JOURNAL OF AEROSOL MEDICINE AND PULMONARY DRUG DELIVERY
Volume 35, Number 0, 2022

Pp. 1–13
DOI: 10.1089/jamp.2021.0059

Preparation and Characterization of Inhalable Ivermectin Powders as a Potential COVID-19 Therapy

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Abstract

Background: Ivermectin has received worldwide attention as a potential COVID-19 treatment after showing antiviral activity against SARS-CoV-2 *in vitro*. However, the pharmacokinetic limitations associated with oral administration have been postulated as limiting factors to its bioavailability and efficacy. These limitations can be overcome by targeted delivery to the lungs. In this study, inhalable dry powders of ivermectin and lactose crystals were prepared and characterized for the potential treatment of COVID-19.

Methods: Ivermectin was co-spray dried with lactose monohydrate crystals and conditioned by storage at two different relative humidity points (43% and 58% RH) for a week. The *in vitro* dispersion performance of the stored powders was examined using a medium-high resistance Osmohaler connecting to a next-generation impactor at 60 L/min flow rate. The solid-state characteristics including particle size distribution and morphology, crystallinity, and moisture sorption profiles of raw and spray-dried ivermectin samples were assessed by laser diffraction, scanning electron microscopy, Raman spectroscopy, X-ray powder diffraction, thermogravimetric analysis, differential scanning calorimetry, and dynamic vapor sorption.

Results: All the freshly spray-dried formulation (T0) and the conditioned samples could achieve the anticipated therapeutic dose with fine particle dose of 300 μg, $FPF_{recovered}$ of 70%, and $FPF_{emitted}$ of 83%. In addition, the formulations showed a similar volume median diameter of 4.3 μm and span of 1.9. The spray-dried formulations were stable even after conditioning and exposing to different RH points as ivermectin remained amorphous with predominantly crystalline lactose.

Conclusion: An inhalable and stable dry powder of ivermectin and lactose crystals was successfully formulated. This powder inhaler ivermectin candidate therapy appears to be able to deliver doses that could be safe and effective to treat the SARS-COV-2 infection. Further development of this therapy is warranted.

Keywords: COVID-19, dry powder aerosol, inhalable ivermectin, lactose, spray drying

Introduction

There has been no threat in recent times of the magnitude of COVID-19 to human survival and economic stability, with >253 million cases and 5.1 million deaths reported globally as of November 15, 2021.[(1)] Encouragingly, several COVID-19 vaccines have been approved, and community immunization efforts of varying efficiency are underway. However, new SARS-CoV-2 variants of concern have emerged, which threaten to reduce the efficacy of current

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vaccines.[2] In addition, having widespread access to vaccines is directly dependent on the economic and political situation in each country.[3,4] Vaccine hesitancy also threatens to undermine the vaccine effort,[5] and even among vaccinated individuals a defined risk of infection, morbidity, and mortality exists.[6] The effectiveness of the immune response in immunocompromised individuals adds further complexity to the situation.[7] Therefore, the need to develop pharmacological treatments for COVID-19 remains a necessity.

Repurposing the antiparasitic drug ivermectin to treat COVID-19 is now the focus of a large clinical trial after demonstration by a number of investigators of its efficacy against SARS-CoV-2 *in vitro*.[8–10] Ivermectin, a semisynthetic product of avermectin, consists of two homologs, H2B1a and H2B1b. It has been an approved drug for human use for >30 years to treat a broad spectrum of parasitic infections.[11] It has demonstrated *in vitro* antiviral activity against several types of viruses in addition to SARS-CoV-2, such as dengue, Venezuelan equine encephalitis, and avian influenza A virus.[12]

The concern with using ivermectin orally or by injection as a therapy for COVID-19 is that achieving the concentrations of ivermectin demonstrated to inhibit SARS-CoV-2 *in vitro* would likely result in dose-limiting side-effects.[8] Despite this, a number of animal and clinical studies have been instigated to study the effect of oral ivermectin against COVID-19. Data are complicated by a high proportion of studies awaiting peer review, possible selection bias, and the lack of completed large randomized control trials; however, preliminary evidence supports a mortality benefit of ivermectin in COVID-19 patients, while indicating the need for further studies to confirm efficacy, dosing regime, and the need for combination therapy.[13]

The delivery of inhaled ivermectin could overcome the issue of dose-limiting toxicity as only small doses are required to achieve very high concentrations in the lungs and respiratory tract. We hypothesize that this will result in rapid and highly efficacious clearing of COVID-19 without the side-effects associated with high oral or parenteral ivermectin. Achieving high concentrations rapidly may also result in improved outcomes for patients with very high viral loads. The net effect of this could be reduced hospitalization and mortality, as well as reduced infectivity.

Even if oral ivermectin is shown to be effective or partially effective in treating COVID-19, pulmonary delivery of ivermectin would be targeted to the site of SARS-CoV-2 entry and key pathology. Direct delivery would conceivably result in more rapid and enhanced efficacy while requiring lower systemic doses. This could have an important role in severe cases where viral replication is already well underway, and in patients on regular medications that interact with ivermectin or who have other contraindications to standard doses of ivermectin.

A pilot study of nebulized ivermectin in rats demonstrated pharmacodynamic concentrations of ivermectin in lung tissue following this route of administration.[14] However, due to the poor aqueous solubility of ivermectin (0.0004% w/v),[15] nebulization required delivery with an ethanoic solution, which would not be suitable for human use. A recent study of inhaled ivermectin lyophilized powder in rats demonstrated safety at low doses of ivermectin ranging between 0.05 and 0.1 mg/kg. Pulmonary toxicity was demonstrated at high doses of ivermectin ≥0.2 mg/kg, with resultant dose-dependent histopathological changes and upregulation of proinflammatory and profibrotic pathways.[16] In addition, a subacute inhalation toxicity study undertaken in rats confirmed that there was no evidence of histological, biochemical, or behavioral toxicity in animals exposed to doses of up to 380 mg/m^3 for 4 h/day 5 days a week for a total of 4 weeks.[17]

This equates to an estimated inhaled dose of 2.48 mg/day or 12.4 mg/[kg·day] 5 days a week for the 4 weeks of the experiment (i.e., $0.380\,\text{mg/L} \times 6.5448\,\text{L} = 2.48\,\text{mg} = 12.4\,\text{mg/kg}$, the value of 6.5448 L was based on 4 h/day with the breathing rate of Sprague Dawley rats being 91.86/min, the minute ventilation 27.27 ± 2.39 mL/min,[18] and the average weight of rats 200 g). Using the customary assumption of 10% deposition of the inhaled dose in the rat and a safety margin of 10× for the maximum dose in humans,[19] the maximum safe dose in a human of 70 kg weight would be 8.68 mg/day (i.e., $12.4\,\text{mg/kg} \times 0.1 \times 0.1 \times 70\,\text{kg}$).

The anticipated therapeutic dose required for pulmonary delivery is $\sim 125\,\mu$g based on the reported concentration value of 5 μM to achieve effective 100% viral clearance; this corresponds to a concentration of 5 μg/mL in the airway surface fluid, with a volume of 25 mL.[20,21] Due to the low therapeutic dose required, the inhaled formulation would need to contain an excipient to improve the handling properties (flowability, capsule and device filling, and emptying) and dispersibility of the powder.[22]

Dry powder inhalers (DPIs) provide several advantages over nebulizers, including portability, accessibility, infection prevention, and economic feasibility.[23] From the limited number of approved inhalable excipients,[24] lactose is the most frequently used excipient for DPIs.[25] Spray drying is a direct single-step approach to formulate dry powders from liquid feed containing one or more substances. Unlike traditional physical mixing techniques, spray drying can offer particle engineering by controlling the process parameters to optimize the particle properties, including size distribution and morphology.[26]

In response to the urgent call of the International Society for Aerosols in Medicine (ISAM) to consider inhaled treatments for COVID-19,[27] we have successfully prepared inhalable crystalline powders of hydroxychloroquine sulfate by jet milling.[28] In this study, we aimed to prepare and characterize an inhalable dry powder of ivermectin as a potential COVID-19 therapy. Spray drying was used to produce a dry powder formulation of ivermectin, with lactose crystals selected as the excipient owing to its validated safety for pulmonary delivery.

Materials and Methods

Materials

GMP-grade ivermectin raw powder was received from Hovione PharmaScience Ltd. (Tapa, Macau). Lactohale® 300 (LH300, alpha-lactose monohydrate) was supplied from DFE Pharma (Goch, Germany); potassium chloride, sodium bromide, and silica gel from Sigma-Aldrich Co. (St. Louis, MO, USA); potassium carbonate anhydrous from Fluka Chemie AG (Buchs, Switzerland), acetonitrile and methanol from Merck KGaA (Darmstadt, Germany), and isopropyl

alcohol (IPA) from Sigma-Aldrich Pty Ltd. (Sydney, Australia). The water used in chemical assays was purified with an SG ultrapure water system (Barsbüttel, Germany).

Powder preparation

Before preparation, the LH300 lactose was conditioned at 80% relative humidity (RH) and 25°C in a desiccator for 7 days over a saturated solution of potassium chloride to ensure maximal crystallinity. Ivermectin was dissolved in IPA at a concentration of 2.4 mg/mL, followed by suspending LH300 lactose at a concentration of 45.6 mg/mL to achieve a weight ratio of drug to excipient at 1:19 (SD ive-lac_T0). For comparison, an ivermectin-only solution and lactose monohydrate-only suspension were prepared in IPA at the same concentrations used in the ivermectin–lactose monohydrate formulation. Dry powders were produced by a B-290 mini spray dryer (Büchi Labortechnik AG, Flawil, Switzerland) connected to a B-295 inert loop. The operation parameters were as follows: 70°C inlet temperature, 38.2 m^3/h aspiration, 601 L/h atomization nitrogen rate, and 15 mL/min solution feed rate.

Powder storage

The spray-dried powders were divided and stored for 7 days in sealed desiccators at 25°C and different RH conditions controlled by saturated salt solutions or silica beads (Table 1). Powder characterization was conducted pre- and poststorage.

Particle morphology

The morphology of raw and spray-dried samples was visualized using scanning electron microscopy (SEM; Zeiss Sigma VP HD, Oberkochen, Germany) at a beam accelerating voltage of 3 kV. The samples were prepared by spreading the particles on a stub followed by coating with gold layer of 30 nm using a Quorum Emitech K550X sputter coater (Kent, United Kingdom).

Particle size

The particle size distribution of the raw and spray-dried samples was measured by laser diffraction using a Mastersizer 2000 equipped with a Scirocco 2000 dry powder dispersion (Malvern Instruments, United Kingdom). Compressed air of 4 bars was applied to produce powder dispersion. The refractive indexes used for the measurements were 1.56 and 1.65 for ivermectin and lactose, respectively.[29,30] The volumetric diameters (D_{10}, D_{50}, and D_{90}) and span [defined as $(D_{90} - D_{10})/D_{50}$] were obtained, and each sample was measured in triplicate.

Table 1. Storage Conditions of Spray-Dried Samples

Sample	*RH (%)*	*RH controlling agent*
SD ive:lac_T0	15	Silica beads
SD ive:lac_43%RH	43	Potassium carbonate
SD ive:lac_58%RH	58	Sodium bromide

RH, relative humidity; SD, standard deviation.

Actual drug ratio and content homogeneity

The actual ratio of ivermectin in spray-dried ivermectin:lactose (SD ive:lac_T0) powder and the homogeneity of the formulation were assessed by randomly sampling 10 specimens from different regions of the powder followed by chemical assay. The actual ratio of ivermectin was calculated by dividing the average of detected ivermectin values by the theoretical ivermectin value (5% wt. of the loaded formulation containing 1:19 ivermectin:lactose). The formulation was considered homogeneous if the content of ivermectin in each single sample was between 85% and 115% of the average content according to British Pharmacopeia.[31] Each sample of 6.5 ± 0.5 mg was added to 10 mL of methanol, shaken and sonicated for 5 minutes to fully dissolve ivermectin, followed by filtering and filling in vials using a polytetrafluoroethylene (PTFE) filter membrane of 0.45 μm pore size to exclude the suspended lactose. The chemical assay method used to quantify ivermectin load is described below.

In vitro *dispersion performance*

The *in vitro* dispersion performance of SD ive:lac samples was evaluated using a next-generation impactor (NGI; Copley Scientific, Nottingham, United Kingdom) connected to a USP metal induction port. The dispersion was carried out in a chamber at controlled RH of $50\% \pm 5\%$. Before dispersion, NGI plates were sprayed with silicone oil (Dry Film Silicone Lubricant; LPS, GA, USA) to prevent particle bounce. Size 3 Vcaps® Plus capsule (Capsugel, NSW, Australia) was filled with 10 ± 0.5 mg of the formulation and aerosolized to the NGI by a medium-high resistance Osmohaler (Pharmaxis Ltd., Sydney, Australia) at a flow rate of 60 L/min for 4 seconds. The aerodynamic cutoff diameters for stages 7 to 1 were 0.34, 0.55, 0.94, 1.66, 2.82, 4.46, and 8.06 μm, respectively. After dispersion, 10 mL of methanol was used to dissolve the ivermectin particles deposited in the capsule, inhaler, adaptor, throat, and NGI stage 1. Five milliliters was used for the other stages.

The collected solution samples were filtered by PTFE filter membrane of 0.45 μm pore size to exclude the suspended lactose. High-performance liquid chromatography was used to chemically analyze the collected ivermectin and determine the fine particle dose (FPD), the recovered fine particle fraction ($FPF_{recovered}$), the emitted fine particle fraction ($FPF_{emitted}$), the median mass aerodynamic diameter (MMAD), and the geometric standard deviation (GSD). The FPF was defined as the mass fraction of aerosolized ivermectin particles <5 μm in the aerosol (FPD) with respect to the mass of ivermectin load recovered in the NGI parts, including the adaptor, capsule, and the inhaler ($FPF_{recovered}$) or with respect to the recovered mass of ivermectin load excluding the capsule and the inhaler ($FPF_{emitted}$). The dispersion was conducted in triplicate.

Chemical assay method

High-performance liquid chromatographer (Shimadzu, Kyoto, Japan) connected to a Phenomenex Luna C18(2) 100 Å 5 μm 4.6×250 mm column was utilized for quantifying the amount of ivermectin for content homogeneity and

in vitro dispersion tests at a detection UV wavelength of 254 nm based on a Pharmacopeial method.[32] The mobile phase contained acetonitrile, methanol, and water (51:34:15 v/v). The standard curve of pure ivermectin in methanol was linear at a concentration range between 0.0005 and 0.6 mg/mL ($r^2 = 0.999$). The run and elution times were 35 and 28.8 minutes, respectively. The injection volume was 20 μL, and the flow rate was 1 mL/min.

Differential scanning calorimetry

The solid-state properties of raw and spray-dried samples were assessed with a differential scanning calorimetry (DSC) instrument (Mettler Toledo, Zurich, Switzerland). Aluminum crucibles of 40 μL were filled with 6 ± 1 mg of each sample and heated from 30.0°C to 350.0°C at a rate of 10.0°C/min under a continuous flow of nitrogen gas at 50 mL/min.

Thermogravimetric analysis

A thermogravimetric analysis (TGA) instrument (Mettler Toledo, Zurich, Switzerland) was used to measure the weight loss of raw and spray-dried samples when heated. Aluminum oxide crucibles of 70 μL were filled with 8 ± 0.5 mg of each sample and exposed to heat at a rate of 10.0°C/min from 30.0°C to 350.0°C under a continuous purge of nitrogen gas at 50 mL/min flow rate.

X-ray powder diffraction

A Siemens D5000 X-ray diffraction instrument (Munich, Germany) connected to copper X-ray radiation at 45 kV and a current of 40 mA was used to evaluate the crystallinity of raw and spray-dried samples. Scan method of 2θ with a scan rate of 0.013°/s from 5° to 50° was used to collect the data.

Raman spectroscopy

A Renishaw inVia Reflex Microscope (Wotton-under-Edge, United Kingdom)—which is supplied with a Leica DMLM microscope and a 2400 g/mm grating and an air-cooled charge-coupled device detector—was utilized to obtain Raman spectra, and examine the solid state of raw and spray-dried samples. A diode-pumped solid-state laser with a wavelength of 532 nm was used as the excitation light. The spectra were acquired with a Leica N Plan 20×/0.40 between 650 and 1750 cm^{-1} spectral range. The laser power, accumulation, and exposure time were 50 mW, 200 scans, and 0.5 seconds, respectively.

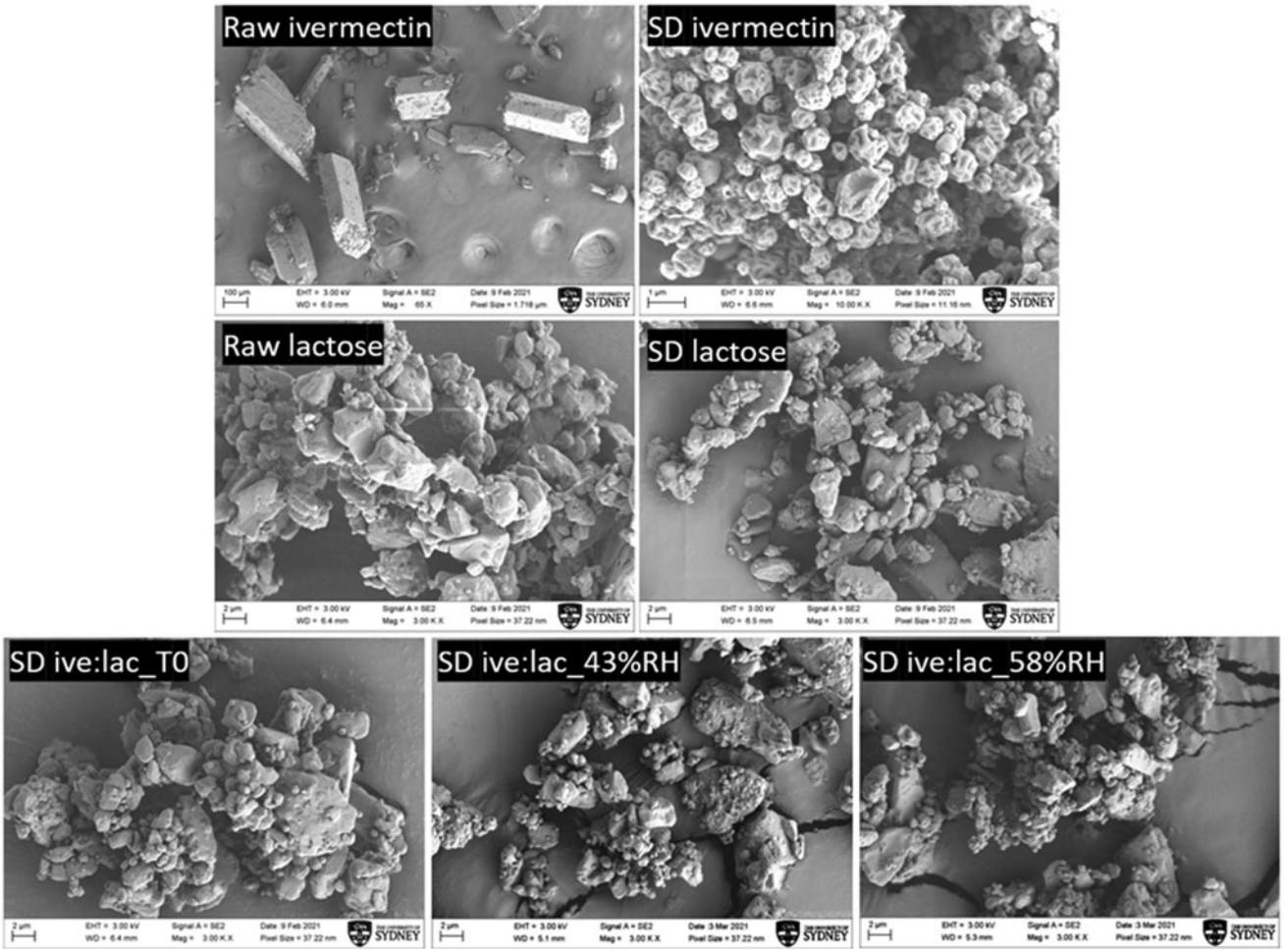

FIG. 1. SEM images of raw and spray-dried samples at a magnification of 65 (raw ivermectin), 10,000 (SD ivermectin), or 3000 (raw, SD lactose, and SD ive:lac samples). SD, standard deviation; SEM, scanning electron microscopy.

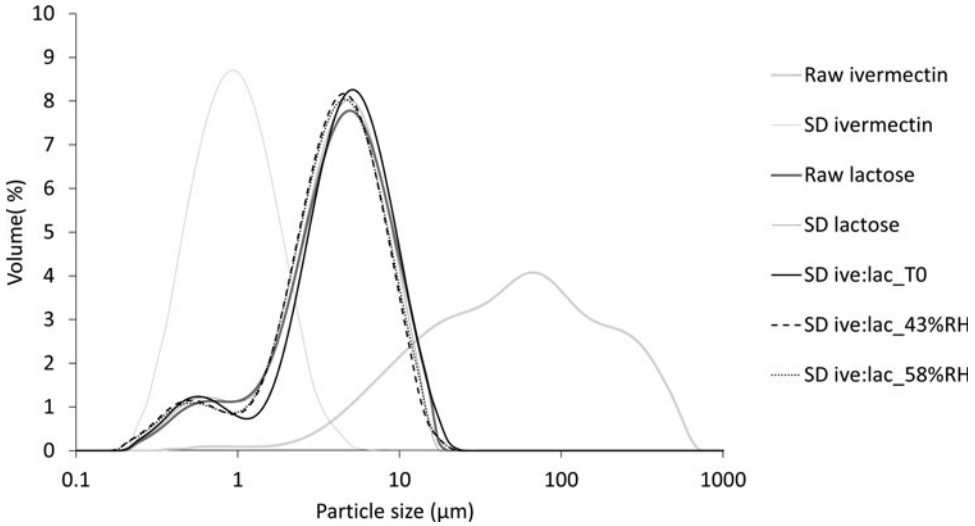

FIG. 2. Volumetric particle size distribution of the raw and spray-dried samples.

Dynamic vapor sorption

The vapor sorption profiles of raw and spray-dried samples were studied by dynamic vapor sorption (DVS) (Surface Measurement Systems, London, United Kingdom) at 25°C. The samples were exposed to two cycles of moisture from 0% to 90% with 10% RH step increase. The mass changes over time were recorded when the dm/dt was <0.002% per minute.

Statistics

The data are displayed as mean ± standard deviation ($n = 3$). One-way analysis of variance with Tukey's multiple comparison test was used to determine the statistical difference between SD ive:lac samples. p-Values of ≤0.05 were considered statistically different.

Results

Particle morphology

Figure 1 represents the SEM morphological characteristics of raw and spray-dried samples. The raw ivermectin powder contained regular-shaped large particles ranging between tens and hundreds of micrometers, while the particles of spray-dried ivermectin alone were very wrinkled with ≤1 μm particle size. Raw lactose, SD lactose, and SD ive:lac particles were all irregular in shape with no major differences between them.

Particle size

Figure 2 shows the volumetric particle size distribution of the raw and spray-dried samples. Raw ivermectin revealed a broad monomodal particle size distribution with a span of 4.95 ± 0.21 and a volumetric median diameter (D_{50}) of 49.7 ± 0.11 μm (Table 2). Spray drying dramatically reduced the value of D_{50} to 0.88 ± 0.08 μm and narrowed the span to 1.70 ± 0.14 (SD ivermectin). Raw and SD lactose showed a similar bimodal distribution of the particles with D_{50} values of 4.05 ± 0.07 and 3.95 ± 0.14 μm, respectively. As the major component of SD ive:lac samples was lactose, their particle distributions and D_{50} values were similar to those of other lactose samples.

Actual drug ratio and content homogeneity

The average of detected ivermectin in SD ive:lac_T0 powder was 91% of the theoretical load of the drug to the excipient with a relative standard deviation (RSD%) of 2.45%. Therefore, it is concluded that the actual ratio of ivermectin to lactose was changed after spray drying, becoming 1:20.9 instead of 1:19 (Table 3). All the 10 samples had a homogeneous amount of ivermectin with a percentage drug content of 96–106.

In vitro *aerosolization performance*

The SD ive:lac samples showed similar dispersion behavior with a significant deposition of ivermectin at stages

TABLE 2. THE VOLUMETRIC DIAMETERS AND SPAN OF THE RAW SPRAY-DRIED SAMPLES ($N = 3$)

Samples	D_{10} (μm)	D_{50} (μm)	D_{90} (μm)	*Span*
Raw ivermectin	7.81 ± 0.11	49.7 ± 0.11	253 ± 9.90	4.95 ± 0.21
SD ivermectin	0.41 ± 0.03	0.88 ± 0.08	1.91 ± 0.26	1.70 ± 0.14
Raw lactose	1.07 ± 0.03	4.05 ± 0.07	9.17 ± 0.13	2.01 ± 0.04
SD lactose	1.01 ± 0.19	3.95 ± 0.14	8.61 ± 0.33	1.93 ± 0.11
SD ive:lac_T0	1.03 ± 0.02	4.32 ± 0.01	9.46 ± 0.03	1.95 ± 0.01
SD ive:lac_43%RH	1.21 ± 0.17	4.34 ± 0.31	9.43 ± 0.66	1.91 ± 0.02
SD ive:lac_58%RH	1.25 ± 0.15	4.39 ± 0.27	9.72 ± 0.43	1.93 ± 0.05

The difference between SD ive:lac samples, raw and SD lactose was not statistically significant ($p > 0.05$).

Table 3. Actual Ratio of Ivermectin to Lactose in the Formulation

	Actual ivermectin	
Theoretical ivermectin	*Content average %*	*RSD%*
5% of the load	91.0 ± 2.23	2.45

Theoretical ivermectin %: the percentage of theoretical load of ivermectin in the sample.
Actual ivermectin %: the amount of the detected ivermectin in the sample relative to amount of the theoretical ivermectin.
RSD%: relative standard deviation = (SD/mean%) × 100.

4, 5, and 6 of the impactor (Fig. 3 and Table 4). The SD ive:lac_T0 and the conditioned powders at 43% and 58% RH had very similar FPD values between 297 and 302 μg, $FPF_{recovered}$ between 68% and 70%, and $FPF_{emitted}$ between 82% and 84%. Consistently, MMAD and GSD were in the range of 1.5 and 2.2 μm, respectively.

Differential scanning calorimetry

DSC thermographs of raw and spray-dried samples are displayed in Figure 4. Raw ivermectin showed a melting peak at 152°C followed by degradation after 300°C. Spray drying converted the crystalline raw ivermectin to an amorphous powder as it showed a glass transition event at 137°C. On the contrary, spray drying did not convert the crystalline raw lactose to an amorphous form as the SD lactose and lactose-containing samples show matched events at 147–148°C and at 216–219°C, which were related to water loss and the melting of alpha lactose monohydrate, respectively.[33]

Thermogravimetric analysis

Figure 5 shows TGA graphs of raw and spray-dried samples. Raw ivermectin lost 4% of the weight when melted at 152°C, presumably due to evaporation of residual solvents (ethanol and formamide), which are used during the purification process of raw ivermectin.[34] It degraded at 300°C. However, SD ivermectin showed no significant weight change until degradation. Raw and SD lactose showed a weight loss of 4% between 120°C and 150°C due to water loss followed by another phase of loss after 220°C related to melting of lactose until degradation. Similarly, SD ive:lac samples showed identical events as they were dominated by the 95% (w/w) of lactose present in the samples.

X-ray powder diffraction

Raw ivermectin was crystalline with several sharp diffraction peaks observed at 6.5°, 9.3°, 11.2°, 12.4°, 13.1°, 14.8°, and 17.4° 2-θ. However, a halo pattern was shown with SD ivermectin, indicating that the powder was amorphous. The crystalline form of lactose did not change by spray drying as both raw and SD lactose displayed matched diffraction patterns with dominant peaks at 12.6°, 16.5°, 19.2°, 19.6°, 20.0°, 21.3°, 23.8°, and 37.6° 2-θ. SD ive:lac samples showed similar patterns to those of raw and SD lactose (Fig. 6).

Raman spectroscopy

The Raman spectra of the raw and spray-dried samples are shown in Figure 7. Both raw and SD ivermectin showed characteristic peaks at 1624 and 1672 cm^{-1} related to the unsaturated lactones with a double bond adjacent to the O group.[35] Spectra obtained from raw ivermectin and after exposure to 90% RH (in the DVS analysis) demonstrated sharp peaks corresponding to the crystallinity of the materials. Spectra acquired from spray-dried ivermectin alone and all the formulations containing ivermectin showed broadening of ivermectin peaks. However, all the peaks related to lactose (846, 871, 1015, and 1082 cm^{-1}) remained sharp (Fig. 8). Interestingly, peaks related to ivermectin even after exposing the formulation to 43%, 58%, and 90% RH remained broad, confirming its amorphous nature.

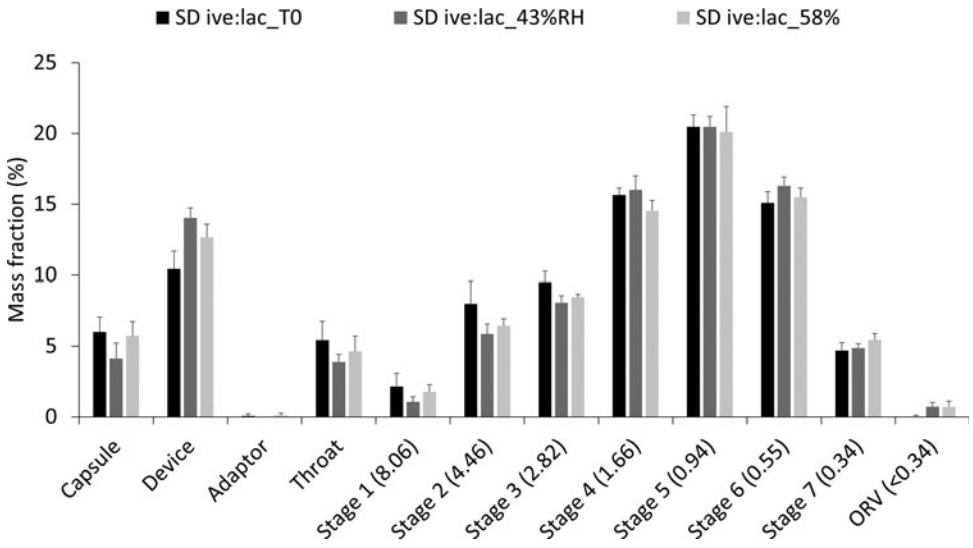

FIG. 3. The dispersion performance of SD ive:lac powders at each NGI stage ($n = 3$). NGI, next-generation impactor.

Table 4. Recovered Ivermectin, Fine Particle Dose, Fine Particle Fractions, Median Mass Aerodynamic Diameter, and Geometric Standard Deviation Values of SD ive:lac Samples ($N=3$)

Samples	*Recovered ivermectin (%)*	*FPD (μg)*	$FPF_{recovered}$ *(%)*	FPF_{loaded} *(%)*	$FPF_{emitted}$ *(%)*	*MMAD (μm)*	*GSD*
SD ive:lac_T0	97.4 ± 2.20	302 ± 13.9	68.4 ± 2.66	60.6 ± 3.08	82.2 ± 1.13	1.42 ± 0.10	2.22 ± 0.09
SD ive:lac_43%RH	95.7 ± 0.29	297 ± 11.3	69.9 ± 1.06	64.5 ± 2.01	84.1 ± 1.03	1.49 ± 0.02	2.25 ± 0.01
SD ive:lac_58%RH	96.7 ± 0.51	297 ± 17.3	70.1 ± 1.43	64.8 ± 1.71	84.4 ± 2.47	1.47 ± 0.02	2.22 ± 0.08

No statistical difference was found between the three samples ($p>0.05$). Recovered ivermectin %: the amount of the recovered ivermectin relative to the theoretical loaded amount of ivermectin in the capsule.

FPD, fine particle dose; FPF, fine particle fraction; GSD, geometric standard deviation; MMAD, median mass aerodynamic diameter.

Dynamic vapor sorption

Raw ivermectin showed low moisture uptake of 0.26 wt.% at 90% RH. However, the mass became smaller by 0.09 wt.% after the desorption, probably due to removal of the solvent residues (ethanol and formamide).[34,36] SD ivermectin was more hygroscopic and absorbed moisture more than the raw ivermectin (3.5 wt.% at 90% RH). Raw and SD lactose showed low moisture sorption of 0.21 and 0.28 wt.%, respectively, with no recrystallization events (Fig. 9). Similarly, SD ive:lac samples showed <0.5% of moisture uptake with no recrystallization events. At 90% RH, the mass of SD ive:lac_T0 increased by 0.43%, and the conditioned samples at 43% and 58% RH displayed increase in mass of 0.38% and 0.31%, respectively (Fig. 10).

Discussion

In this study, ivermectin and lactose crystals were co-spray dried to produce an inhalable dry powder. Evidence is mounting, which suggests that ivermectin may be an important drug in the fight against COVID-19. The potential benefits of ivermectin may be enhanced by targeted drug delivery that may better achieve the drug concentrations required for antiviral activity *in vivo*. We hypothesized that direct respiratory delivery of ivermectin as a dry powder could ensure delivery of the therapeutic concentration to the lungs to overcome the limitations associated with the systemic delivery route.

The required therapeutic dose was calculated to be 125 μg based on the concentration reported to attain 100% *in vitro* viral clearance. Low-dose drug deployment paradigms require mixing with excipients for enhanced inhaled delivery.[22] Thus, lactose was selected owing to its safety, stability, and compatibility with most drugs.[25] However, it is known that spray drying of solution containing lactose would generate unstable amorphous powders with a strong tendency for recrystallization.[37] Our recent studies established that spray drying of suspended lactose crystals in isopropyl alcohol (IPA) with or without drugs can maintain the crystallinity of lactose.[38] Therefore, the formulation was prepared as a suspension containing lactose crystals and dissolved ivermectin in IPA.

Preliminary studies (data not included) were conducted to optimize the combination that can deliver FPD near the anticipated therapeutic dose (125 μg), taking into consideration an *in vitro/in vivo* correlation of lung doses for DPIs being 1.5.[39] In brief, formulations with different ratios of ivermectin to lactose (e.g., 1:4, 1:19, and 1:49) were spray dried and assessed for *in vitro* dispersion performance at different capsule-loaded doses (e.g., 5, 10, and 20 mg).

Based on the results, the ratio of ivermectin to lactose and the capsule-loaded dose were set at 1:19 and 10 mg, respectively. After spray drying, the actual ratio of ivermectin to lactose was changed and became 1:20.9 instead of 1:19. This may be because some of the small drug-only particles escaped the cyclone collection. In scale-up production,

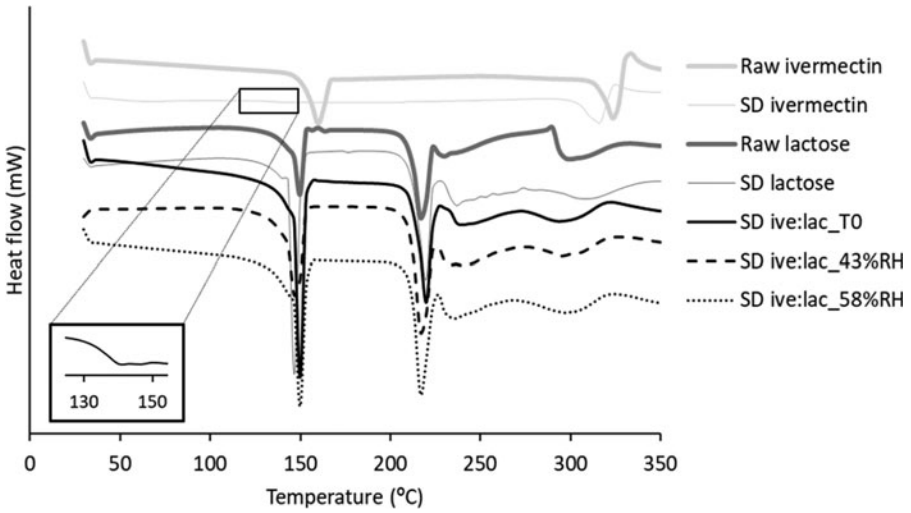

FIG. 4. DSC thermograms of raw and spray-dried samples. DSC, differential scanning calorimetry.

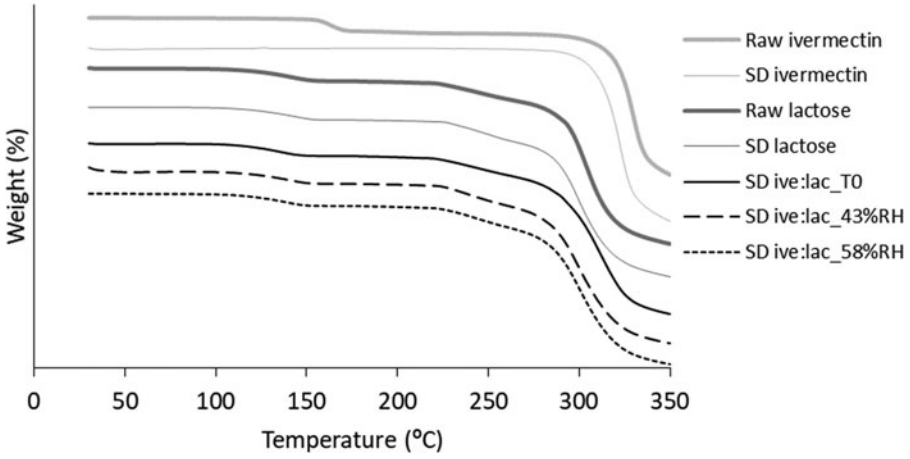

FIG. 5. TGA thermograms of raw and spray-dried samples. TGA, thermogravimetric analysis.

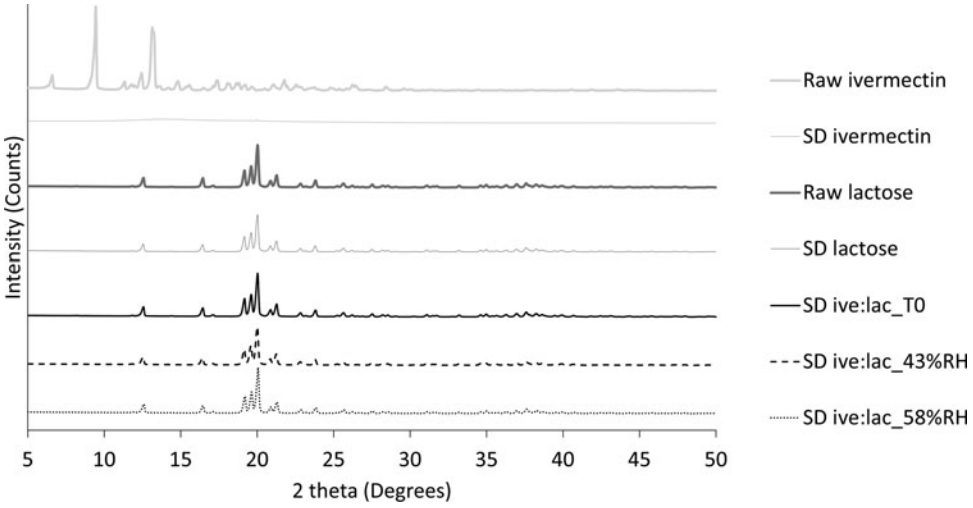

FIG. 6. XRPD graphs of the raw and spray-dried samples. XRPD, X-ray powder diffraction.

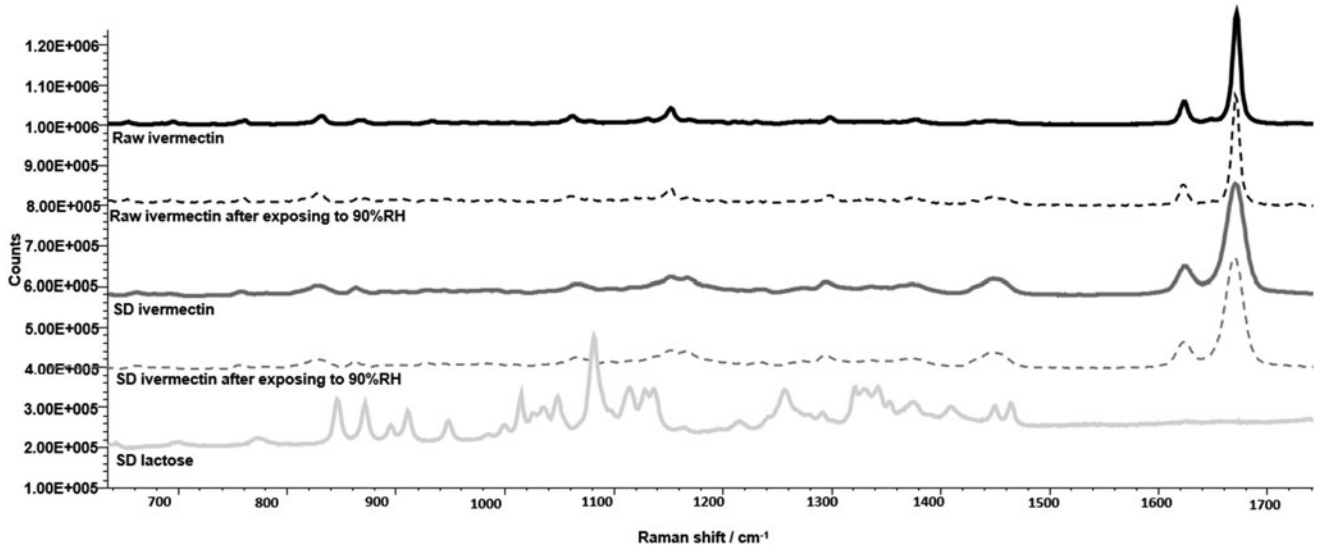

FIG. 7. Raman spectra of raw and SD ivermectin and lactose before and after exposing to 90% RH. RH, relative humidity.

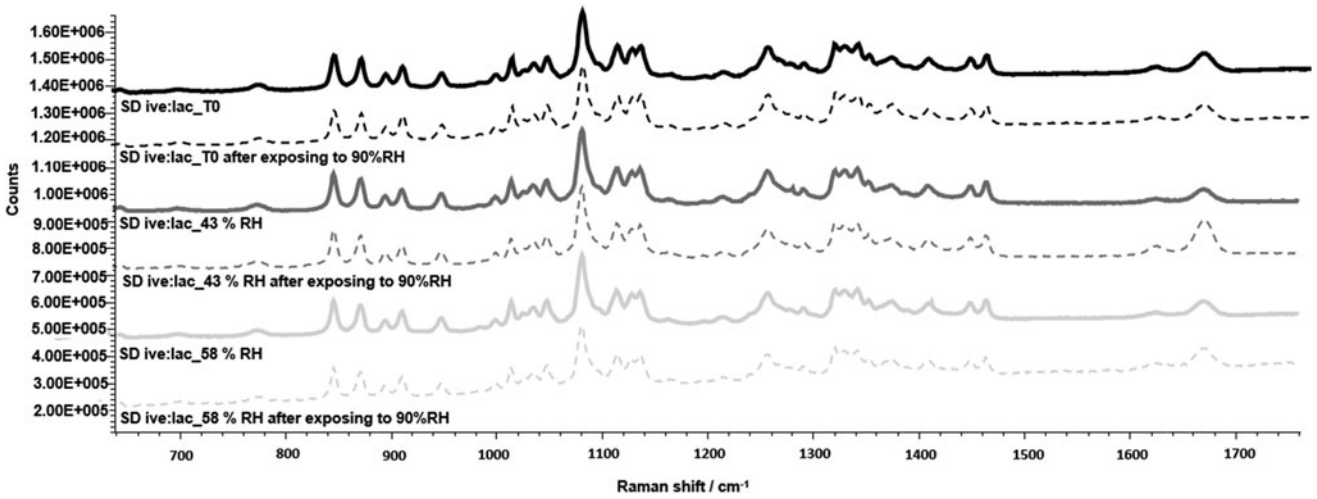

FIG. 8. Raman spectra of SD ive:lac samples before and after exposing to 90% RH.

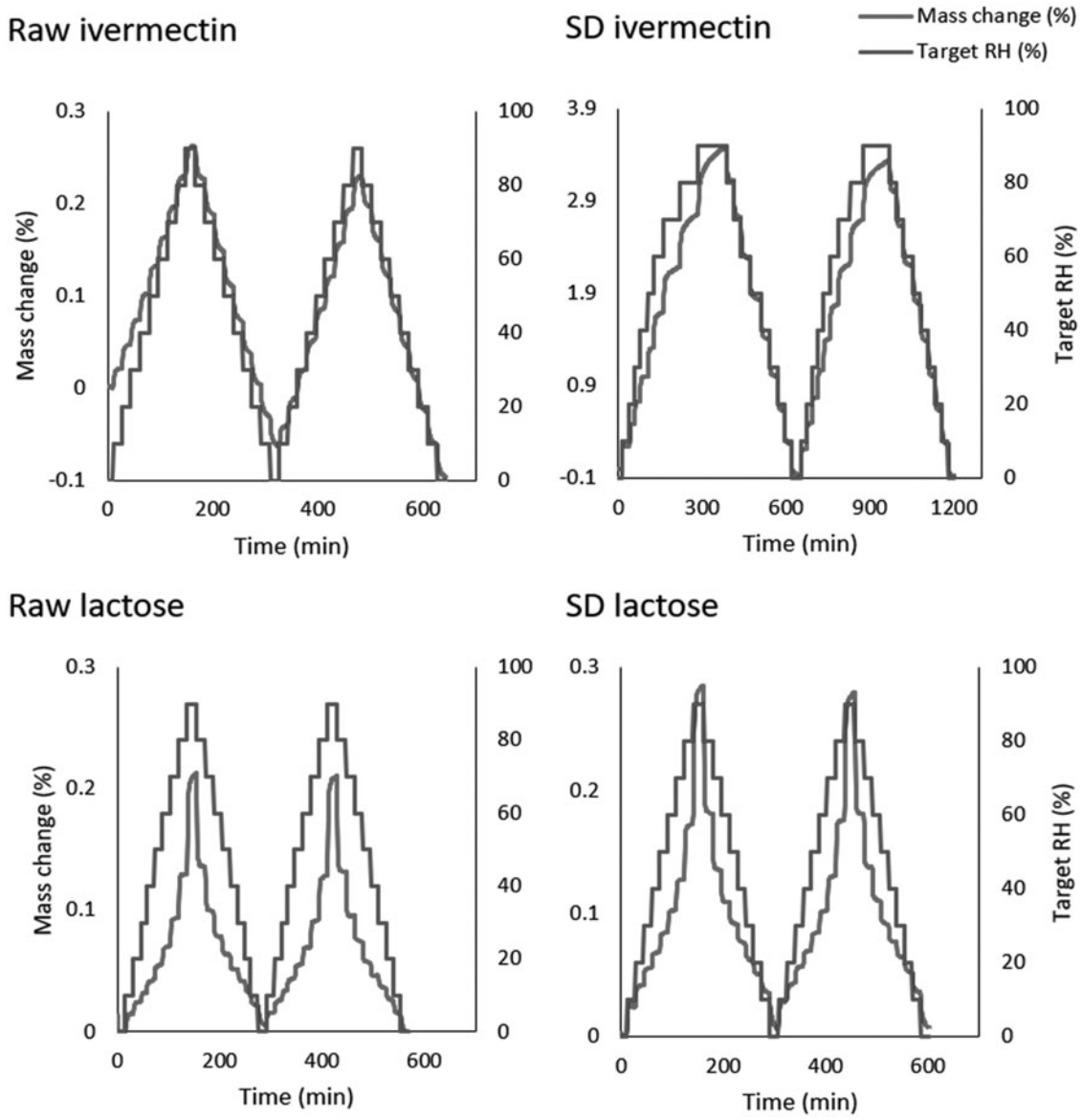

FIG. 9. Moisture sorption profiles of raw and SD ivermectin and lactose.

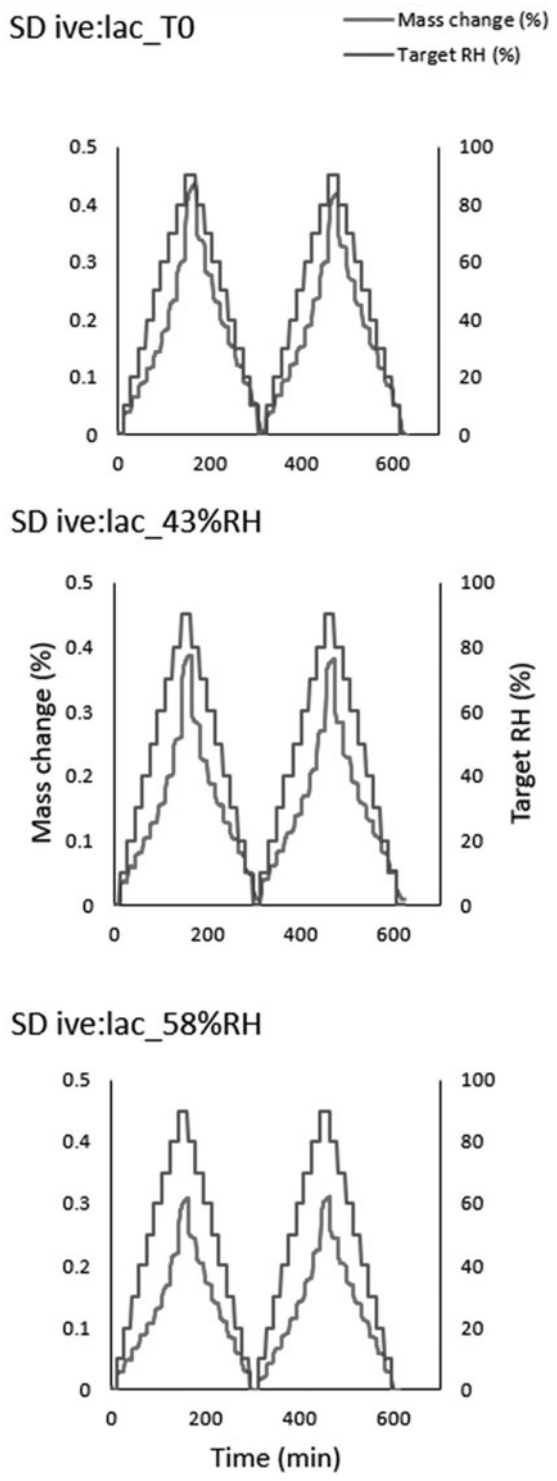

FIG. 10. Moisture sorption profiles of SD ive:lac samples.

collection of finer particles is possible using higher efficiency cyclones with a smaller cutoff size.[40,41]

The crystallinity of raw ivermectin and lactose was confirmed as they showed sharp peaks in Raman spectra, X-ray powder diffraction (XRPD), and DSC patterns that are consistent with the crystalline form of lactose in the literature.[34,35,38] However, SD ivermectin alone was amorphous, showing broad peaks in Raman spectra, a glass transition event in DSC, and a halo diffraction in XRPD. In general, during spray drying, dissolved materials tend to become amorphous, whereas the undissolved or suspended materials remain crystalline.[38]

Since our formulation contained only a low quantity of ivermectin with lactose crystals being the bulk, XRPD and DSC were unable to confirm the crystallinity of the drug as the diffraction peaks and the thermal events were only attributed to the lactose. Thus, Raman spectroscopy was used to confirm that ivermectin was in an amorphous state as peak broadening of the drug is consistent with the transition of material from crystalline to amorphous form.[42] Amorphous materials are sensitive to moisture, and often require some protective coatings or packaging to prevent them from recrystallization. However, ivermectin remained amorphous even after the samples were conditioned at moderate RH or exposed to extreme moisture at 90% RH, thus confirming its stable nature.

The vapor sorption profiles of the materials were consistent with the findings of XRPD and Raman spectroscopy. Raw ivermectin was crystalline, and the increase in its mass was very low (0.26%) due to its hydrophobicity. However, SD ivermectin absorbed 13 times more moisture than the raw ivermectin (3.5% wt. compared with 0.27% wt.) after being amorphorized after spray drying. Interestingly, SD ivermectin did not show any recrystallization events despite being exposed to two cycles of humidity sorption–desorption. It is possible that moisture uptake occurred in the amorphous regions during relaxation but not necessarily induced recrystallization, which depends on molecular mobility and thermodynamics.[43] SD ive:lac samples showed a minor mass change of <0.5% with no recrystallization despite the fact that ivermectin was amorphous. This can be attributed to the predominantly crystalline lactose, and the hydrophobicity and low quantity of the amorphous drug in the samples. Consequently, no change would be expected after long-term storage.

The characterization results of SD ive:lac_T0 and the two conditioned samples showed a similarity in their particle morphology, volumetric size distribution, and aerosol performance. Based on our previous studies involving both drug and lactose assays,[44] spray drying of suspensions containing lactose crystals and a dissolved hydrophobic drug in isopropyl alcohol produced mostly drug-coated lactose particles along with certain droplets containing dissolved drug only, which resulted in submicron drug-only particles. It is not possible to have lactose-only particles as the particles must be accommodated inside the spray droplets, which contain dissolved drug. Therefore, the smaller particles that appeared to adhere or bind to the bigger particles are likely ivermectin-only or small lactose particles coated with ivermectin, attaching to the surface of the larger lactose particles coated with ivermectin.

The anticipated therapeutic dose of 125 μg can be achieved by all three samples as they showed FPD of *ca.* 300 μg. No statistical difference was found between their aerosol performance as all showed $FPF_{emitted}$ *ca.* 83%, $FPF_{recovered}$ *ca.* 70%, MMAD *ca.* 1.5 μm, and GSD *ca.* 2.2. This therapeutic dose is likely to be safe based on the safety and tolerability of intranasal ivermectin in pigs at dose of 0.2 mg/kg, spray-dried ivermectin in rats at dose of 140 mg/kg, and the results of the subacute ivermectin

inhalational study.[17,14,45] In addition, the lipophilic nature of ivermectin leading to its large volume of distribution and the high drug exposure in lung tissue that has been noted in other nonrodent animal pharmacokinetic studies may be able to attain and sustain the therapeutic concentrations in human.[46,47]

Ultimately, the preparation described in this study will need to be tested in SARS-CoV-2 nonhuman primate animal models,[48] which recapitulate the cellular targets and host responses seen in humans as only such experimental paradigms will truly identify potential acute and chronic lung toxicity resulting from ivermectin or lactose and define any therapeutic benefit, which may reflect not only cellular viral uptake inhibition and direct antiviral effects but also modulation of the host response to the virus.

Conclusion

A stable and inhalable dry powder formulation of ivermectin with lactose as the excipient was successfully prepared as a potential COVID-19 treatment. The formulation could achieve the anticipated therapeutic dose for respiratory delivery with FPD of 300 μg when inhaled through a medium-high resistance Osmohaler at 60 L/min. Further investigation of its safety and efficacy to be used in humans should be conducted.

Authors' Contributions

J.D., S.K., and H.K.C. provided study initiation. A.H.A. contributed to powder preparation and storage. A.H.A. and P.T. performed particle morphology analysis. A.H.A. and D.K. carried out Raman spectroscopy analysis. A.H.A., W.-R.K., D.K., S.K., J.D., W.J.B., and H.-K.C. contributed to data analysis and interpretation. All authors contributed to the critical revision of the article and approved the final article.

Acknowledgments

A.H.A. is financially sponsored by Jazan University (Saudi Arabia). The authors thank the Australian Centre for Microscopy and Microanalysis at The University of Sydney for their technical assistance. H.-K.C. is grateful to The Jack and Robert Smorgon Families Foundation for their financial support as a gift to the University of Sydney.

Author Disclosure Statement

J.D., H.K.C., and A.H.A. are inventors of a provisional patent (Australian application number: 2021902130) on inhaled ivermectin filed by the University of Sydney.

Funding Information

Jack & Robert Smorgon Families Foundation provided financial support as a gift to the University of Sydney for this study.

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Received on November 16, 2021
in final form, January 25, 2022

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