SAFER VACCINES AND ANTIBIOTICS MANUFACTURED THROUGH USE OF NEAR-CRITICAL OR SUPERCRITICAL FLUIDS

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This paper is dedicated to the memory of Dr. Jose Luis Valdespino-Gomez, who, together with his collaborators in Mexico, pioneered the introduction of inhalable wet mist measles vaccine.

Near-critical and supercritical fluids have been used successfully in the manufacture of vaccine, antibody and antibiotic microparticles by Carbon-dioxide Assisted Nebulization with a Bubble Dryer® (CAN-BD). In CAN-BD two fluid streams, a drug, antibody, or vaccine solution or suspension, and supercritical or near-critical CO_2, are intimately mixed in a low dead volume tee. These mixed fluid streams are then rapidly expanded through a flow restrictor to atmospheric pressure where the micro-bubbles and micro-droplets, formed in the plume, are dried by mixing with warm nitrogen at 50-65 °C. The resulting free flowing stable microparticles, containing vaccine virus or drug particles imbedded in sugars, amino acids, and/or other excipients, are within a respirable size range (1-5 μm) and have less than 2% residual moisture. These powder properties are ideal for developing a needle-free delivery system that addresses the Grand Challenges in Global Health Initiative #3. In response to this challenge, we developed unit-dose packaging that preserves powder properties by protecting them from moisture, oxidants and UV exposure as well as a low cost "active" dry powder inhaler to disperse the Edmonston-Zagreb measles vaccine. This inhalable vaccine has demonstrated 2-year storage stability at 2-8 °C, high virus titers, the ability to create a robust immune response and challenge protection in Rhesus macaques. Our continuing work includes CAN-BD anti-tuberculosis antibiotic microparticles for needle-free, combination tuberculosis therapy. Unit-dose dry powder vaccines and antibiotics have the potential to provide easy to use, stable products with improved safety profiles.

INTRODUCTION

Carbon dioxide Assisted Nebulization with a Bubble Dryer® (CAN-BD), technology is ideally suited to develop new dry powder vaccines and biopharmaceutical microparticles for needle-free pulmonary delivery strategies and unit-dose packaging. CAN-BD processed microparticles have powder properties (size, moisture content, stability and potency) that were developed specifically for pulmonary delivery. The needle-free pulmonary delivery strategy was developed in response to the Grand Challenges in Global Health Initiative #3 [1]. This initiative was designed to create new technologies for the administration of vaccines. Traditional syringe and needle administration is associated with increased risk of infections

due to human immunodeficiency virus (HIV), Hepatitis B (HBV), and C (HCV) virus resulting from needle-stick injuries, inappropriate re-use of needles or syringes and the challenges of proper needle disposal [2,3]. Needle-free delivery methods have the potential to improve vaccination and drug delivery safety, while unit-dose packaging may reduce vaccine and antibiotic wastage. Vaccine wastage is the excess number of vaccine doses wasted, as the number of persons actually vaccinated is less than the number of doses used or destroyed [4]. Vaccine wastage can be as high as 70% depending on the country and nature of the delivery system. The more vaccination is based on outreach, the higher the vaccine wastage. Major contributing factors to vaccine wastage are loss of potency during transport through the cold chain, drug expiration, and opened but unused doses [5]. The unit-dose needle-free administration of stable dry powder vaccines and biopharmaceutical microparticles may mitigate many of the factors contributing to vaccine wastage.

CAN-BD has been used successfully to produce a variety of dry, active powders of vaccines and small molecule pharmaceuticals in a size range suitable for pulmonary delivery including Edmonston-Zagreb (EZ) measles vaccine, Hepatitis B vaccine, human papilloma virus (HPV) vaccine, polyclonal IgG, and several anti-tuberculosis (anti-TB) antibiotics [6]. We have prepared a fine dry powder of HPV vaccine that showed no detectable degradation of the HPV capsid protein during CAN-BD processing and subsequent storage for one year at 2 to 8 °C by SDS-PAGE analysis. This paper explores the development of inhalable EZ measles vaccine and inhalable antibiotic microparticles (i.e., inhalable TB-drugs) intended to be used in the treatment of TB. Both measles vaccination campaigns and the treatment of TB share several challenges, which include product contamination, difficult to reach populations, wastage, the potential for needle-stick injuries, and difficulties associated with sharps disposal [2-5,7-10]. Many of these challenges may be overcome with the use of a pulmonary delivery strategy that incorporates unit-dose packaging. The promise of pulmonary delivery for measles vaccination is evident from the nearly 4 million people who were vaccinated with wet mist aerosols with no reported serious adverse events [11]. The potential therapeutic advantages of inhalable TB treatment are based on the success of aerosol antibiotic drug delivery in cystic fibrosis patients, the pulmonary route of infection of Mycobacterium tuberculosis (Mtb), and the large population of HIV and TB co-infected individuals [12-14].

In each step of development for powder properties, packaging and delivery we have designed the CAN-BD dried EZ measles vaccine to reduce major contributing factors to vaccine wastage as well as improve safety. The EZ measles vaccine dry powder is formulated with <2% moisture content. Beyond preserving powder aerosolization and deposition properties, stabilization via residual moisture reduction is an important factor in preserving potency by preventing microorganism growth and other degradation [15]. To ensure the moisture content remains <2% and that the vaccine is not exposed to UV, oxidants or other contaminants, we package each dose in tightly sealed aluminum foil-polymer film laminate blister packs. Multiple blister packs are sealed in a foil overwrap with molecular sieve desiccant. This foil laminate unit-dose blister pack improves the safe use of each dose by mitigating the dangers of UV, oxidant inactivation and general contamination. Lastly, EZ vaccine dry powder is administered through a low cost dry powder inhaler (e.g., the PuffHaler®), providing an alternative to traditional subcutaneous injections.

The development of the TB drug formulations for capreomycin, kanamycin and isoniazid were modeled on the successful powder properties of the EZ measles vaccine dry powder. The CAN-BD dried antibiotic powders are similarly packaged in unit-dose, tightly sealed foil laminate blister packs with minimal moisture content. However, in treating TB by

inhalation, the particle size requirements are more stringent, and the doses delivered larger, for the TB drug microparticles. These antibiotic microparticles are targeted largely to the alveolar space, where Mtb enters in the early stages of infection and also to poorly vascularized lesions and granulomas which harbor bacilli in protective microenvironments that often elude conventional therapy [16-20]. The powder properties were tested using the PuffHaler as a prototype administration device.

CAN-BD is a unique blend of supercritical/near-critical microparticle forming techniques and spray drying. The CAN-BD process for creating microparticle vaccines and biopharmaceuticals is advantageous because the dense gas is not used to precipitate the solute of interest, but rather as a solvent nebulization enhancer. Unlike many supercritical processes that require the solvent and supercritical or near-critical fluid to be miscible [21], CAN-BD is not limited by this requirement, as organic solvents are not necessary to increase the solubility of the dense gas in the vaccine or biopharmaceutical solution of interest. [6]. The solute can be dissolved in a biologically stabilizing solvent such as water with buffers, stabilizers and excipients. Use of biologically stabilizing formulations may preserve native protein secondary structure, a potentially important factor for improving long-term storage stability of proteins in a dry solid form [22]. Further, due to the relatively low processing temperatures used in CAN-BD one may expect less decomposition of thermally labile drugs.

To provide an effective alternative to traditional syringe-based vaccines and biopharmaceuticals, one must develop inhalable vaccine and antibiotic powders with fine particle fractions suitable for pulmonary delivery, low moisture, stability, potency and the potential to manufacture powders to meet worldwide demands (2008: ~ 300 million doses per year for measles vaccine). Pulmonary delivery of vaccines and biopharmaceuticals requires specific fine particle fractions and dispersibility. The aerodynamic particle requirements for pulmonary delivery are particles in the 1 to 5 µm range, preferably with a 1 to 3 µm range for delivering particles to the alveolar or deep lung region [23,24]. Aerodynamic particle size distribution, particle shape and powder dispersion characteristics dictate the performance of inhalation devices [24,25]. To meet regulatory requirements, measurements of aerodynamic particle size distribution and deposition are usually done using an Andersen Cascade Impactor (ACI) and a Dosage Unit Sampling Apparatus (DUSA). ACI experimental results provide fine particle fraction (FPF). The FPF measured is an indication of where the powder is likely to deposit in the lung upon inhalation, and is affected by dispersion of the powder. Results from DUSA provide emitted dose (ED). ED is the mass percentage of particles leaving the dry powder inhaler (DPI) system and available for inhalation. Particle shape, geometry and surface morphology are examined using a Scanning Electronic Microscope (SEM).

Moisture content analysis and the monitoring of crystal structure are important for evaluating powder stability and the success of packaging strategies. Powder moisture content is analyzed using coulometric Karl Fischer titration. Karl Fischer titration is a technique used to measure the percent moisture remaining in the powder. Measuring the percent moisture before and after shipment of the microparticles gives an understanding of both the inherent moisture content and how well the packaging works to protect the powder from moisture ingress. Changes after shipping provide a reasonable estimate of expected problems in worldwide distribution. Similarly, evaluating the structure of the microparticles using X-ray diffraction (XRD) ensures that the powders remain unchanged (crystalline or amorphous) throughout the storage and usage period.

The reformulated dry powder live-attenuated measles virus vaccine was manufactured by the Serum Institute of India Limited (SIIL). Dry powder vaccines must also demonstrate adequate potency to achieve the desired immune response. These vaccine qualities are established by examining 50% cell culture infective dose ($CCID_{50}$) assays, T-cell immune responses, PCR and plaque neutralization assays and challenge results from animal studies. Comparing the ($CCID_{50}$) assay potency measured in India by SIIL after bulk powder production with that measured after the powders were sealed in blister packs and shipped from Pune, India to Boulder, Colorado ensured that the packaging strategy preserves potency. The ($CCID_{50}$) method is based on observation of cytopathic effect (CPE) after viral infection of a susceptible cell culture.

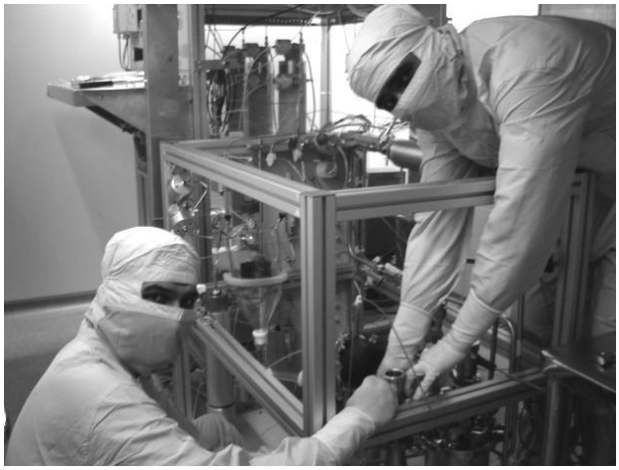

Figure 1: GMP version of CAN-BD installed at Serum Institute of India (Pune, India) for production of myo-inositol based inhalable dry powder measles vaccine for NHP toxicity study and Phase I clinical trials.

The myo-inositol stabilized EZ measles vaccine [26] is in the process of commercialization with Phase I clinical trials planned to begin in 2010. Fig. 1 is a GMP lab-scale version of CAN-BD installed at SIIL and used to prepare samples for animal toxicity and human clinical trials.

MATERIALS AND METHODS

The CAN-BD equipment and setup are shown in Fig. 2. Additional information can be found in reference [6]. In this process, the drug or vaccine is dissolved in a solvent (usually water, but it can also be a water-organic mixture, or a pure organic solvent). Both the drug solution and the nebulization fluid are pumped through 1/16 in. outer diameter (OD) stainless steel tubing into the inlets of a tee (Alltech 0.25 mm bore stainless steel tee (p/n 30771)) at 83 bars. Either Isco piston pumps for CO_2 or conventional HPLC pumps for the drug solution were used. Once in the tee the solutions are mixed intimately. The mixture of dense gas and the drug solution flow from the tee through a 75 µm inner diameter, 10 cm-long, fused silica

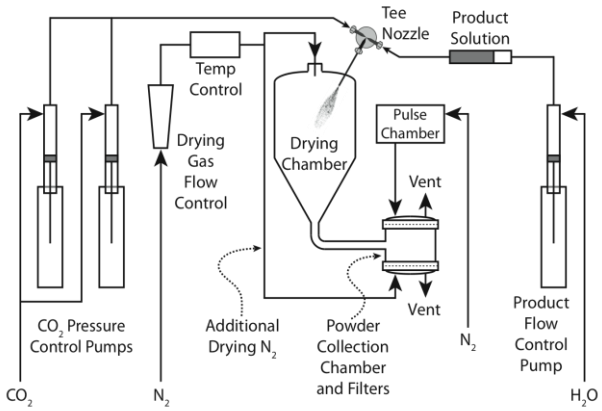

Figure 2: Schematic diagram of CAN-BD system

restrictor (Polymicro TSP075375) and form a wet aerosol plume upon the rapid expansion of the compressed CO_2 into a glass drying chamber maintained at or near atmospheric pressure. Particles are formed when the plume containing micro-bubbles and micro-droplets is rapidly dried by warm nitrogen (50-65 °C) pumped into the glass-drying chamber at 30 L/min. Particles are collected downstream of the drying chamber in a double filter apparatus that has two 0.45 µm filters on either side of a glass chamber. The top filter is pulsed at regularly timed intervals to release powder onto the bottom filter where the powder undergoes additional drying with warm nitrogen pumped at 30 mL/min through the bottom filter at the end of processing.

The Fine Particle Fraction (FPF) and Emitted Dose (ED) were determined according to USP Chapter <601> using an Andersen Cascade Impactor (Westech, Marietta, Georgia) and a Dosage Unit Sampling Apparatus (DUSA), respectively. Tests were conducted at relative humidity below 15%. Blisters remained sealed until peeling back the lid immediately before use. The powders were then dispersed into the reservoir/spacer before being sampled through a mask by the ACI or DUSA. In these procedures, the powder aerosol in the reservoir is drawn into the ACI or DUSA at 28.3 L/min. In the ACI procedure, particles are separated by aerodynamic diameter on sequential stages and gravimetrically analyzed to quantitate the FPF [27]. The ED is gravimetrically determined by analyzing a single filter in the DUSA. When particles are dispersed from the foil laminate blister pack in the inhaler, a portion of the particles flows with the gas stream, while some remain adhered to the walls of the blister pack and on the walls of the other inhaler parts. The DUSA filter captures only what is emitted from the reservoir. As an alternative, total organic carbon (TOC) analysis, which is insensitive to moisture, can be used for quantitation of FPF and ED.

Scanning electron microscopy (SEM) was performed with a JEOL model # JSM-6480LV (LVSEM), a thermal emission SEM for the EZ measles placebo and a JEOL model #JSM-7401 (FESEM) for the TB drugs operating between 5 and 10 kV with a filament current of about 0.5 mA. Powder samples were deposited on carbon conductive double-sided tape, and then most of each sample was tapped off to leave a thin layer of microparticles. Samples were coated with a gold layer using a sputter coater operated for 30 seconds at a sputtering current of 40 mA.

The moisture content of the powders was determined by extraction of the water into methanol and measuring the concentration by the Karl Fischer coulometric titration method (Denver Instruments, Model 260 and 275KF). USP Chapter <921> was followed and tests were conducted at humidity below 15% RH.

Crystallinity of the powders was analyzed using powder X-ray diffraction (pXRD) scanning from 5º – 45º at a wavelength of 1.54 Angstroms on a Scintag PAD-5 X-ray diffractometer in continuous-scan mode. The step size was 0.02 and the scan rate was 2° per minute.

Potency pre- and post-shipping was established using the 50% cell culture infective dose ($CCID_{50}$) assay. Virus is added to cells cultured in liquid media in microtiter plate wells. A single test unit consists of the entire cell layer in a well. After the virus is added to test units, it is serially diluted down the microtiter plate (B.D. Davis). Each test unit is scored as infected or not infected. The virus titer is determined by the Spearman-Karber method for calculation of $CCID_{50}$ [28,29].

RESULTS AND DISCUSSION

Measles, a highly contagious virus is a leading cause of death for children worldwide [30,31]. In 2000 there where an estimated 733,000 measles deaths. In 2008, all UN member states reaffirmed their commitment to achieving a 90% reduction in measles mortality by 2010 [31]. There are number of challenges to achieving and sustaining high levels of measles vaccine coverage and population immunity, including population growth, demographic changes, conflict, political instability, and public perceptions of vaccine safety [32]. To achieve the measles mortality reduction goal, progress in delivering measles vaccines to the world's children by increasing vaccination coverage is essential. While needle-free administration of the EZ measles vaccine cannot overcome all of the challenges of mass vaccination, it does address public perceptions of vaccine safety by eliminating the fear of needles, the pain of injection, the cost and complexity of sharps disposal, and needle-stick injuries to healthcare workers [33]. Additionally, due to the stability of dry powders, the lack of water in the inhalable formulation and the foil laminate blister packaging of unit-doses, transport to remote regions may prevent wastage from unused doses and contamination, allowing for greater population coverage. Below we describe the characterizations of the powder properties and a low cost dry powder inhaler proposed for a needle-free inhalable administration of the EZ measles vaccine.

The microparticle geometric sizes and surface morphologies of EZ measles vaccine placebo powders were analyzed by SEM. Figure 3 shows the SEM image of particles of the myo-inositol-based placebo formulation prepared by CAN-BD at 50°C.

Emitted Dose (ED) was gravimetrically determined using PuffHaler to disperse EZ measles vaccine microparticles from foil laminate blister packs. EZ measles vaccine microparticles, from each manufacturing batch, were processed and packaged into foil laminate blister packs by SIIL in Pune, India. The foil laminate blisters were shipped to the University of Colorado, where the PuffHaler was used to disperse the EZ measles vaccine. The ED data is dependent on the properties of the inhalation device and operator usage.

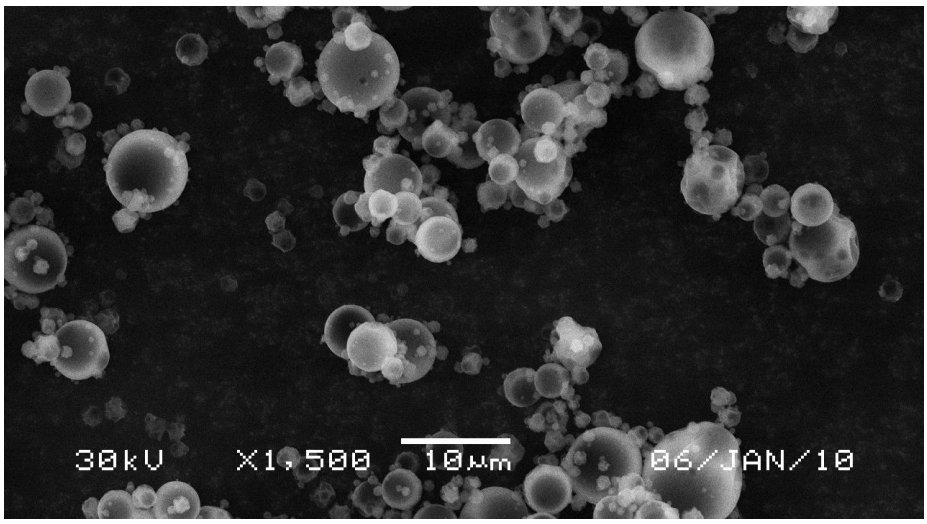

Figure 3: LVSEM picture of myo-inositol based inhalable dry powder measles placebo produced by CAN-BD.

Operator-dependent variation is a well-known challenge with dry powder inhalers (DPIs) [34]. The next generation PuffHaler will target improvements in user-dependent variation. However, to establish the ED properties post-shipping, and ensure that the blister packaging strategy was successful in preserving powder properties, one operator analyzed eight individual manufacturing batches of inhalable EZ measles vaccine microparticles as dispersed from the PuffHaler. The range of ED values was between 40.8 and 51.4% ED with an average value of 46.2% ED and a standard deviation of 4.3% (see Fig. 4). These ED values post shipping are comparable to those measured at SIIL and demonstrate the reproducibility of the manufacturing process and preservation of powder properties after long distance shipping.

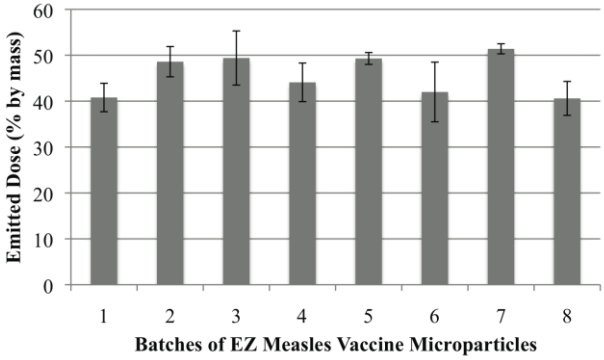

Figure 4: Emitted Dose Data: 8 separate manufacturing batches of the EZ measles vaccine were sealed in foil laminate blisters and shipped to the University of Colorado. Blisters from each batch were dispersed into the reservoir of the PuffHaler before being sampled through a mask by the DUSA. Each batch was analyzed in triplicate. The ED results in the 40-50% range demonstrate the success of the foil laminate blister packs in preserving powder properties during long-term shipping.

We developed the PuffHaler (Fig. 5) as a low cost, simple active dry powder inhaler that can disperse powder agglomerates to generate high emitted doses in response to Grand Challenges in Global Health Initiative #3. The PuffHaler incorporates a special polyethylene film that contains an anti-static agent in the polymer film of the reservoir. This anti-static material greatly improved the emitted dose of our device by reducing the powder that remained adhered to the walls of the reservoir. This antistatic polymer was sourced due to its

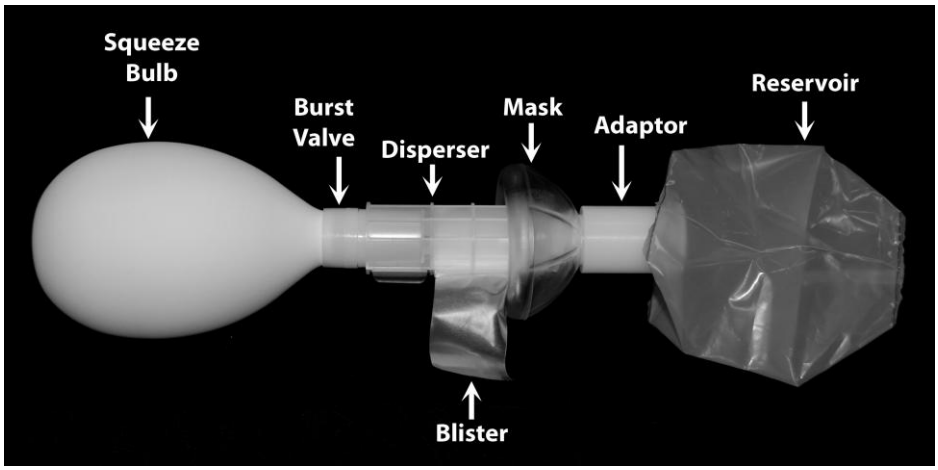

Figure 5: The PuffHaler dry powder inhaler with individually labeled components.

ability to maintain antistatic properties at relatively high humidity.

Moisture content of the EZ measles vaccine microparticles sealed in foil laminate blister packs was analyzed by Karl Fischer titration. EZ measles vaccine microparticles were processed and packaged into foil laminate blister packs by the Serum Institute of India Limited (SIIL). The EZ measles vaccine powders were analyzed for moisture content at SIIL before shipping to the US, after arriving at the University of Colorado, and lastly (two months later) after domestic shipping to the East Coast and back to the University of Colorado. Average moisture content results are reported in Fig. 6.

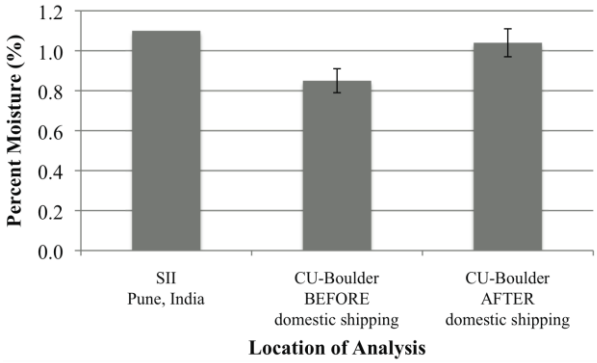

Figure 6: Shipping comparison study of moisture as analyzed by KF of Batch 71. Each bar is the average of 3 measurements, which consisted of two blisters each.

The lack of major deviation in water content illustrates the success of the foil laminate blister packs in preventing moisture ingress. Batch 71 is representative of typical EZ measles vaccine microparticle moisture values that have been shipped from SIIL and analyzed at the University of Colorado. The range of moisture content in 15 batches of EZ measles vaccine microparticles manufactured at SIIL was between 1.1 and 1.7% moisture with an average moisture content of 1.4% and a standard deviation of 0.2%.

Stability during shipping was also demonstrated by the comparative analysis of potency by 50% cell culture infective dose ($CCID_{50}$) assay results from 8 batches of the EZ measles vaccine. The $CCID_{50}$ potency of the 8 batches was analyzed by SIIL before shipping and upon arrival at the University of Colorado. The difference in $CCID_{50}$ values obtained between the two analysis locations was analyzed for outliers and also for significant differences. When these difference values are subjected to a F-test they produce a value of 0.66, which indicates the differences in the values are not significant and thus the stability results are essentially the same before and after shipment from India.

The crystallinity of a placebo of the vaccine powder was analyzed using powder X-ray diffraction (pXRD); the placebo formulation was shown to be amorphous. Previous pXRD spectra of the stock myo-inositol used in the formulation show significant crystallinity, while the placebo shows no peaks above the background. The amorphous quality of these powders is likely due to the rapid drying process of CAN-BD. The average residence time of the micro-bubbles and micro-droplets of solution in the drying chamber has been estimated, from chamber volume and flow rate calculations, to be a few seconds [35]. This drying time may be sufficiently fast to prevent crystallization and produce amorphous microparticles [36].

Potency and immune response were demonstrated by analysis of T-cell immune responses, and PCR and plaque neutralization assays of samples from Rhesus Macaques that had been dosed with dry powder measles vaccine aerosols generated with the PuffHaler. These studies demonstrated that the EZ measles vaccine, stabilized with myo-inositol and dried by CAN-BD, then inhaled from PuffHaler can induce immune responses that are at least equivalent to the responses induced by subcutaneous injection using the commercial SIIL

vaccine suspension. Macaques that received dry powder vaccine by inhalation had higher copy numbers of measles RNA in their lung lavage, higher neutralizing antibody titers, and a more robust T-cell immune response than animals that received vaccine by either nasal delivery or injection. Fourteen months after immunization, the same macaques were protected against measles when challenged with wild-type Bilthoven strain measles virus. These results demonstrate that the measles dry powder vaccine can be delivered effectively by inhalation from the low-cost PuffHaler with foil laminate blister packs.

To combat TB, a disease that kills 1.3 million people a year, we are developing unit-dose, inhalable, antibiotic microparticles for use as a primary and combined therapy approach to treating TB and multiple drug resistant TB (MDR-TB) [37]. Lack of patient compliance during lengthy treatment regimens is a major contributing factor in the development of MDR-TB [38,39]. Because inhalable dry powders can be administered by health-care workers with minimal training and weigh less than combined vaccines and diluents, they are transport-friendly and may reach more remote patients, making compliance more probable. Moreover, as CAN-BD antibiotic powders are also needle-free, they improve safety for both administrators and patients by eliminating needle-sticks and blood-borne infections from needle re-use. These antibiotic powders have fine particle fractions from the PuffHaler as great as 21.6% less than 3.3 µm aerodynamic diameter, which are ideal for alveolar deposition. By depositing antibiotics in the deep lung, one may be able to reach TB lesions that lack a strong blood supply, and may also increase the targeted dose to the lung airspace and tissue while reducing systemic side effects and, potentially, the length of treatment. Using inhalable antibiotics in combination with more traditional treatment may provide a two-pronged attack strategy for MDR-TB. Failure to sterilize all bacterial populations often results in the development of resistant strains [18-20]. Increasing the targeted dose in the lung airspace and targeting bacteria in protective microenvironments such as those in granulomas may prevent the development of MDR-TB by ensuring that threshold doses of antibiotics reach bacteria populations that are difficult to target by traditional methods.

Figure 7 shows the ACI results for fine particle fraction (FPF) and Emitted Dose (ED) results using PuffHaler to disperse capreomycin, isoniazid and kanamycin microparticles from foil laminate blister packs. Capreomycin, isoniazid, and kanamycin were processed at the University of Colorado, Boulder and packaged into foil laminate blister packs at Aktiv-Dry LLC. The antibiotic microparticles were analyzed at Aktiv-Dry LLC for fine particle fraction (FPF) and emitted dose ED. The formulation of isoniazid is still being developed with an emphasis on the improvement of its homogeneity, FPF and ED. However, the % FPF <3.3 µm is

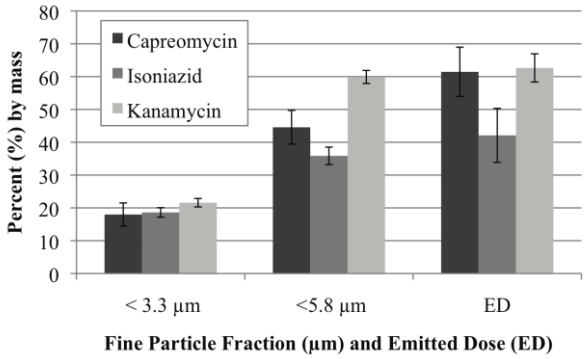

Figure 7: The Fine Particle Fractions and Emitted Doses for capreomycin, kanamycin and isoniazid. The goal of the antibiotic formulations is to have a large ED and a large FPF <3.3 µm. While isoniazid has a lower FPF <5.8 µm than the other antibiotic formulations the FPF <3.3 µm is comparable.

comparable with the other TB drug formulations.

The moisture content of the capreomycin and kanamycin microparticle formulations was analyzed via Karl Fischer titration. The capreomycin formulation contained 1.52% moisture with a standard deviation of 0.10%. The kanamycin formulation contained 2.7% moisture with a standard deviation of 0.42%.

The shape and morphology of capreomycin, isoniazid and kanamycin were investigated using SEM. As the capreomycin and kanamycin particles get larger they tend to have a crumpled morphology. The smaller particles are more spherical. The SEM of isoniazid shows textured spheres, crystals, and some cube like particles in a range of sizes. However, the amorphous textured spheres are the dominant particle morphology.

The crystallinity of capreomycin, kanamycin and isoniazid microparticles formulated with 20% L-leucine were analyzed using powder X-ray diffraction (pXRD) scanning from 5º – 45º on a Scintag PAD-5 X-ray diffractometer. Capreomycin and kanamycin formulated microparticles are amorphous in character while the isoniazid microparticles show significant crystallinity.

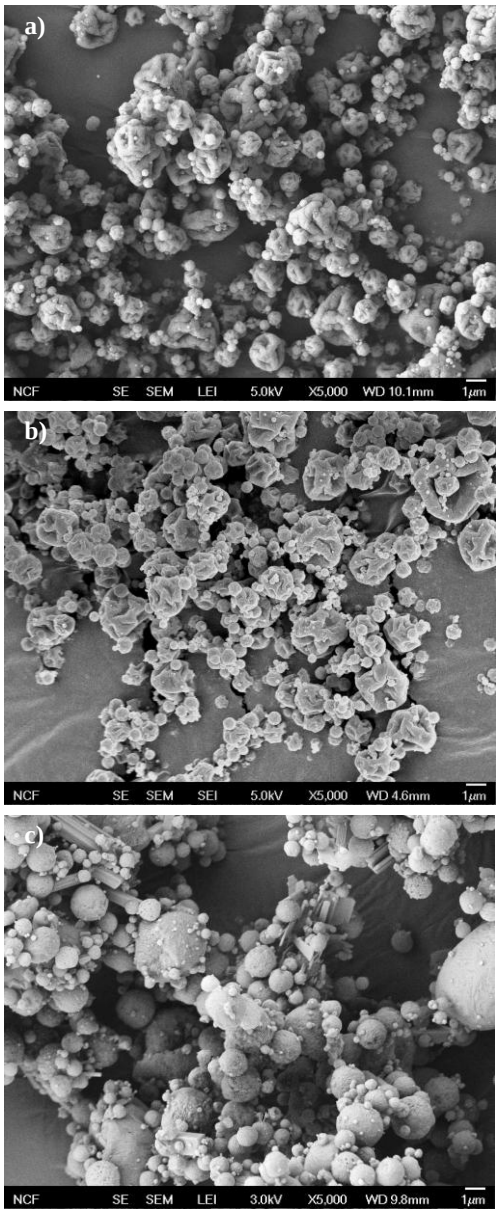

Figure 8: FESEM images of a) Kanamycin b) Capreomycin c) Isoniazid. The antibiotics were formulated with 5 to 5.63% total dissolved solids and consisted of 80% antibiotic with 20% L-leucine. The antibiotics were dried at 60˚ C and 83 bars with a flow rate of 0.5 mL/min.

CONCLUSIONS

CAN-BD is a powerful

supercritical/ near-critical technique for creating and drying microparticles of vaccines, and antibiotics. Allied with protective unit-dose packaging and a low cost dry powder inhaler CAN-BD microparticle processing can make a strong impact on shifting the immunization and treatment paradigm toward a needle-free solution. The immunization and live measles virus challenge results in Rhesus macaques treated with inhalable dry powder measles vaccine demonstrate the effectiveness of pulmonary vaccine delivery. The impressive powder properties of the anti-TB antibiotics combined with the important packaging technologies developed for EZ measles study may pave the way for new pulmonary treatments that reduce treatment time and the dangers associated with traditional syringe delivery, while increasing coverage to high risk populations.

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