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ORIGINAL RESEARCH ARTICLE

Optimization and Scale Up of Spray Dried CPZEN-45 Aerosol Powders for Inhaled Tuberculosis Treatment

Ian E. Stewart[1] · Phillip G. Durham[2] · Jacob M. Sittenauer[3] · Aranza P. Barreda[3] · Grayson W. Stowell[4] · Carmella Moody[4] · Jeffery B. Mecham[1] · Catherine Simpson[4] · Sharon Daily[4] · Sara E. Maloney[1] · Mark D. Williams[3] · Diana Severynse-Stevens[4] · Anthony J. Hickey[1]

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Abstract

Purpose Tuberculosis (TB) remains one of the most serious diseases caused by a single organism. Multiple (MDR) and extensively (XDR) drug resistant disease poses a threat to global health and requires new drugs and/or innovative approaches to treatment. A number of drugs have been proposed as inhaled therapy for TB, frequently prepared by spray drying. CPZEN-45 is a novel anti-tubercular drug that has poor oral bioavailability but has shown promise when administered via inhalation.

Methods Excipient-free CPZEN-45 HCl has been spray dried into a powder with physicochemical characteristics, aerodynamic particle size distribution, and delivered dose suitable for consideration as an inhaled product.

Results The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of the powder delivered using a RS01 inhaler were 2.62 ± 0.04 µm and 1.76 ± 0.09, respectively. Additionally, the powder was physically and chemically stable after storage at ambient conditions for >1.5 years with particle size similar to freshly manufactured product. Overages in spray dried powder were recycled the powder and resprayed into drug product likewise resulting in negligible change in quality thus allowing for further preclinical characterization as necessary. CPZEN-45 was scaled up using pilot-scale manufacturing equipment where the density of the powder was increased to facilitate larger delivered doses without affecting the aerodynamic performance properties.

Conclusion The spray dried powders were suitable for pharmacokinetics, efficacy and preclinical toxicology studies. The final method of manufacture may be used directly for CGMP particle manufacture to support IND and Phase I clinical trials and beyond.

Keywords aerosol · inhaled drugs · spray drying · scale-up · tuberculosis

Introduction

Tuberculosis remains a serious threat to global health. An increase in the incidence of disease is the unfortunate outcome of the global pandemic of COVID-19 [1–3]). While approaching 2 million individuals present with disease annually a significant proportion have drug resistant disease requiring challenging and complicated treatment regimens [4]. New drug treatments are being developed with some success but also presenting a new set of challenges with respect to safety [5].

New drugs will always be the solution of choice to treat tuberculosis. However, some new drugs may require alternative routes of administration if they are not orally bioavailable. CPZEN-45, a caprazamycin derivative, is a potent drug

Ian E. Stewart and Phillip G. Durham contributed equally to this work.

✉ Anthony J. Hickey
ahickey@rti.org

[1] Technology Advancement and Commercialization, RTI International, Durham, NC, USA

[2] Department of Pharmacoengineering and Molecular Pharmaceutics, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[3] CritiTech Particle Engineering Solutions, Lawrence, KS, USA

[4] Social, Statistical, and Environmental Sciences, RTI International, Durham, NC, USA

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candidate that targets, WecA A (a mycobacterial phosphoroglyosyltransferase involved in arabinogalactan biosynthesis a fundamental process in lipid cell wall biogenesis), a new mechanism of action [6, 7]. Consequently, it is not subject to cross-resistance with any existing drugs.

Inhaled therapies for the treatment of tuberculosis were first adopted in response to streptomycin resistance over 70 years ago [8]. The advent of new drugs over the following 20 years led to this approach being abandoned. In the 1990s, the incidence of AIDS/HIV resulted in a significant increase in tuberculosis in immunocompromised patients. As a result, inhaled therapy was adopted again most notably with the administration of the cytokine, IFN-gamma [9]. Subsequently, the field of inhaled therapy for tuberculosis has seen increasing interest globally [10].

Dry powder inhalers have been employed widely for the delivery of drugs to treat asthma and chronic obstructive lung disease [11]. Antibiotics generally require high dose delivery. The advent of spray drying as a method of manufacture of dry powders suitable for pulmonary delivery makes therapeutic dose delivery possible [12, 13]. Low density particles produced by spray drying are highly dispersible because of the small interparticulate forces. Consequently, it is easier to disperse large doses of low density particles than high density particles where aggregations occurs due to large interparticulate forces. Notably, tobramycin is the best commercial example of this formulation approach [14]. Capreomycin sulfate, normally administered parenterally, has also been clinically evaluated [15].

The following studies, uniquely in our experience, describe not only the initial formulation approach to the preparation of CPZEN-45 dry powders but also the transition through scale-up to show the way in which the product can be transferred from one apparatus to another and the metrics that establish the robustness of the process. We also describe a "recycling" or re-spray drying of the CPZEN powder a) to facilitate development of a drug with limited availability and b) showcase the unique stability of CPZEN-45 after undergoing spraying, dissolution, and respraying processes.

Materials and Methods

Materials

Powder Formulation Materials

CPZEN-45 HCl drug substance was synthesized and provided by Zhejiang Hisun Pharmaceutical Co. (Taizhou, China) and Institute for Microbial Chemistry (Tokyo, Japan). CPZEN and CPZEN HCl salt have aqueous solubilities of 1.1 mg/mL and 14.6 mg/mL, respectively.

Spray Drying and Powder Manufacture

Bench Scale

Microparticles of CPZEN-45 were spray dried using a Buchi B-290 with a two fluid nozzle (inner orifice = 0.7 mm, outer orifice = 1.5 mm) and standard cyclone utilizing a grounding cable connected to the collection vessel. Typical batch sizes ranged from 500 mg – 4 g CPZEN-HCl API. A small digital hygrometer/thermometer was placed at the air inlet of the spray dryer where relative humidity (RH) and temperature were monitored during spray drying. Typical values were 20–35% RH and 20–27°C. The aspirator, inlet temperature, peristaltic pump speed, and atomizing gas (Ultra High Purity N_2; Airgas, NC, USA) flow rate were set to 100%, 190°C, 5 mL/min (15% on B-290), and 1052 L/h (50 mm on B-290 rotameter), respectively. Solutions of spray dried CPZEN-45 consisted of 10 mg/mL active pharmaceutical ingredient (API) in deionized (DI) water. Resulting outlet temperatures varied between 85 and 95°C. Powders were collected from the collection vessel/lid and stored under silica desiccant inside a desiccator (RH ~ 20–45%; silica desiccant outside of powder vials). Percent recovery was calculated as a ratio of initial API mass to recovered dry powder mass.

Pilot Scale

Scaled up manufacture of CPZEN-45 was performed on a SD30 Spray Dryer. Typical batch sizes ranged from 7.5–340 g (see Tables SI-1 and SI-2). Similar variables to the Buchi-B290 were adjusted including aspiration rate, N_2 atomization rate, solution concentration and feed speed, and inlet temperature. These conditions are summarized in Tables S1-S2 in the Supplemental Materials along with results for yield, particle size via laser diffraction, moisture content, and bulk/tapped density.

Storage Description

Results comparing CPZEN-45 dry powder characteristics before and after storage were gathered after 18–24 months. Large quantities of powder were initially spray dried and stored in amber glass bottles sealed with Parafilm. During the storage period, the powder bottles were stored with small desiccant pouches (McMaster, Atlanta, GA, USA) in insulated foam boxes under ambient conditions). When required, the powders were shipped domestically under similar conditions. No other precautions were taken.

Physicochemical Characterization

Particle Morphology Characterization and Geometric Size Distribution

Double-sided copper or carbon tape were secured to an aluminum scanning electron microscopy (SEM) stub. A small amount (< 1 mg) of CPZEN-45 HCl powder was deposited on the stubs and subsequently sputter coated with Au/Pd (Hummer Sputtering System, Anatech Ltd., Union City, CA) for 2 mins. Micrograph images were captured using a Quanta 200 SEM (FEI, Hillsborough, OR) and geometric diameters were determined from these images by averaging ImageJ software measurements of individual particles (n = 200).

Thermal Analysis and Moisture Content

Thermogravimetric analysis (TGA) was performed using a Q50 instrument from TA Instruments (New Castle, DE, USA). A portion of solid (3–5 mg) was loaded onto a platinum TGA sample holder and heated under nitrogen gas from 30°C to 500°C at 5°C/min to monitor weight loss as a function of temperature. Results were extracted using TA Instruments' Universal Analysis 2000 software.

To perform differential scanning calorimetry (DSC; Q200, TA Instruments, New Castle, DE, USA), solids (<5 mg) were first loaded into aluminum pans and crimped. The experiment was performed by ramping to 150°C using a rate of 5°C/min.

CPZEN-45 API and spray dried powder were analyzed for moisture content by Karl Fischer Titration (K-F) using a Mettler Toledo V30 Compact Volumetric Karl Fischer Titrator (Mettler Toledo, Columbus, OH, USA). Samples were analyzed using 25–30 mg of material per replicate (n = 3).

Crystallinity

Approximately 50 mg of CPZEN-45 was loaded onto a flat, low-diffraction silicon wafer for X-ray powder diffraction (XRPD) analysis via D8 XRD (Bruker AXS Inc., Germany). Scanning was completed from 5 to 65° 2θ at intervals of 0.02° 2θ with a 2 second dwell time at 40 kV and 40 mA using a copper anode beam source (0.154 nm wavelength). Jade version 9.6 software (Materials Data Inc., Livermore, CA, USA) was used to process the resulting patterns.

Powder Density

Bulk / tapped density was tested using an Agilent 350 Tapped Density Tester and a modified USP <616> Method I to account for small sample volumes. Results can be seen in Tables S1-S2.

Stability Assay and Analysis

Assay and related substance testing of initial and stored stability samples was completed at Alera Labs (Durham, NC, USA). Samples were stored under the following stability conditions: 25°C/60% RH and 40°C/75% RH and samples pulled periodically for testing. Testing was conducted using a Agilent 1100/1200 HPLC system with DAD (200 nm) under the following chromatographic conditions: injection volume = 5 µL, column = Agilent Zorbax SB-C18, 4.6 × 150 mm, 3.5 µm with ambient column temperature, flow rate = 0.8 mL/min, mobile phase A and B = 0.01% TFA and acetonitrile respectively, gradient program = 0 min −5% MPB, 25min – 100% mobile phase B, 26 min – 100% mobile phase B, 26.1–5% mobile phase B, and run time = 31 mins. Chromatographic purity was assessed for both the CPZEN-45 API and spray dried powder in capsules stored under identical conditions. These controlled conditions are separate from the 1.5 years of room temperature conditions (simply an amber vial of powder left on a laboratory shelf) used to assess aerodynamic stability.

Aerosol Characterization

Laser Diffraction

A Mastersizer 2000 or 3000 equipped with the Sirocco 2000 dry powder dispersion system was used to acquire laser diffraction data. Approximately 100–150 mg of powder was loaded onto the sample tray and used to generate three sets of data per sample for averaging. A feed ratio of 40% and dispersive air pressure of 2 bar were used to feed the powder into the instrument. The software was set to begin measuring for 5 seconds once the obscuration limit was reached (0.5–10).

Inertial Impaction

Aerodynamic particle size distribution (APSD) data for spray dried CPZEN-45 was generated using a Next Generation Impactor (NGI, MSI Corp., MN, USA) and following USP <601> guidelines. Powder was loaded into a size #3 hydroxyproylmethylcellulose (HMPC) capsule at a nominal weight of 10 mg and subsequently placed into either a RS00 or RS01 inhaler (Plastiape, Italy). Stages of the NGI were precoated with 1% w/w silicone oil in hexanes and the preseparator was filled with 15 mL deionized water. Once the hexanes evaporated, the NGI was assembled, the capsule pierced by actuating the inhaler, and the inhaler was placed in the mouthpiece adaptor connected to the NGI. Vacuum was pulled through the system at 60 L/min for 4 seconds to disperse the powder along the stages of the impactor. The capsule, inhaler, and NGI stages were washed with 10 mL

deionized water to collect CPZEN-45 in solution. A UV spectrometer (SynergyMX, Biotech, Winooski, VT) was used to quantify CPZEN-45 at a wavelength of 264 nm to construct APSD data sets. NGI runs were completed in triplicate. Mass median aerodynamic diameter (MMAD) was calculated by plotting the cumulative percentage of mass of CPZEN-45 deposited in the NGI stages (y – axis), using a probability scale, against the corresponding cutoff diameter (x – axis) and applying a log-linear fit on either side of 50% cumulative mass. Geometric standard deviation (GSD) was calculated by the square root of the ratio of the particle size one standard deviation above and below the median particle size (84th and 16th percentile, respectively, or 1 and − 1 on a probit scale). Fine particle fraction of the nominal (FPF_N) and emitted (FPF_{ED}) dose were calculated as a ratio of the sum of drug mass collected below 4.46 µm (Stage 3 to the micro-orifice filter) to the mass loaded in the capsule and the mass collected at the inlet of the NGI and below, respectively.

Delivered Dose

USP General Chapter <601> was used as a reference in conducting delivered dose experiments. A dry powder inhaler (DPI), loaded with a #3 HPMC capsule containing 10 mg or 30 mg nominal CPZEN-45 mass, was connected via mouthpiece to a Dosage Uniformity Sampling Apparatus (DUSA; MSP Corp., MN, USA) and Apparatus B in USP <601> was constructed. It was determined that a flow rate of 90 L/min was necessary to create a pressure drop of 4 kPa across the DPI. The inhaler was actuated and vacuum applied for 1.3 seconds corresponding to 2 L of air. The DUSA tube containing a 47 mm glass fiber filter was capped and the contents washed with 1:1 MeOH:DI water and shaken vigorously for at least 30 seconds. The contents were collected and filtered through a 0.45 µm nylon syringe filter. CPZEN-45 masses were determined by HPLC at 200 nm (UV detector, Agilent Zorbax SB-C18 column, 4 mm × 150 mm × 3.5 µm) with 0.01% TFA and acetonitrile as mobile phases A and B.

Respraying CPZEN-45 Powder

Recycling and respraying CPZEN-45 powder followed identical experimental, characterization, and analytical techniques as described above using both the Buchi-B290 and SD30.

Results

Spray Drying and Powder Manufacture

The recovery for bench scale spray drying of CPZEN-45 was 65.7 ± 3.4% (n = 22). Pilot scale batches (using SD30) resulted in yields of 67.9 ± 6.8% (n = 20).

Physicochemical Characterization

Particle Morphology Characterization

Figures 1A and 1B present SEM images of spray dried CPZEN-45 microparticles at different magnifications. Spray dried microparticles were primarily corrugated, collapsed hollow spheres. Representative images from the SD30 pilot scale spray dryer powder are shown in Figs. 2A and 2B. The bench scale hollow spheres appear to have collapsed to a greater extent than the pilot scale.

Thermal Analysis and Moisture Content

Thermal degradation data for the API and spray dried products of CPZEN-45 are shown in Fig. 3. Degradation temperatures listed below were gathered at the maxima of the weight loss derivative curves. The API (squares, solid line) did not show signs of temperature induced weight loss until 230°C followed by another event at

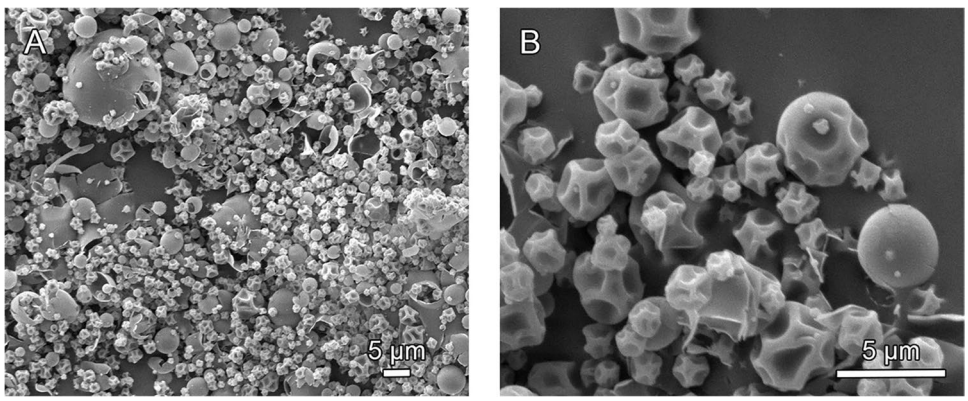

Fig. 1 **A** and **B**: SEM images of spray dried CPZEN-45 microparticles spray dried using a Buchi B290.

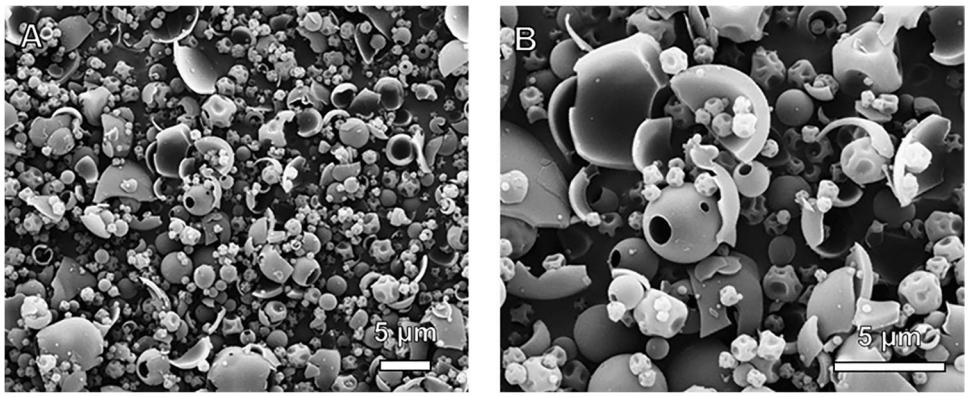

Fig. 2 **A** and **B**: SEM images of spray dried CPZEN-45 microparticles spray dried using SD30 (spray dry run SD24).

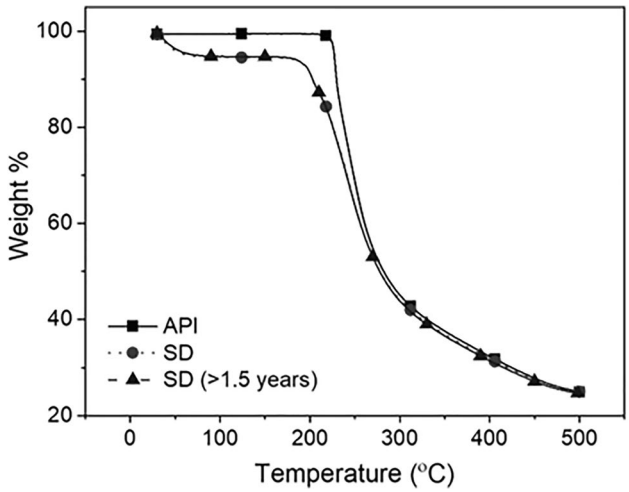

Fig. 3 Thermograms for CPZEN-45 API (square markers, solid line) and freshly spray dried (SD; circle markers, dotted line) and > 1.5 year-old spray dried material (triangle markers, dashed line).

244°C. Spray dried CPZEN-45 (circles, dotted line) similarly showcased two major degradation events at 200°C and 245°C preceded by an event below 100°C associated with a 5.48% weight loss likely due to unbound moisture [16]. No indication of significant weight loss associated with solvent evaporation was seen with the API. Moisture contents were confirmed by KF analysis: API and spray dried CPZEN-45 contained 0.91 ± 0.08% and 5.94 ± 0.09% water, respectively. After >1.5 years of storage under ambient conditions samples of spray dried CPZEN-45 did not show changes in its TGA profile (triangles, dashed line in Fig. 3) and no additional moisture uptake was evident after KF analysis: 5.67 ± 0.02%. Finally, Fig. SI-1 shows DSC results for the API and spray dried CPZEN-45 indicating minor shifts in exothermic events.

Crystallinity

Figure 4 shows XRPD diffractogram data for CPZEN-45 HCl API (squares, solid line) and spray dried CPZEN-45 (both fresh [circles, dotted line], and stored >1.5 years [triangles, dashed line]). Prior to spray drying, the drug substance is crystalline, as indicated by the sharp peaks, but the spray dried powder is amorphous and maintain its amorphous character as indicated by the broad pattern typical for amorphous material.

Chromatographic purity of the drug and the spray dried product were also assessed via HPLC. Table I shows the chemical stability of both the API and drug product over 12–18 months at accelerated aging conditions. Both the API and spray dried materials maintained purity over at least 12 months at 25°C/ 60% RH. Only slight degradation was seen over 6 months at 40°C/75% RH.

Aerosol Characterization

Laser Diffraction

Initial laser diffraction experiments were performed to characterize volume particle diameter. Figure 5A and C show laser diffraction data for freshly sprayed, >1.5 year stored, and resprayed CPZEN-45 powder. The cumulative percent of the particle size distribution undersize, by volume, referred to in the right y-axis, is shown as the sigmoid plot on the graph, with dashed lines indicating d_{10}, d_{50}, and d_{90} points. For all three powders, the d_{10}, d_{50}, d_{90}, and span values were similar, falling between 1.38–1.45 µm, 3.18–3.20 µm, 7.25–7.80 µm, and 1.82–2.02 µm, respectively. There appears to be a modest increase in span for the resprayed powder. The results are summarized in Table II. Since diffraction is a volume-based measurement a large number of smaller particles is required to get the same volume as a smaller number of large particles. The deceptive size distributions in Figs. 1 and 2 average out to a smooth volume distribution in Fig. 5.

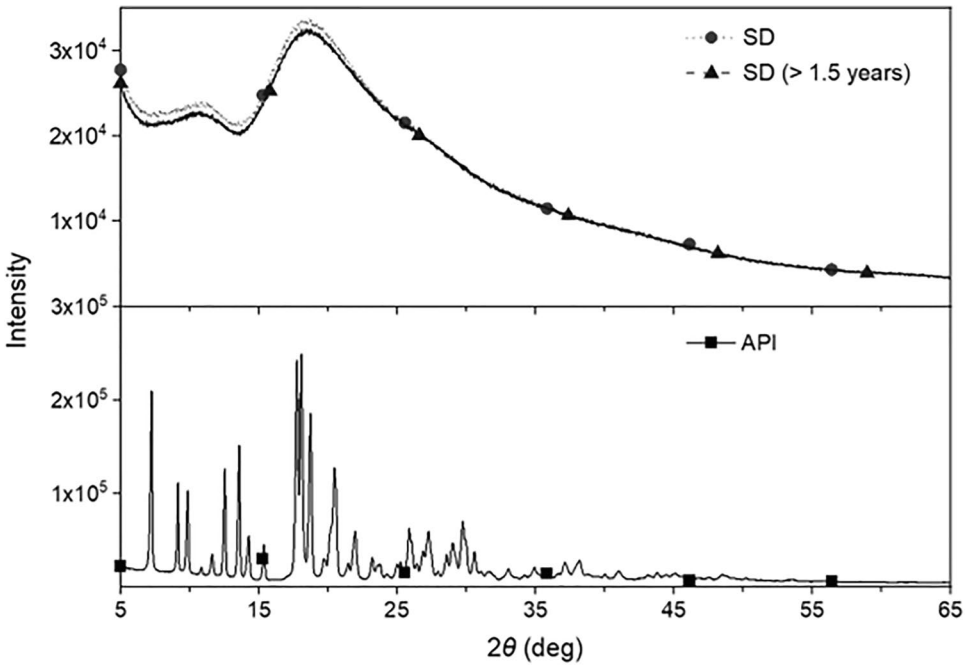

Fig. 4 XRPD diffractograms for CPZEN-HCl API (squares, solid line) and fresh (circles, dotted line) and > 1.5 year stored (triangles, solid line) spray dried CPZEN-45.

Table I Chromatographic Purity of CPZEN-45 Drug Product and Substance (in Capsules) Determined *via* HPLC After Aging Under Accelerated Conditions

	25°C/ 60% RH		40°C/ 75% RH	
	CPZEN-45 API (%)	SD CPZEN-45 in Capsule (%)	CPZEN-45 API (%)	SD CPZEN-45 in Capsule (%)
t = 0 Months	99.5	98.7	99.5	98.7
t = 3 Months	99.4	97.4	99.4	97.8
t = 6 Months	98.7	97.2	98.4	96.6
t = 9 Months	99.2	95.8	–	–
t = 12 Months	99.5	98.0	–	–
t = 18 Months	99.5	–	–	–

Small Scale Delivered Dose and Inertial Impaction

Delivered dose uniformity (DDU) was assessed for #3 HPMC capsules filled with both 10 mg and 30 mg total powder mass. When 10 mg was used, 7.1 ± 0.4 mg (n = 6) CPZEN-45 were delivered. The DDU results were similar when the dose was tripled to 30 mg total powder where 21.4 ± 0.6 mg (n = 6) CPZEN-45 were delivered.

NGI results for laboratory scale spray dried powder from the Buchi B290 are shown in Fig. 6 (for fresh [left bars] and > 1.5 years stored [right bars]) and Fig. 7 (resprayed material). Freshly sprayed powder exhibited MMAD, GSD, and FPF_{ED} of 2.62 ± 0.04 μm, 1.76 ± 0.09, and 72.0 ± 1.48%, respectively. Powder stored under ambient conditions for >1.5 years exhibited MMAD, GSD, and FPF_{ED} of 2.67 ± 0.16 μm, 1.78 ± 0.03, and 69.9 ± 0.58%, respectively.

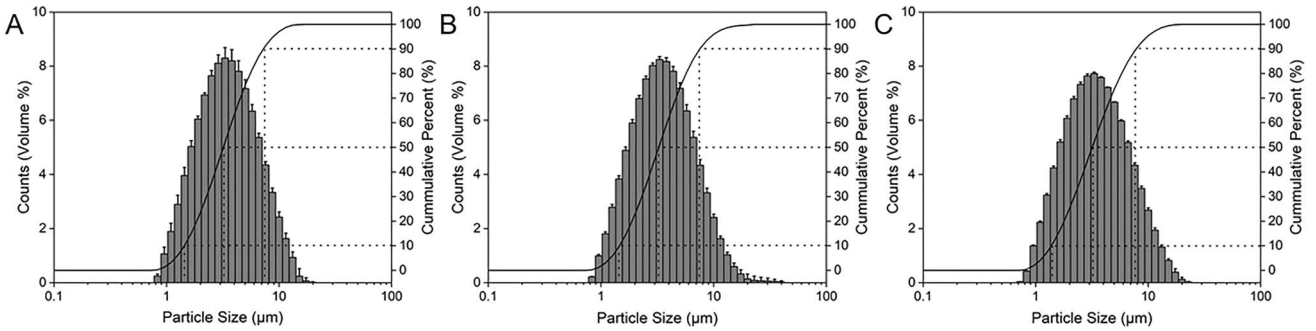

Fig. 5 Laser diffraction data for CPZEN-45 bench scale spray dried powder. From left to right: **A** freshly sprayed, **B** > 1.5 years storage, and **C** resprayed powder.

Table II Summary of Laser Diffraction Data for Freshly Sprayed, >1.5 Years Storage, and Resprayed Powder (n = 3, Mean ± SD

	Freshly Sprayed	>1.5 Years Storage	Resprayed
d10 (μm)	1.45 ± 0.06	1.46 ± 0.02	1.38 ± 0.01
d50 (μm)	3.20 ± 0.05	3.26 ± 0.05	3.18 ± 0.04
d90 (μm)	7.25 ± 0.20	7.53 ± 0.27	7.80 ± 0.15
Span (μm)	1.82 ± 0.10	1.86 ± 0.09	2.02 ± 0.02

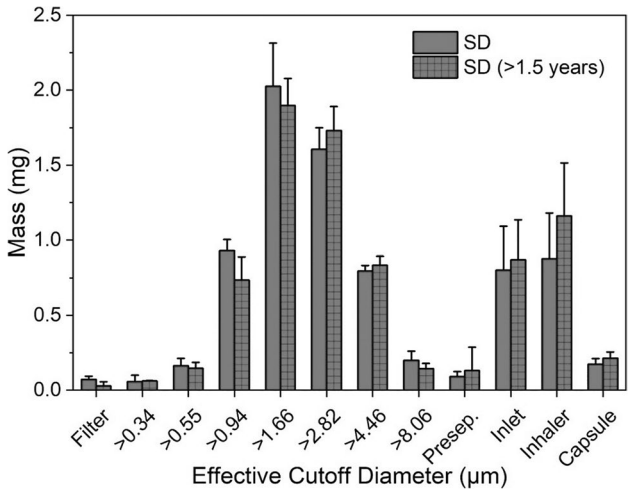

Fig. 6 APSD comparison for freshly spray dried CPZEN-45 powder (left, solid bars) and spray dried powder stored under ambient conditions for >1.5 years (right, checkered bars). N = 3 for each data set.

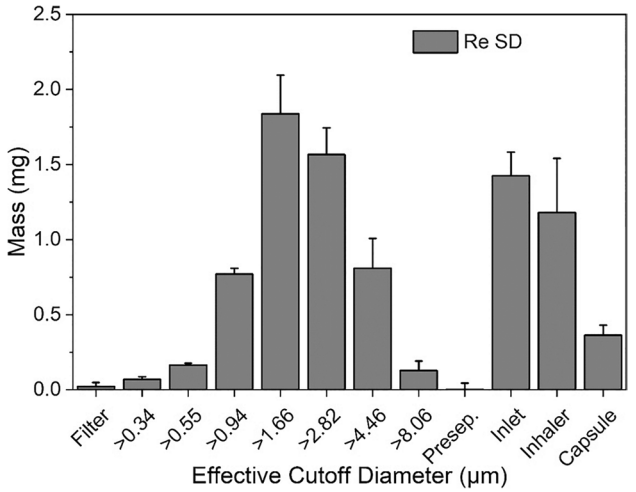

Fig. 7 APSD comparison for resprayed CPZEN-45 (n = 3).

Resprayed powder exhibited MMAD, GSD, FPF_{ED} of 2.60 ± 0.09 μm, 1.78 ± 0.09, and 67.3 ± 4.47%, respectively.

Scale Up Aerodynamic Characterization and Comparison to Lab-Scale

Aerodynamic particle size distributions (APSD; measured by Next Generation Impaction [NGI]) were similar between

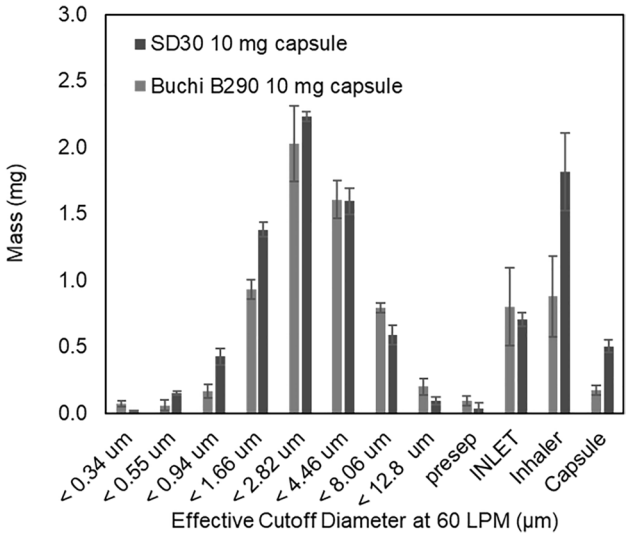

Fig. 8 APSD Comparison between Bench-scale (left, gray) and Optimal Pilot Scale Conditions (right, black) using 10 mg total powder mass in #3 capsule and RS00 inhaler.

the Buchi B290 (RTI) and optimal SD30 spray drying conditions when a 10 mg nominal dose was used. This resulted in a MMAD of 2.74 ± 0.11 μm (GSD = 1.85 ± 0.11) and 2.23 ± 0.07 μm (GSD = 1.76 ± 0.74), respectively (Fig. 8). Note that due to the low density of the initial CPZEN-45 powder, 10 mg was the maximum fill weight of a #3 HPMC capsule and this comparison was performed to ensure no drastic physicochemical or aerodynamic parameters were altered. Indeed, moisture content (~3–4% via Karl Fischer analysis) remained similar and the powders were both amorphous. Once optimal spray drying conditions were established to increase particle density and therefore total powder mass in capsule (above in Methods and below in Discussion), n = 4 SD30 runs (runs SD19 and SD22–24) were completed to assess batch variability with respect to ED and APSD following 25 mg nominal CPZEN-45 capsule fill. First, in Fig. 9, a RS00 inhaler with #3 capsule was filled with 10 mg and 30 mg spray dried powder from a representative pilot scale batch to confirm APSD similarity (batch SD19). Both the 10 mg and 30 mg fill (total powder mass) exhibited similar APSD profiles and MMAD/GSD data: 2.23 ± 0.07 μm/1.76 ± 0.06 and 2.40 ± 0.10 μm/1.82 ± 0.02, respectively. Once this behavior was observed, there was confidence in proceeding with large scale batch production to demonstrate batch to batch replication. Delivered dose values as a function of total powder fill for four SD30 scale-up batches are shown in Table III. Since there is no excipient, these data were generated by simply weighing the capsule and inhaler before and after actuation on the NGI (60 LPM, 4 seconds). Delivered dose data using the USP method are discussed above and correlate well to the results here using this

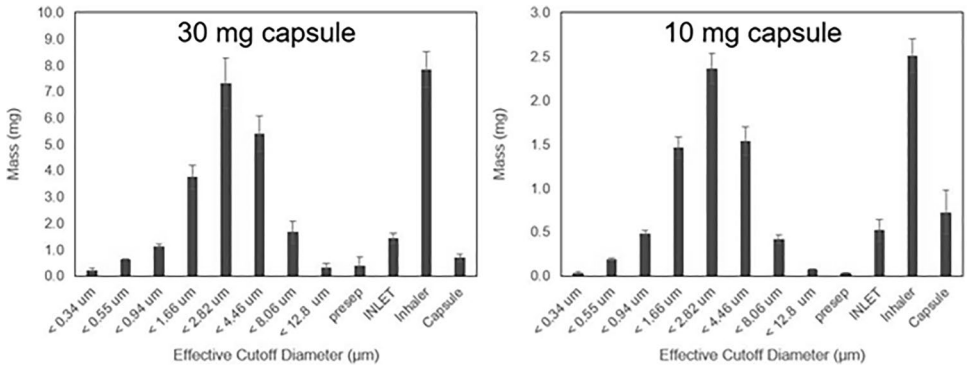

Fig. 9 Comparison between SD30 large scale CPZEN-45 powder at different capsule powder load weights 30 mg (left) and 10 mg (right).

Table III Delivered Dose Data for Four Replicate Pilot Batches (25 mg #3 HPMC Fill Weight; NGI Operated at 60 LPM for 4 Seconds Using RS00 mod. 8 Inhaler)

Development Scale-Up Batch	Delivered Dose (Total Mass)
SD19	78.8 ± 3.8%
SD22	84.4 ± 4.5%
SD23	76.7 ± 10.2%
SD24	83.7 ± 6.5%

abbreviated method. Delivered dose is roughly 75–85% for all batches based on a 25 mg fill weight. Figure 10 depicts corresponding APSD data for n = 3 NGI experiments per batch. Particle sizes for all four batches fell between 2.4–2.5 µm. Table IV displays data for CPZEN-45 masses collected after performing NGI on scale up batches SD19, SD22, SD23, and SD24, respectively (using non-validated methods; briefly CPZEN-45 free base, accounting for moisture, was used as the standard curve during UV spectroscopy readings at 264 nm to find stage masses similar to methods discussed above). These tables include metrics for the batches including MMAD, GSD, ED, fine particle fraction of the emitted dose (FPFED; ratio of mass collected <4.46 µm to mass collected emitted from the inhaler) and fine particle dose (FPD; total mass collected <4.46 µm; stage 3 and below from the NGI at 60 LPM). Note delivered doses are likely slightly lower here than in Table III considering CPZEN-45 free base + moisture free nominal mass was used in addition to NGI stage losses, non-validated collection inefficiencies, etc. Table V summarizes several other CPZEN-45 powder characteristics from these four scale-up batches supporting their similarity. Finally, the batch powder was compared using two different inhalers: RS00 and RS01 (Fig. 11). This was to ensure APSD metrics were similar in moving from the RS00 to an updated RS01, both with 25 mg CPZEN powder (both from Plastiape but the latter more suitable for product development). Both inhalers showed similar APSD with RS00 MMAD = 2.40 ± 0.10 µm and GSD = 1.82 ± 0.02 (notably same data as in Fig. 10) and RS01 MMAD = 2.54 ± 0.03 µm and GSD = 1.82 ± 0.02.

Discussion

CPZEN-45 has been developed as an inhaled product intended to be delivered via an RS01 dry powder device. The drug product is produced by spray drying of the drug substance from an aqueous solution without the aid of

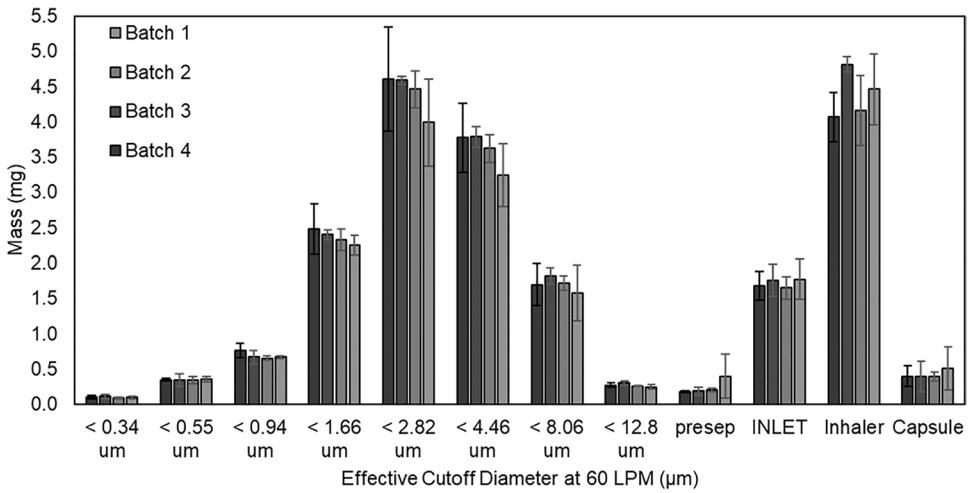

Fig. 10 APSD comparisons for four replicate scale up batches (25 mg #3 HPMC fill weight; NGI operated at 60 LPM for 4 seconds using RS00 mod. 8 inhaler). From right to left: Batch 1 = SD19 (right-most bars), Batch 2 = SD22 (second bars from right), Batch 3 = SD23 (second bars from left), and Batch 4 = SD24 (left-most bars).

Table IV NGI Cascade Impactor Results for Four Replicate Pilot Batches

Batch	SD19		SD22		SD23		SD24	
Component	Average (3 samples) mg	St.Dev	Average (3 samples) mg	St.Dev	Average (3 samples) mg	St.Dev	Average (3 samples) mg	St.Dev
Capsule	0.40	0.07	0.40	0.07	0.39	0.21	0.40	0.15
Inhaler	4.16	0.49	4.16	0.49	4.82	0.11	4.07	0.35
Inlet	1.65	0.15	1.65	0.15	1.76	0.23	1.68	0.20
Presep	0.21	0.03	0.21	0.03	0.19	0.06	0.18	0.01
< 12.8 μm	0.26	0.01	0.26	0.01	0.30	0.03	0.27	0.03
< 8.06 μm	1.72	0.10	1.72	0.10	1.82	0.11	1.70	0.30
< 4.46 μm	3.63	0.20	3.63	0.20	3.79	0.14	3.77	0.49
< 2.82 μm	4.46	0.26	4.46	0.26	4.59	0.06	4.60	0.74
< 1.66 μm	2.33	0.16	2.33	0.16	2.40	0.07	2.49	0.36
< 0.94 μm	0.66	0.04	0.66	0.04	0.67	0.09	0.76	0.10
< 0.55 μm	0.34	0.05	0.34	0.05	0.35	0.08	0.35	0.02
< 0.34 μm	0.10	0.01	0.10	0.01	0.11	0.03	0.11	0.02
Sum	19.91	1.01	19.91	1.01	21.19	0.14	20.38	1.77
MMAD (μm)	2.40	0.02	2.45	0.02	2.47	0.02	2.41	0.01
GSD	1.82	0.01	1.78	0.01	1.79	0.03	1.79	0.01
FPD (<4.46 μm)	11.51	0.69	11.51	0.69	11.91	0.34	12.09	1.71
DD (%) (free base)	66.73	3.37	66.73	3.37	69.47	1.15	69.20	9.21

Table V CPZEN-45 Powder Characteristics for Four Scale-Up Batches

Development Scale-Up Batch	d_{10} (μm)	d_{50} (μm)	d_{90} (μm)	Yield (%)	Moisture Content (%)	Crystallinity
SD19	0.805	2.49	5.65	61.86	3.52	Amorphous
SD22	0.814	2.47	5.63	74.94	3.53	Amorphous
SD23	0.847	2.48	5.58	71.86	3.25	Amorphous
SD24	0.818	2.49	5.66	74.19	4.38	Amorphous

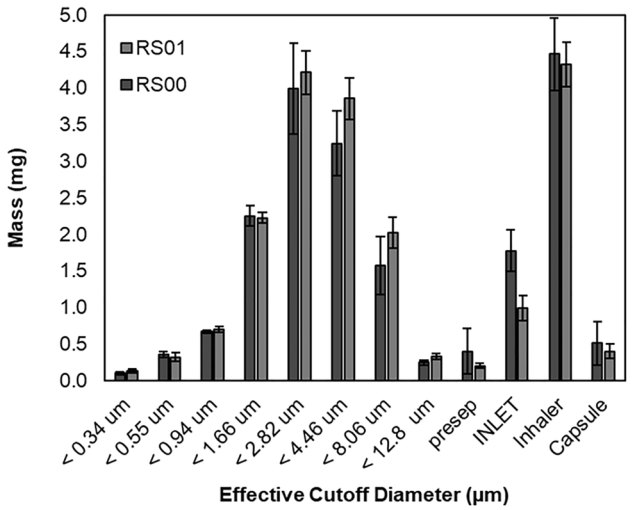

Fig. 11 SD30 batch SD19 spray dried CPZEN-45 powder APSD using two dry powder inhaler versions: RS00 (left) and RS01 (right).

excipients, solubilizing agents, or pH adjustments. Spray drying has been conducted at bench scale and pilot scale with little disruption in product characteristics. During development, physicochemical properties of the CPZEN-45 spray dried powder such as morphology, crystallinity, melting point, water content, and degradation features were assessed and proved suitable for further drug development. Aerodynamic properties including particle size and delivered dose were analyzed and indicated the drug could be delivered at respirable particle sizes.

Initial spray drying development has proceeded through a bench-scale spray dryer, Buchi B-290. This spray-drying method is widely used in the pharmaceutical industry as a strategy for dry powder production for inhaled products in addition to amorphous material manufacture [12]. For inhaled products, it is generally believed that the drug product needs to have an aerodynamic diameter between 1 and

5 µm to be considered inhalable [17–19]. There are several variables that can be tuned during the spray drying process to produce microparticles for inhaled products. Briefly: the drug substance is dissolved or suspended in a solvent that is fed to a heated nozzle via peristaltic pump. An atomizing gas is fed concurrently through the nozzle (typically N_2 for pharmaceutical development) under pressure to shear the liquid API feed and generate droplets. These droplets are quickly evaporated and solid drug particles are precipitated. Input conditions such as precursor solids concentration, atomizing gas pressure, precursor feed rate, and inlet temperature dictate output conditions such as droplet evaporation rate and outlet temperature which in turn dictate particle morphology, size, and moisture content that ultimately determine aerodynamic properties.

Previous development of a CPZEN-45:capreomycin combination inhaled anti-TB product was used as a guide [20]. The experimental conditions outlined above yielded a drug product with physicochemical and aerodynamic properties deemed suitable for an inhaled product: amorphous, low density microparticles. We were also able to show the powder is stable for >1.5 years and can be redissolved and re-spray dried (to facilitate further characterization) without adverse impact on aerodynamic properties.

CPZEN-45 microparticles produced at the bench scale were intended to be delivered from capsules by a dry powder inhaler: RS00 from Plastiape. However, only ~10 mg of this powder could fit in the capsules and it was necessary to increase the density of the powder to increase fill weight in capsules and thus ultimately the per capsule dose to the patient. This would also have the effect of lowering the number of patient capsule consumption and presumably increasing patient compliance.

The spray-drying process was transferred to a pilot scale production facility (CritiTech PES, Lawrence, KS) The goals of the project were to: 1) conduct spray drying (SD) runs on the Buchi B-290 spray drying systems to transfer CPZEN-45 manufacturing methods, 2) scale-up the spray drying process of CPZEN-45 on the SD30 spray drying system, and 3) increase density of spray dried material to increase capsule loading.

Small-scale Buchi B-290 runs of CPZEN-45 conditions were adopted as the basis for the large-scale system conditions. SD1 was conducted at 10 mg/mL and similar conditions to the reference material run, except with the high performance (HP) cyclone. The HP cyclone was employed when materials were limited to improve yield by more efficiently collecting fine particles (<1 µm). Following the run, it was determined that although the material produced was promising, moving forward with the HP cyclone (additional collection of fines) may not be representative/scalable. Therefore, SD2–SD4 were run using the standard Buchi cyclone. SD2 used conditions duplicating feed concentration, feed rate, atomization rate, and outlet temperature from SD1 but with the standard cyclone. SD3 used conditions duplicating feed rate, atomization rate, outlet temperature, and cyclone from SD2 but with 20 mg/mL feed concentration. SD4 used conditions duplicating feed rate, atomization rate, outlet temperature, and cyclone from SD2 and SD3 but with 30 mg/mL feed concentration.

With no substantial differences observed between SD2, SD3, and SD4 samples, the 30 mg/mL feed concentration from SD4 was selected to move into scale up SD30 runs due to improved throughput/manufacturing rate.

SD5, SD6, and SD7 runs on the SD30 spray dryer were focused on nozzle selection, with the SU1A nozzle from SD7 selected for use in future runs. SU1A was selected when off-line testing showed that the low feed rate of 20 mL/min resulted in inconsistent atomization when using the SU2 and SU4 nozzles; though 20 mL/min is a high flow rate for the Buchi lab scale equipment it is considered low using the SD30 pilot scale spray dryer. SD8 run tested decreased inlet/outlet temperatures, with the goal of producing more homogeneous pea/raisin morphology and increased bulk and tap density; SD8 exhibited increased bulk density. Therefore, SD9 used the same inlet/outlet temperature setpoint, but increased atomization energy, again an improvement in bulk and tap density was observed following the parameter changes in SD9. SD10 run tested decreased atomization energy, to determine if there was a correlation between atomization energy and bulk and tap density. SD11 decreased the atomization temperature; however, this change did not seem to influence the desired product attributes as results were similar to SD9. SD12 tested the SU-HTE91C nozzle. This nozzle uses the same fluid cap as the SU1A, but uses an air cap which supplies higher levels of atomization gas. As a result, inlet temperature had to be increased to retain the outlet temperature of ~82°C. SD12 exhibited similar properties to SD9 and SD11, but with a decreased PSD and slightly increased B/T density. SD13 tested increased atomization energy. This change with the SU-HTE91C nozzle did not show improvement in materials produced. SD14 decreased feed concentration to 20.02 mg/mL This change did have an appreciable effect on the product. Following this run, it was theorized that the increased atomization gas volume associated with the SU-HTE91C nozzle may "overwhelm" all other parameter adjustments. Therefore, moving away from the SU-HTE91C nozzle was deemed necessary.

At this point, with the data produced in runs SD5–SD13, there was a large enough data set to conduct statistical analysis of the results and parameter adjustments with JMP statistical analysis software. Trends suggested that increased atomization energy and decreased outlet temperature using the SU1A nozzle would result in increased bulk and tap density while retaining PSD characteristics. SD15 increased atomization energy to the maximum pressure (107–108 psig)

and decreased the outlet temperature to 79–80°C. Additionally, the feed concentration was increased to 39.91 mg/mL with the hope of increasing shell thickness. The resulting material had a similar PSD to previous runs but an increase of ~15% in bulk density and capsule fill weight. The conditions and results were then added to the JMP model and improved the "fit", supporting the accuracy of the model.

Due to decreasing outlet temperatures, additional testing was conducted to insure the SD15 material was amorphous in nature before moving to SD16. Testing included X-ray powder diffraction and differential scanning calorimetry. Results showed material to be amorphous, therefore, another pilot batch was conducted, run SD16, with the same sample feed concentration and atomization gas pressure as SD15, but decreased the inlet/outlet temperature to achieve an outlet temperature of 70°C. SD16 exhibited similar PSD to previous runs but with an increase of 32% in bulk density and an increase of 12% capsule fill weight. Interestingly, SD16 results closely resembled the result from SD1 on the Buchi, which produced the highest density material of the entire project.

SD17 produced at decreased outlet temperature (60°C) resulted in the increased tapped density and capsule fill weights. SEM micrographs seemed to show a decrease in broken particles and capsule fill weights exhibited an increase of ~7%. SD18 produced at decreased outlet temperature (50°C) resulted in similar tapped density to SD16 and SD17 but increased capsule fill weights. The material appeared to have less static and flowed better than SD16 and SD17. The water content increased significantly (+47%) from SD17. The moisture content of the spray dried particles is summarized in Tables SI-1 and SI-2. Moisture content of the particles is expected to increase as the outlet temperature decreases."The yield decreased ~10% from SD17. Due to the loss in yield and concerns that the lower outlet temperature (50°C) may cause crystallization, the outlet temperature was increased to 60°C for the remaining runs.

SD19 produced with increased feed rate (30 mg/mL *vs.* 20 mg/mL of all previous runs) and outlet temperature of 60°C resulted in increased bulk and tapped density but slight decrease in fill weight. The material was noted as easily compacted, holding the shape of the spatula during capsule filling. The water content decreased to 3.52% from 3.96% and yield increased ~10% when compared to SD18. The capsule fill weight was very similar to SD17 but with different density and PSD. SD20 was produced with increased drying gas rate (50 *vs.* 40 cfm of all previous runs) and did not produce the results that are generally anticipated when increasing the drying gas rate. Yield did not improve even though Dv90 and span increased. The water content decreased to 2.67% from 3.52%. Micrographs seem to show more intact spheres, suggesting more efficient drying. This may explain the increase in Dv90 while Dv10 remained effectively unchanged.

SD21 and SD22 were produced with the goal of duplicating drying conditions and material characteristics of runs SD17 and SD19, respectively. Both runs reproduced well, with slight differences observed attributed to sample-to-sample variation and method variability. Capsule fill weights of SD21 and SD22 were virtually identical to the previous runs results. With the confidence in the repeatability of the system and conditions, SD19/SD22 conditions were selected to test at larger scale due to increased manufacturing rate made possible by the higher feed rate. Therefore, SD23 and SD24 were produced using the conditions identified in SD19 and duplicated in SD22 but at an approximately 3x batch size increase (23.5 and 23.9 g *vs.* 7.5 g of previous batches). Scale-up went smoothly with yields >70%, particle size distributions unchanged from previous runs, and capsule fill weights virtually identical to SD19 and SD22 results. The only difference between the two runs was the use of a 10 L catchpot in SD24 instead of the 1 L catchpot used on previous runs. This catchpot (similar to a sollection vessel) was used to give confidence in future larger scale runs that material characteristics would remain unchanged with the system modification required for >100-g batches.

Conclusion

Drug resistant tuberculosis remains a serious component of the global health threat posed by this disease. New drugs are essential to the treatment of the disease but many are not orally bioavailable. CPZEN-45 is an example of a drug candidate that requires an alternative route of administration. Spray dried powders were prepared from drug alone in aerodynamic particle sizes, as estimated when delivered from a capsule based RS01 inhaler, suitable for pulmonary delivery. The physicochemical characteristics showed the powder was amorphous but stable on storage under conventional regulatory storage conditions. As such the powder is remarkably robust both in quality and performance. The spray dried powders were suitable for pharmacokinetics, efficacy and preclinical toxicology studies. The final method of manufacture may be used directly for CGMP particle manufacture to support IND and Phase I clinical trials and beyond.

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Author Contributions

• I.E. Stewart, P.G. Durham and A.J. Hickey made substantial contributions to the conception, experimental design and final data interpretation of the bench scale spray drying work;

• I.E. Stewart, P.G. Durham, J. Mecham and S. Maloney were responsible for data acquisition, analysis, and initial data interpretation of the bench scale spray drying work;

• I.E. Stewart, JM Sittenauer, A.R. Barreda, G.W. Stowell, CD Moody, C. Simpson, S. Daily, M.D. Williams, D Severynse-Stevens, A.J. Hickey made substantial contributions to the conception, experimental design and final data interpretation of the pilot-scale scale spray drying work;

• JM Sittenauer, A.R. Barreda, and M.D. Williams were responsible for the acquisition, analysis, and initial interpretation of data for the pilot-scale spray drying;

• I.E. Stewart, P.G. Durham, G.W. Stowell, J.M Sittenauer, M.D. Williams and A.J. Hickey drafted the manuscript;

All contributors were given the opportunity to revise the manuscript and gave final approval of the version to be published.

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Declarations

Conflict of Interest None of the authors have a financial interest in the work described beyond receiving funding from the NIAID for its conduct. All work was conducted as a not-for-profit activity.

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