, we selected four representative powders prepared from placebo formulations under the same processing conditions. The water contents of post spray drying powders were: mannitol 1.28%; mannitol with polyvinyl pyrrolidone (PVP) 1.94%; trehalose 5.40%; and MCTD 1.45% (Table 1). The water absorption tendency was different between the trehalose

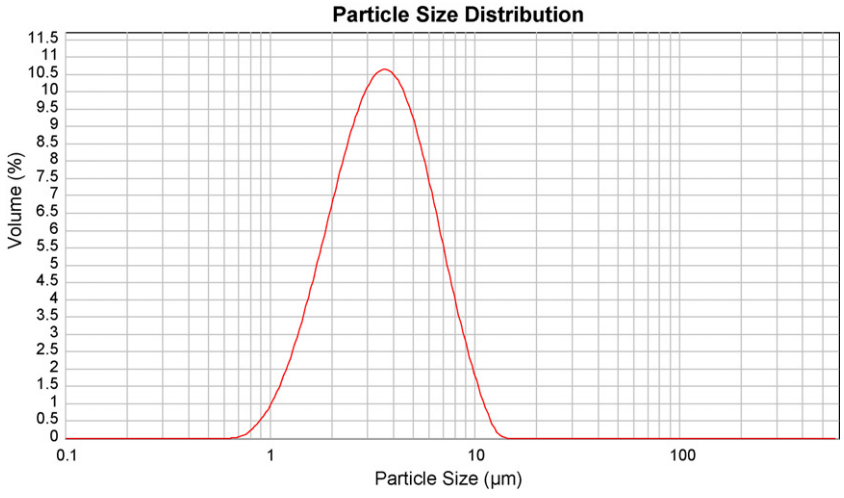

Fig. 3. The particle distribution of MCTD powder. Particle size distributions were measured by laser diffraction (Mastersizer 2000). The polydispersity of the powder was expressed by the span. Span = $[D(v, 90) - D(v, 10)]/D(v, 50)$, where $D(v, 90)$, $D(v, 10)$ and $D(v, 50)$ are the equivalent volume diameters at 90%, 10% and 50% cumulative volume, respectively.

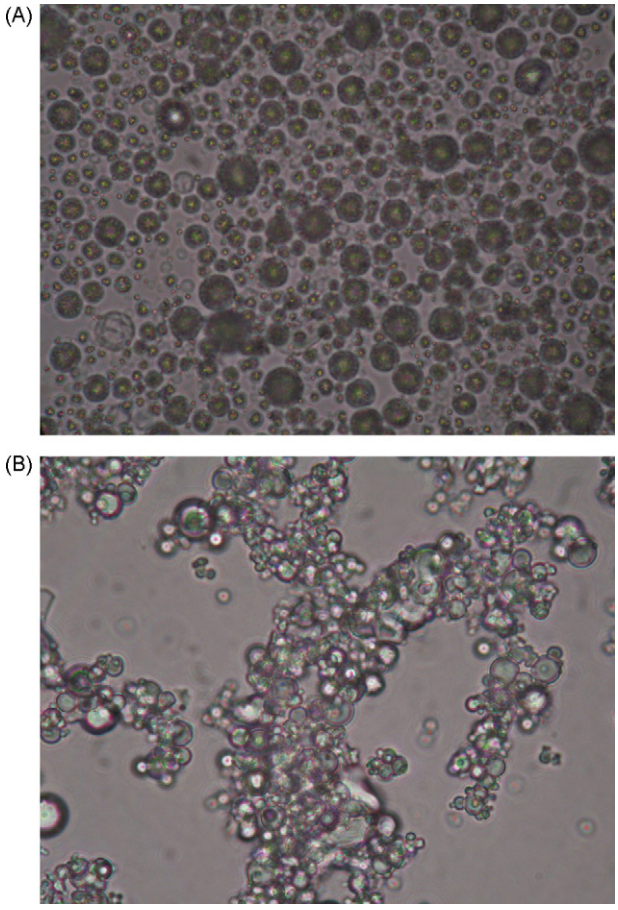

Fig. 4. (A) Microscope image (1000×) of particle generated from mannitol-based formulation. The powder was re-suspended in anhydrous methanol at 20 mg/mL. (B) Microscope image (1000×) of particle generated from trehalose formulation. The powder was re-suspended in Halocarbon 0.8 oil at 20 mg/mL.

and mannitol-based formulations (Fig. 5). After 2 h in 70% humidity, the water content of trehalose powder increased 61% (from 5.40% to 8.70%, w/w), and after 4 h, trehalose powder absorbed 37% more water. The total weight of water increased 120.9% (from 5.40% to 11.93%). The moisture saturation stage occurred after 4 h for trehalose powder. The mannitol-based formulations (mannitol, mannitol with PVP and MCTD) had high resistance to water absorption. Mannitol alone did not have any increase in moisture content during 6 h under high humidity exposure, and MCTD powder only increased from 1.45% to 1.80% in water content during this period.

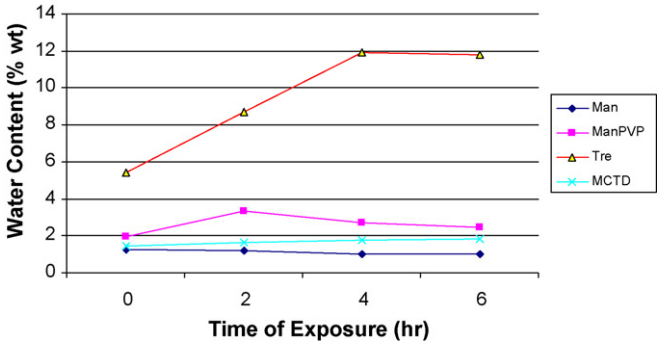

Fig. 5. Increase in water content of dry powder formulations exposed to 70% relative humidity.

After exposure at high humidity of 70%, the T_g of mannitol-based powder (MCTD) did not show significant change within 6 h. Trehalose powder, however, showed apparent difference in thermodynamic properties: first, after 2 h exposure at high humidity, its T_g shifted down to 38.76 °C then, after 4 h and 6 h the melting point was reduced to around 102 °C (Fig. 2B), and the exposed powder appeared crystallized.

3.5. Recovery and stability of different spraying lots

The effect of spray drying on the infectivity of AERAS-402 in 10 different formulations is shown in Fig. 6, where the titer before drying is compared with the titer after spray drying. The titer changes were expressed in log loss per milligram solid or powder. The total amount of pre-spray drying solid was from the weight of lyophilization of the pre-spray drying mixture.

All formulations except MCTD suffered at least a 1.5 (maximum 4.9) log loss of the viral infectivity. For the MCTD formulation, the loss in virus titer after spray drying was only 0.83 log. The largest decreases in virus activity by median Tissue Culture Infective Dose ($TCID_{50}$) test were for the trehalose and leucine formations. Although adding sucrose, inositol, or PVP, or adding PBS buffer could increase the survival of virus during the spray drying process, the $TICD_{50}$ of live AERAS-402 was still over 1 log decreased at the end of preparation. Most formulations also resulted in low process yield at less than 10%, while mannitol-based formulations

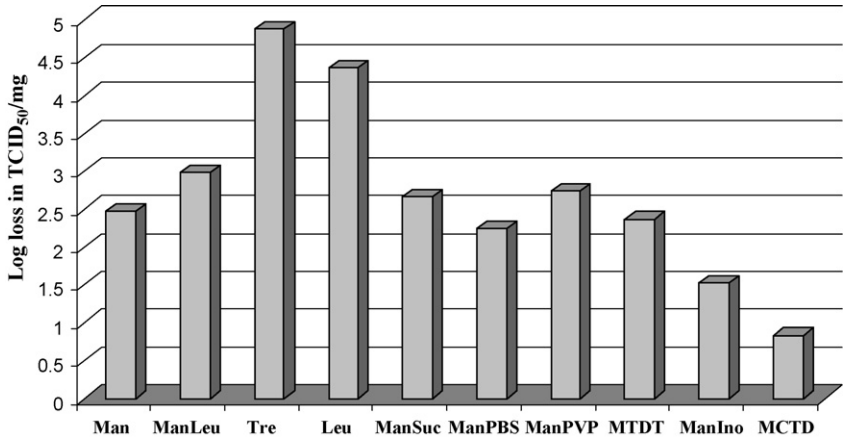

Fig. 6. The $TCID_{50}$ recovery (log loss) of AERAS-402 in different formulations during spray drying process. The TCID50 recovery is expressed as the infectivity loss of AERAS-402 between pre-spray drying and post spray drying samples. Titer changes are expressed in log loss per milligram solid or powder. Man = mannitol; ManLeu = mannitol mixed with leucine; Tre = trehalose; Leu = leucine; ManSuc = mannitol mixed with sucrose; ManPBS = mannitol in PBS buffer; ManPVP = mannitol mixed with PVP; MCTD = mannitol–cyclodextrin–trehalose–dextran; ManIno = mannitol mixed with inositol; MTDT = mannitol–trehalose–dextran–tween 80.

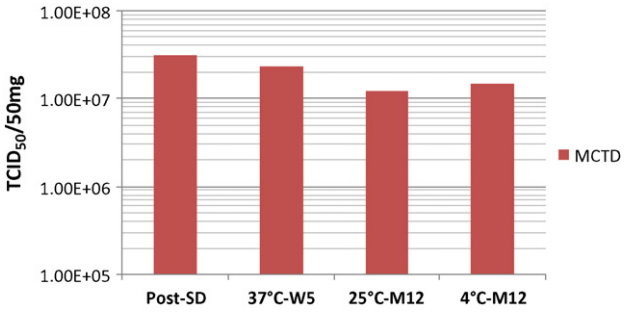

Fig. 7. The stability of AERAS-402 spray drying samples at 4 °C, 25 °C and 37 °C. The stability study of AERAS-402 spray lot with the candidate MCTD formulation was conducted for 5 weeks at 37 °C, 12 months at 25 °C and 4 °C. The change in virus activity is expressed as log loss of virus infectivity by the $TCID_{50}$ test. Post-SD = post spray drying.

could reach more than 30% solid yield at the end of spray drying processing (partial data shown in Table 1).

The stability study of AERAS-402 spray samples using the MCTD formulation showed that they could be stored at 4 °C and 25 °C for 12 months without significant change in $TCID_{50}$ titer. After storage at 37 °C for 5 weeks, the loss of virus activity was only 0.12 log (Fig. 7).

4. Discussion

Stabilizing excipients are used before spray drying to prevent degradation during processing and storage. Disaccharides are amongst the most frequently used excipients, with trehalose being a particularly common selection [23,33–38]. However, the trehalose and sucrose-based powders are more hygroscopic, picking up moisture during handling in the laboratory environment that leads to degradation in physical properties of the powder and reduces the ease of dispersion [33–35,37]. The sensitivity of powders to moisture uptake is important because the aerosol physical properties of inhalable dry powders are strongly dependent on moisture content; too much water can cause particle agglomeration, leading to reduced respirability.

Leucine and mannitol-based formulations are the least hygroscopic. Mannitol is stable as a powder and resists moisture resorption at relatively high humidities. These characteristics make it an ideal substance to encapsulate for inhalation, for diagnostic and therapeutic purposes [19,34,35,38–40]. The inhalation of dry-powder mannitol alone causes a marked increase in MCC (mucociliary clearance) in the whole right lung and in all lung regions in both asthmatic and healthy subjects [39,41–45]. Inhalation of dry-powder mannitol was well tolerated by all subjects and induced only a mild cough which was reproduced on the control day [39,45]. This increases the advantage of using a mannitol-based spray drying formulation in the development of powder form vaccines. With the processing conditions used in the present study, the moisture content of the trehalose-based formulation was higher than the other tested formulations, while mannitol-based formulations typically resisted water absorption, even when exposed to condition of high humidity, which will benefit the future applications in vaccine storage and clinical trials.

The glass transition temperature of the dry formulations is also strongly dependent on water content; just a few percent increases in the water content of sugar-based formulations can decrease the T_g by several tens of degrees Celsius [35]. Higher moisture content also results in decreased viral stability [34,35]. Immobilization of the labile materials in amorphous glass is believed to be advantageous to maintain the activity of the incorporated molecules [46]. The resistance to crystallization can be evaluated by measuring the glass transition temperature, which is the temperature at which the transition from the glassy to the rubbery state or from a low molecular mobility to a high molecular mobility (and therefore, higher risk of crystallization) occurs. PVP and albumins are known to increase the glass transition temperature, which means that the formulations can be exposed to higher ambient temperatures before the glass transition occurs [35,47,48]. However, PVP as a stabilizer in the tested formulation did not appear to prevent loss of virus activity during the spray drying process.

Dextran has also been shown to prevent crystallization of spray-dried and freeze-dried excipients [24,50]. Lung delivery of aerosolized dextran is well tolerated and has potential therapeutic benefit in the treatment of cystic fibrosis [51]. Therefore, the mannitol-based formulation used in the present study, MCTD, includes two kinds of dextran as components. This formulation could increase the glass transition temperature of trehalose from 50.55 °C to 97.09 °C. The formulation also generates a dry powder that inhibits re-crystallization of stabilizing sugars, preventing inactivation of incorporated labile materials, and its glass transition temperature does not decrease during storage at high humidity. Equally important as low hygroscopicity in formulation selection, since water molecules are known to increase the molecular mobility, is a high and non-shifting glass transition temperature during storage. With glass transition temperature of the formulations occurring at about 50 °C and higher, the powders and microparticles should be physically stable at temperatures up to about 40 °C, as long as the powders are protected from moisture ingress. As mentioned above, the MCTD formulation showed no detectable decrease in IP < 5 μm after storage at 37 °C for 28 days. The higher T_g values measured for this formulation suggest that enhanced long-term thermostability may be possible.

At this stage of formulation development, results show that MCTD is a leading candidate for both live virus and placebo selections. MCTD is not only conducive to forming easily dispersed microparticles in dry processing, but also appears to be a good stabilizer formulation for the AERAS-402 vaccine virus. Combinations of small and high molecular weight sugar stabilizers help achieve optimized viral processing and storage stability, while mitigating the negative particle forming properties of trehalose. The other tested formulations did not retain activity as well as the MCTD formulation during the spray drying process, or at 37 °C in the 5 weeks stability test. Additional studies are planned to precisely identify and optimize the key excipients in the current MCTD-based formulations. It may be possible to add amino acids to some of the formulation components. Studies identifying the optimal concentration that balances the opposing objectives of maximizing virus stability and minimizing powder hygroscopicity must still be conducted.

AERAS-402 is a new Ad35 vectored TB vaccine that is highly immunogenic in animals and humans and is currently entering human clinical Phase IIB proof of principle efficacy trials. This new dry powder formulation of the AERAS-402 TB vaccine has potential as a very affordable easily delivered version of this TB vaccine for use in the developing world. Aerosol delivery of this vaccine also has the potential for increased protection due to direct stimulation of immunity in the lung compared to vaccines delivered by the parenteral route.

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