# MICRONIZATION OF MEASLES VACCINE AND siRNA BY CAN-BD FOR AEROSOL DELIVERY BY AIR EXPANSION OF POWDERS WITH A PUFFHALER™

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## INTRODUCTION

Powders produced by $CO_2$-Assisted Nebulization with a Bubble Dryer® (CAN-BD) are used in developing the PuffHaler™, an inexpensive air-activated dry powder inhaler (DPI) (1) that utilizes silicone rubber pressure release valves. This active inhaler incorporates a detachable holding chamber and mask to make the aerosol cloud available to infants, toddlers and uncooperative subjects who cannot use a passive DPI.

## METHODS

Dry powders of measles vaccine formulations and siRNA were prepared by CAN-BD (2-7) at 50 °C. Fine particle fractions (FPF) of live virus and placebo powders delivered from the Aerolizer (Schering) were measured using an Andersen Cascade Impactor (ACI). Performance of the Puffhaler system using placebo powders, as measured by FPF and emitted dose (ED), was characterized at adult flow rates as well as infant respiratory patterns using a variant of the method described by Janssens *et al.* (8). Water content was measured by Karl Fischer coulometric titration. Measles vaccine potency was measured by a standard plaque assay (9). Material and particle crystallinity was analyzed using powder X-ray diffraction. Material and particle glass transition temperatures were determined using a Perkin-Elmer, Diamond Differential Scanning Calorimeter (DSC).

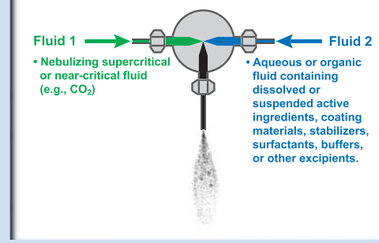
## PUFFHALER DESCRIPTION

The PuffHaler depends upon squeezing a pliable bottle to pop a polymeric pressure release valve and disperse a dose of microparticles. An air-filled bottle (660 cc), fitted with one or two valves in series, is manually squeezed to generate a pressure of ~14 kPa, which opens the valves. A volume of ~160 cc flows through the valves in less than 0.1 second and there is an accompanying audible pop and transient vibration. The first valve (Nike) must be stiffer than the second (Seaquist) if two valves are used. As the valves open, the compressed air disperses a bolus of dry microparticles into a detachable reservoir, which is fitted with a permeable mask. The detached, collapsible reservoir/mask containing the aerosol cloud is gently pressed on the face of the subject, who inhales the aerosol over the span of up to 30 seconds. The aerosol generator can be re-used hundreds of times, while the mask/reservoir is disposable and not re-used to treat different subjects in order to prevent disease transmission. Older cooperative subjects may inhale a single breath from the reservoir through a mouthpiece.

## REPRESENTATIVE VACCINES AND PHARMACEUTICALS MICRONIZED BY CAN-BD

- **Vaccines:** live attenuated measles virus vaccine, influenza live virus vaccine, hepatitis B surface of antigen (HBsAg) vaccine
- **Oligonucleotides:** siRNA
- **Antibodies:** PRIMATIZED® anti-CD4, human IgG, anti-human lambda light chain
- **Enzymes:** $\alpha_1$-antitrypsin, trypsinogen, lactate dehydrogenase, lysozyme, insulin, alkaline phosphatase
- **Sugar excipient stabilizers:** myo-inositol, trehalose, mannitol, sorbitol, lactose, sucrose
- **Antibiotics:** moxifloxacin hydrochloride, tobramycin sulfate, amoxycillin, doxycycline, cefazolin, ciprofloxacin hydrochloride, amikacin, capreomycin, rifampin
- **Other:** phytosterols, PEG, PVP, hydrolyzed gelatin, sodium chloride, DPPC, salbutamol
- **Components in formulations:** buffers (tricine, sodium or potassium phosphate, sodium acetate, sodium citrate), surfactants (palmitic acid, stearic acid, Tween 20, Tween 80, Pluronic F68), amino acids (arginine, alanine, histidine, leucine, methionine), and metal chelating agents (EDTA, DTPA)

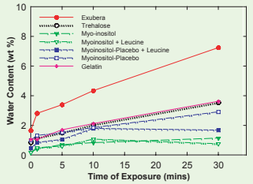
## LOW DEAD VOLUME TEE FOR CAN-BD




## PRINCIPLES OF THE CAN-BD PROCESS

- In CAN-BD, dense $CO_2$ and a liquid aqueous solution or suspension are intimately mixed in a low volume mixing tee at room temperature and 83 bar.
- The mixture as an emulsion is rapidly expanded through a flow restrictor (ID of 75 to 380 µm) into a drying chamber at near atmospheric pressure to generate aerosols of microbubbles and microdroplets.
- Warm nitrogen gas is used to maintain the drying chamber at near ambient temperatures (usually below 60 °C) to dry the aerosols and generate dry powders. With myo-inositol based formulations, residual water can be < 0.5%.

**Kinetics of Water Uptake at 70% Relative Humidity**




| Formulation ID | | Fine Particle Fractions (% of loaded mass) < 5.8 µm | Fine Particle Fractions (% of loaded mass) < 3.3 µm | Glass Transition Temperature (Tg) Onset (°C) | Glass Transition Temperature (Tg) Midpoint (°C) | Moisture Content (%) |
|---|---|---|---|---|---|---|
| M50 | Placebo | 44 ± 2 (n=4) | 19 ± 2 (n=4) | 61 ± 2 (n=4) | 65 ± 4 (n=4) | 1.0 ± 0.3 (n=4) |
| | Active | 50 ± 1 (n=2) | 21 ± 2 (n=2) | 53 | 61 | – |
| M35man15 | Placebo | 43 | 19 | 60 | 61 | 0.9 |
| | Active | 51 ± 5 (n=2) | 25 ± 4 (n=2) | 46 ± 5 (n=4) | 53 ± 4 (n=4) | 0.8 ± 0.6 (n=4) |
| M25man25 | Placebo | 22 | 9 | 50 | 51 | 0.8 |
| M15man35 | Placebo | 44 | 17 | 52 | 55 | 0.9 |
| M35S15 | Placebo | 48 | 20 | 46 | 50 | 1.3 |
| M25S25 | Placebo | 35 | 20 | 33 | 38 | 1.4 |
| M15S35 | Placebo | 43 | 19 | 36 | 41 | 0.5 |
| M50L2 | Placebo | 44 ± 1 (n=3) | 20 ± 2 (n=3) | 64 ± 4 (n=3) | 67 ± 2 (n=3) | 0.8 ± 0.1 (n=3) |
| | Active | 46 ± 11 (n=2) | 27 ± 5 (n=2) | 49 ± 1 (n=2) | 57 ± 1 (n=2) | 0.8 ± 0.2 (n=2) |

Myo-inositol based placebos are amorphous as determined by XRD.
n = number of replicate powder lots tested. If not indicated, only one powder lot was tested.
The error (±) is the standard deviation of the "n" replicate powder lots tested.
With a little more aggressive drying during CAN-BD processing powders of myo-inositol based formulations with < 0.5% moisture content were produced.

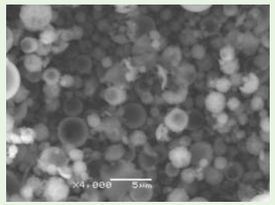
## GRAND CHALLENGES IN GLOBAL HEALTH

***GRAND CHALLENGE 2:*** **Thermostable vaccines** ***GRAND CHALLENGE 3:*** **Needle-free vaccines**

### GENTLY DRIED LIVE VIRUS VACCINES

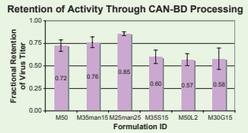
- **Dr. Albert Sabin (1983): "Immunization by inhalation of aerosolized measles vaccine provides a procedure that could make such a mass immunization program possible, especially in parts of the world where measles contiues to be a serious problem..."**
- **CAN-BD may now offer the particle synthesis technology that will enable us to realize Dr. Sabin's prediction of 21 years ago.**
- **Earlier field studies[10-13] of wet mist pulmonary delivery of live attenuated measles virus in Mexico showed that aerosol immunization led to a lower attack rate, 0.8%, than sub-cutaneous injection, 14%.**
- **Advantages of formulating vaccines as inhalable dry powders**
  - **1) Glassy sugar solid matrices give greater stability to sensitive biologicals than aqueous formulations.**
  - **2) Simple needle-free devices can dispense individual doses with no cross-contamination.**
  - **3) Powder aerosols generated by active dry powder inhalers offer narrow particle size distributions.**

## Inhalable Dry Powder Measles Vaccine Formulation Development



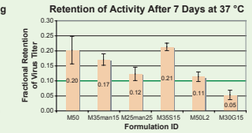
Scanning electron microscopy (SEM) image of particles of a myo-inositol based measles vaccine formulation formed at 50 °C from an aqueous solution containing 11% total dissolved solids.

**Retention of Activity Through CAN-BD Processing**




| Formulation ID | Number of replicates | Mean | Std Dev | Std Err Mean |
|---|---|---|---|---|
| M50 | 8 | 0.72 | 0.18 | 0.06 |
| M35man15 | 5 | 0.76 | 0.13 | 0.06 |
| M25man25 | 2 | 0.85 | 0.03 | 0.02 |
| M35S15 | 4 | 0.60 | 0.15 | 0.07 |
| M50L2 | 5 | 0.57 | 0.15 | 0.07 |
| M30G15 | 3 | 0.58 | 0.21 | 0.12 |

**Retention of Activity After 7 Days at 37 °C**

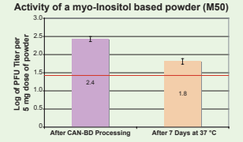



| Formulation ID | Number of replicates | Mean | Std Dev | Std Err Mean |
|---|---|---|---|---|
| M50 | 4 | 0.20 | 0.09 | 0.05 |
| M35man15 | 5 | 0.17 | 0.04 | 0.02 |
| M25man25 | 2 | 0.12 | 0.03 | 0.02 |
| M35S15 | 2 | 0.21 | 0.02 | 0.01 |
| M50L2 | 3 | 0.11 | 0.03 | 0.02 |
| M30G15 | 3 | 0.05 | 0.03 | 0.02 |

**Legend of Formulation IDs**

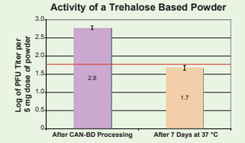
M50 = 50 g/L myo-inositol, other components*
M35man15 = 35 g/L myo-inositol, 15 g/L mannitol, other components*
M25man25 = 25 g/L myo-inositol, 25 g/L mannitol, other components*
M15man35 = 15 g/L myo-inositol, 35 g/L mannitol, other components*
M35S15 = 35 g/L myo-inositol, 15 g/L sorbitol, other components*
M25S25 = 25 g/L myo-inositol, 25 g/L sorbitol, other components*
M35S15 = 15 g/L myo-inositol, 35 g/L sorbitol, other components*
M50L2 = 50 g/L myo-inositol, 2 g/L leucine, other components*
M30G15 = 30 g/L myo-inositol, 15 g/L gelatin, other components*
*Other components = 25 g/L gelatin (except for M30G15), 16 g/L arginine-HCl, 1 g/L alanine, 2.1 g/L histidine, 3.5 g/L lactalbumin hydrolysate, 3 g/L tricine, pH 6.5 - 7.0

**Activity of a myo-Inositol based powder (M50)**




Myo-inositol based powder formulations survive 7 days at 37 °C with less than 1 log loss of activity.

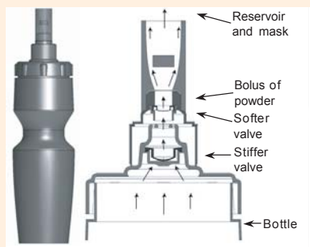
**Activity of a Trehalose Based Powder**




Trehalose based powder formulations do NOT survive 7 days at 37 °C with less than 1 log loss of activity.

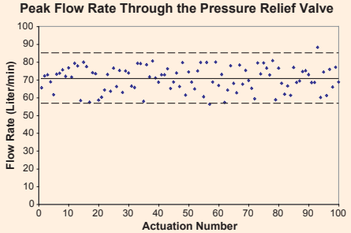
## The PuffHaler Active DPI Development

Plume of CAN-BD generated powder dispersed from the PuffHaler.



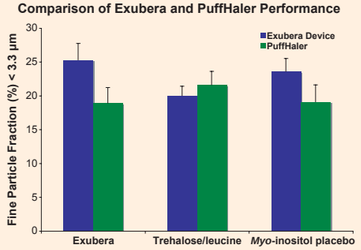

The PuffHaler active DPI, with valves and bottle aerosolizer (images at left), and detachable reservoir and mask with aerosolized dose being emitted (at right) upon gently squeezing the collapsible bag reservoir.

**Peak Flow Rate Through the Pressure Relief Valve**



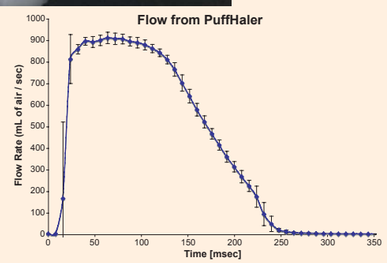

The PuffHaler shows good reproducibility over multiple actuations. After 100 tests, only one run was outside the range of +/- 20% of the mean flow rate and no noticeable decrease in performance was observed.

**Comparison of Exubera and PuffHaler Performance**




The PuffHaler has shown performance similar to Nektar/Pfizer's Exubera device when tested with three different powders, and measuring FPF.

**Flow from PuffHaler**



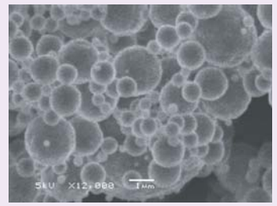

The flow profile out of the PuffHaler, and the dispersive energy it provides to powders, is consistent across runs. The above figure shows the average flow rate over time for 10 actuations.

## CONCLUSIONS

- A stabilization, nebulization, and drying method (CAN-BD) has been presented that can manufacture dry powders of vaccines, oligonucleotides, proteins, enzymes, antibodies,and other drugs without unacceptable degradation.
- Antibodies, vaccines and enzymes retain activity during CAN-BD processing and long-term storage when appropriately buffered and stabilized with high purity sugars, surfactants, and/or other excipients.
- Drying requires only seconds at near-ambient conditions of temperature and pressure.
- Fluid ratios, pressures, and solute concentrations determine particle size (usually 1 - 5 µm) for optimal pulmonary delivery.

## siRNA

Scanning electron microscopy (SEM) image of pure siRNA particles formed at 50 °C from a 10% aqueous solution.



Scanning electron microscopy (SEM) image of particles of siRNA in a *myo*-inositol based formulation formed at 50 °C from a 10% aqueous solution (50 g/L siRNA, 50 g/L *myo*-inositol).

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- Additional references: *www.AKTIV-DRY.com.*

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