Article published online: 2022-12-27

The Study of Spray-Freeze-Drying Technique for Development of Novel Combination pMDIs, Part II: *In Vitro* and *In Vivo* Evaluations

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Pharmaceut Fronts 2022;4:e275–e283.

Abstract

The mometasone furoate (MF) and formoterol fumarate dihydrate (FF) inhalable microparticles prepared by different methods, such as micronized active pharmaceutical ingredients (APIs), microparticles of APIs prepared by spray-freeze drying technique (SFD APIs), and phospholipid microparticles of APIs prepared by SFD (SFD Lip-APIs), showed different inhaled drug delivery characteristics. Study on the physicochemical characteristics of those microparticles and the effect of matrix excipients on pharmacokinetic (*PK*) behaviors of inhalable microparticles is helpful for the development of new methods for inhalable microparticles with excellent performance of inhalation characteristics. In this study, the crystal state of the microparticles was investigated by powder X-ray diffraction and differential scanning calorimetry. The density was investigated by a bulk density method. The suspension and dispersion characteristics were determined by observing its state in hydrofluoroalkane (HFA). Meanwhile, the *PK* behaviors of SFD Lip-APIs in beagle dogs were also investigated by airway administration to evaluate the effect of phospholipids on drug release. The results indicated that the presence of phospholipids prevents the formation of solid bridges bonding to each other during SFD of pure drug solutions. In comparison to the conventional micronized microparticles, inhalable drug–phospholipid microparticles were easily dispersed and suspended in HFA. The embedded drugs were in a crystal state that endowed a better physical stability, and most interestingly, have similar *PK* behavior to the control (a mixed solution of MF/FF), suggesting that the phospholipids, as matrix excipients, had no effect on absorption. Given above, our designed SFD phospholipid microparticles may represent an efficient carrier for pulmonary delivery of MF and FF for further clinical treatment.

Keywords
- inhalable microparticles
- spray-freeze-drying
- characterization
- airway administration
- pharmacokinetic

received
April 6, 2022
accepted
September 27, 2022

DOI https://doi.org/10.1055/s-0042-1758388.
ISSN 2628-5088.

Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

Introduction

Our previous work has described the usage of the spray-freeze-drying (SFD) technique in the preparation of inhalable microparticles of mometasone furoate (MF) and formoterol fumarate dihydrate (FF).[1] In the reported study, pressurized metered dose inhalers (pMDIs) were prepared from three typical inhalable microparticles, including conventional micronized active pharmaceutical ingredients (APIs), SFD-prepared microparticles of APIs (SFD APIs), and SFD-prepared phospholipid microparticles of APIs (SFD Lip-APIs). Aerodynamic characterization and delivered dose content uniformity assay suggested that SFD Lip-API pMDIs have an excellent performance compared with the other two.

The preparation process of SFD Lip-APIs is based on particle engineering technology that directly results from the formation of solid microparticles from a solution, suspension, or emulsion. A representative example of particle engineering in the field of inhalants was the usage of the Pulmosphere technology that is an emulsion-based spray-drying process to generate microparticles with a low density and small particle size.[2,3] Phospholipids are usually used as surfactants in the technology. The technology has been applied by Novartis Pharmaceuticals for the creation of the TOBI Podhaler product for the treatment of infections caused by *Pseudomonas aeruginosa* in patients with cystic fibrosis.[4,5] For PulmoSphere™ formulations, the feedstock comprises a submicron oil-in-water emulsion; therefore, size distribution of emulsion droplets inevitably had a significant effect on the production yield, morphology, diameter size, encapsulation efficiency, and pharmacokinetic (*PK*) behavior of the microparticles, making the quality control of the process complex. Moreover, the usage of organic solvents in the process also causes a severe concern of the environmental contamination risk. However, inhalable microparticles prepared by SFD could avoid the problems mentioned above and, inspiringly, can achieve similar performance to pMDI. In the SFD process, instantaneous freezing of the atomized droplets maximized the retention of the initial droplet structures, thus, enables the possibility of preparation of microparticles, according to the expected size, shape, density, and surface characteristics.[6,7]

Our previous work focused on the preparation of novel combination pMDIs by the SFD technique.[1] Following this, the typical inhalable microparticles produced by this process were evaluated in this study. In this work, physical characteristics of APIs, SFD APIs, and SFD Lip-APIs, including their morphology, thermal properties, density, as well as suspension and dispersion in hydrofluoroalkane (HFA), were compared. For SFD Lip-APIs, distearoylphosphatidylcholine (DSPC) was used as an excipient, as in a previous report. Phospholipid matrix had the potential to rapidly hinder drugs from contacting with body fluids. Phospholipid can also act as a surfactant that may poorly increase solubility of a drug and even affect permeation absorption.[8] Considering that, an *in vivo* kinetics study of airway drug delivery was also performed using beagle dogs as animal models and the concentration–time curve of MF/FF in the blood was also detected by a HPLC-MS method. Our data suggested that SFD Lip-APIs achieved good inhaler performance, and had similar rates and degrees of absorption to a mix solution of MF and FF.

Materials and Equipment

Materials and Equipment *In Vitro*

In this study, the following pieces of equipment were used: a Zeiss Gemini 300 scanning electron microscope (Carl Zeiss AG, Germany); a Bruker D8 advance PXRD (Bruker, Germany); a TA Q2000 DSC (Waters, United States); a vacuum crimper (Shenghua, China); a filler machine (Shenghua, China); a DF316 50 µL valve (Aptar, China); a glass bottle (Bormioli Rocco, Italy).

1,1,1,2-Tetrafluoroethane (HFA134a) was purchased from XueHui Refrigeration Equipment Co., Ltd. Micronized drug FF was purchased from Guangzhou Greensyn Co., Ltd., with a purity of 99.6% (batch No. FF/101/17–18). Micronized drug MF was purchased from HuNan YuXin Pharmaceutical Co., Ltd., with a purity of 99.6% (batch No. KS-200801). Batch P-008–189 was obtained by SFD APIs where the purity of MF was 95.45% and purity of FF was 4.55%. Batch P-008–179 was obtained by SFD Lip-APIs where the purity of MF was 23.48% and purity of FF was 1.12%. DSPC was purchased from Shanghai LangXu Biotechnology Co., Ltd. with a purity of 98% (batch No. 20210501).

Materials and Equipment *In Vivo*

MF-d_3 (TLC Pharmaceutical Standards Ltd., Canada), batch No. 2240–065A2, had a chemical purity of 99.7% and an isotope purity of 99.5%. Ammonium acetate and methanol were of HPLC grade and the other reagents were of analytical grade.

In addition, the following equipment was used: a QTRAP 5500 triple quadrupole linear ion trap composite mass spectrometer (AB Sciex Pte., Ltd., United States), a Nexera XR ultrafast liquid chromatography system (Shimadzu Corporation, Japan), a Sigma 3–18K high-speed centrifuge (Sartorius Stedim Biotech GmbH, Germany), a TOLEDOTCS-150 Electronic platform scale (Mettler, Switzerland), a powder mist delivery kit (Beijing Huirong He Technology Co., Ltd., China), and an aerosol delivery kit (Shanghai Yuyan Scientific Instrument Co., Ltd., China).

Preparation of Inhalable Microparticles by SFD

The mixed drug solution of MF and FF was prepared by dissolving MF and FF in 80% (v/v) aqueous dioxane solution with a mass ratio of 115:5.5. The feedstock was prepared as follows. To a solution of FF and calcium chloride ($CaCl_2$) in water was added DSPC and MF during high shear stirring. The principal excipients comprise a 2:1 molar ratio of DSPC to $CaCl_2$. The ratio of the micronized MF and FF is 115:5.5 (W:W). Then, high-pressure homogenization was performed for five times to obtain the feedstock.

The above-mentioned drug solution and feedstock were respectively transported to an atomizing nozzle through a peristaltic pump, and the atomized droplets were quickly

frozen in liquid nitrogen. Then, they were transferred to a freeze-dryer to obtain SFD APIs and SFD Lip-APIs.

Morphological Characterization

A small quantity of samples for testing was put on the sample table with double-sided glue, coated with gold using an ion sputtering apparatus, and observed under a scanning electron microscope (SEM).

Powder X-Ray Diffraction

The crystal structure of microparticles was investigated using powder X-ray diffraction (PXRD) on a Bruker AXS D8 advance. Tube pressure was 40 kV, the tube current was 40 mA, Cu Kα target ray scanning was used, the divergence slit was 0.6 mm, the sora slit was 4.0°, the conversion range 2θ was 2° to 40°, the conversion step was 0.02°, and the speed was 8°/min.

Differential Scanning Calorimetry

The melting point and endothermic peak due to the melting were analyzed by differential scanning calorimetry (DSC) on a TA Q2000 DSC. Under the protection of nitrogen, the samples for testing were placed on an aluminum plate and heated from 30 to 300°C at a rate of 10°C/min.

Bulk Density Determination

Inhalable microparticles were used in pMDIs; therefore, there was no need to evaluate the fluidity according to the ratio of loose to tight density. Rather, this work investigated the loose density to indirectly reflect the difference in particle density. Briefly, different inhalable microparticles were placed in glass bottles with a volume scale, and the microparticle mass consisting of the same volume of 0.5 mL was recorded and measured three times in parallel. Then, the bulk density was calculated.

Suspension and Dispersion of Inhalable Microparticles in HFA Environment

Micronized APIs, SFD APIs, and SFD Lip-APIs were placed in 30 mL glass bottles that were crimped and filled with HFA134a to prepare the pMDIs, and the corresponding theoretical filling volumes were 2.42, 2.42, and 9.79 mg/mL. Then, the particles were dispersed by ultrasound. After shaking for 5 seconds, the suspension and dispersion states were recorded at a preset time point.

Animals

Beagle dogs of ordinary grade, two males and two females, with a weight range of 11.8 to 13.1 kg (Jiangsu Yadong Experimental Animal Research Institute Co., Ltd., China) were used in this study. The feeding environment was as follows. The dogs were kept in stainless steel dry dog cages, with a volume of $100 \times 100 \times 96$ cm (length × width × height) and one dog per cage. The animals were ration fed and allowed to drink freely, and the environmental temperature was 16 to 26°C, the relative humidity was 40 to 70%, and the light was alternated with darkness every 12 hours.

Evaluation of the Airway *PK* Characteristics of Inhalable Microparticles

Selection of Administration Method

During airway administration, the main factors that affected the dose were the ventilation, drug concentration in the air, aerodynamic particle size, and administration time.[9] There were also differences in ventilation between the different individual animals, and the same individual also caused changes in ventilation due to the influence of external factors during drug administration.[10] It is impossible to adjust the same ventilation volume of awake animals, and to measure the ventilation volume online in real time during the inhalation administration process. The ventilation volume difference will not only affect the deposition behavior of inhalable microparticles, but also lead to changes in the administered dose. In addition, due to the significant differences in the anatomical structures of the larynx and trachea between beagle dogs and humans, it is not meaningful to use animal tests to evaluate the aerodynamic characteristics of the inhalable microparticles. Therefore, in this study, the inhalable microparticles were directly injected into the trachea instead of inhalation administration of the aerosol. To further reduce the dose variability, MF/FF solution was used as a control, and when the drugs entered the airway in a molecular state, there was no dissolution process, allowing us to scientifically evaluate whether phospholipids in the inhalable microparticles could hinder the release and absorption of the drug.

Airway Administration of the SFD Lip-APIs

The dosage of inhalable microparticles was 1 mg per dog. Each dog was given 0.4644 mg MF and 0.0222 mg FF according to drug content of inhalable microparticles. In addition, 1 mg of SFD Lip-APIs were accurately weighed with an electronic analytical balance and poured into the powder spray delivery kit. The dogs were anesthetized by isoflurane inhalation, the trachea was intubated, and 1 mg of SFD Lip-APIs was sprayed into the airway.

Airway Administration of a Mixed Solution of MF and FF

The dosage of drug solution was 200 μL per dog. Each dog was given 0.4644 mg MF and 0.0222 mg FF according to drug concentration of the solution. An appropriate amount of MF and FF was weighed and dissolved with DMSO and 0.9% sodium chloride for injection, which was prepared by a mixed solution containing MF 2.3 mg/mL and FF 0.111 mg/mL (DMSO content was 20%). Then, 200 μL of the mixed solution was poured into the aerosol delivery kit. The dogs were anesthetized by isoflurane inhalation, intubated, and sprayed with 200 μL of the mixed drug solution of MF and FF.

The *PK* behavior of the inhalable phospholipid microparticles for airway administration was studied by a double crossover trial (►**Table 1**).

Liquid Chromatography-Mass Spectrometry (HPLC-MS/MS) Conditions

An X Bridge C18 chromatographic column was used (2.1×50 mm, 3.5 μm), where the column temperature was

Table 1 Two-cycle animal administration

Number (sex, body weight)	M01 (♂, 12.5 kg)	M02 (♂, 11.8 kg)	F01 (♀, 13.1 kg)	F02 (♀, 12.2 kg)
The first cycle	Inhalable microparticles	Drug solution	Inhalable microparticles	Drug solution
Cleaning period[a]				
The second cycle	Drug solution	Inhalable microparticles	Drug solution	Inhalable microparticles

[a]Cleaning period: 7 days.

Note: Before and after administration for 5, 15, 30, 45 minutes, and 1, 1.5, 2, 4, 6, 8, 12, and 24 hours of each animal, blood was collected from the forelimb vein for approximately 1 mL, placed in a heparin anticoagulation tube, centrifuged at 2,000 rpm for 10 minutes at 4°C, and the plasma was separated and stored at −80°C in a refrigerator before testing.

40°C and the automatic injector temperature was 4°C. Mobile phase A consisted of a water solution (containing 2 mmol/L ammonium acetate), while mobile phase B consisted of a methanol solution (containing 2 mmol/L ammonium acetate). A gradient elution procedure was performed.

An electrospray ion source was used as the ion source, where a source voltage of 5.0 kV was used for positive ion detection; the first gas pressure in the collision chamber was 413.69 kPa and the second gas pressure in the collision chamber was 448.16 kPa. The air curtain pressure was 206.84 Pa. The scanning mode was multiple reaction monitoring mode, where the scanning time was 0.1 second, nitrogen gas was used, and the temperature was 400°C. The selected reaction ions for monitoring the tested substance and the internal standard are shown in ►**Table 2**.

Determination of Drug Concentration in Plasma Sample of Beagle Dogs by HPLC-MS/MS Method

A 30-μL dog plasma sample was placed in a 1.5-mL EP centrifuge tube (Eppendorf, Germany). Then, 10 μL of internal standard working solution (50% methanol aqueous solution containing internal standard MF-d_3 [5 μg/mL] and 420 μL of methanol) was added. Then, it was swirled to mix well and centrifuged at 4°C, 12,000 × *g* for 10 minutes. The supernatant (300 μL) was placed in a 1.5-mL EP tube, added 300 μL of water, and the mixture was mixed violently by using a vortex. Then, 150 μL of the diluted solution was placed into an injection bottle, and 2.0 μL of which was used for HPLC-MS/MS analysis. The linearity, specificity, relative recovery, precision, and stability all met the analysis requirements.

Table 2 Monitored ion pairs of the reaction

Compound	Parent ion (*m*/*z*)	Sub ion (*m*/*z*)
MF	521.2	355.1
	521.2	373.2
FF	345.2	121.1
	345.2	149.1
MF-d_3	524.2	355.2
	524.2	373.2

Abbreviations: FF, formoterol fumarate dihydrate; MF, mometasone furoate.

PK Study

PK parameters of MF and FF in the plasma sample of beagle dogs treated with inhalable microparticles (Group 1) and drug mixed solution (Group 2) were estimated by WinNonlin software (Pharsight, Delaware, United States). The main *PK* parameters were calculated by the noncompartment model.

Results

Morphological Characteristics of the Inhalable Particles

SEM images of micronized MF and FF showed massive particles with edges and corners (►**Fig. 1**), which may be drug crystals with different sizes and shapes. Many small and fragmented particles were also observed, which may be related to mechanical comminution. Moreover, a certain degree of aggregation between the microparticles was also found.

SEM images of SFD APIs exhibited good spherical integrity, where the surface of the microparticles was smooth, there were no obvious pores, and the diameters of the single microparticles were mostly smaller than 1 μm (►**Fig. 1**). Unlike the local aggregation of micronized APIs, there was universal aggregation between the SFD-drug-inhalable microparticles.

SEM images of SFD Lip-APIs showed that although the microparticles were spherical, their integrity was not as good as that of SFD APIs, and their surface was uneven with obvious pores. Several individual microparticles were observed in the field of vision, and the dispersion between the microparticles was relatively good, and the adhesion between the microparticles was improved significantly.

PXRD

As shown in ►**Fig. 2**, there were obvious diffraction peaks in micronized MF, indicating a large number of crystals with characteristic 2θ angles of 9.240°, 12.989°, and 15.971° (►**Fig. 2**). Micronized FF also had obvious diffraction peaks; there were a large number of crystals with characteristic 2θ angle of 5.530°.

The characteristic diffraction peaks of the SFD APIs disappeared, showing a diffusion peak, which indicated that they had an amorphous form. The SFD Lip-APIs also showed a diffusion peak, indicating that the drug was possibly amorphous, or did not show a characteristic diffraction peak because it was coated with a phospholipid.

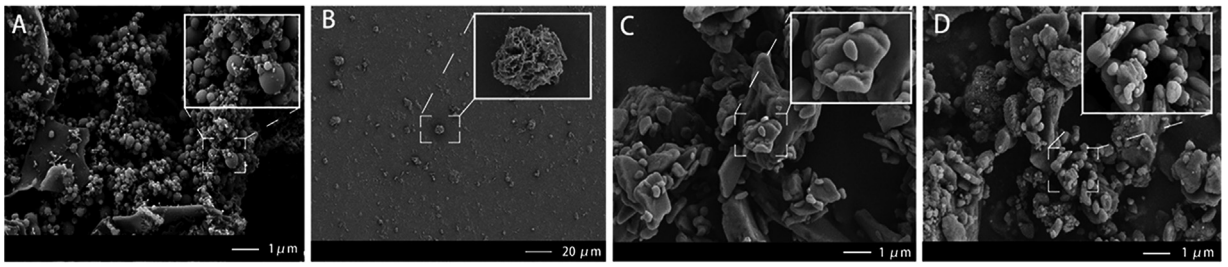

Fig. 1 SEM images of (**A**) SFD APIs, (**B**) SFD Lip-APIs, (**C**) FF, and (**D**) MF. API, active pharmaceutical ingredient; FF, formoterol fumarate dihydrate; MF, mometasone furoate; SEM, scanning electron microscopy; SFD, spray-freeze-drying.

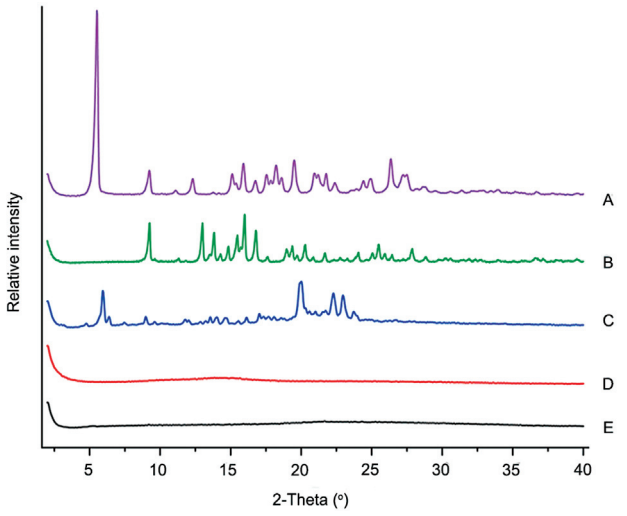

Fig. 2 PXRD results of (**A**) micronized FF, (**B**) micronized MF, (**C**) DSPC, (**D**) SFD APIs, and (**E**) SFD Lip-APIs. API, active pharmaceutical ingredient; DSPC, distearoylphosphatidylcholine; FF, formoterol fumarate dihydrate; MF, mometasone furoate; PXRD, powder X-ray diffraction; SFD, spray-freeze-drying.

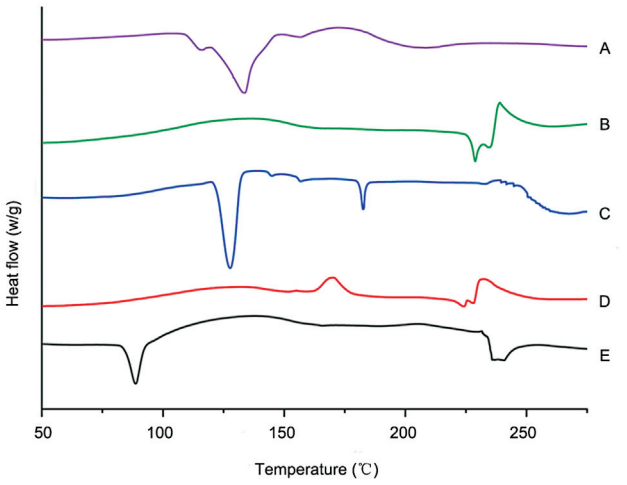

Fig. 3 DSC results of (**A**) micronized FF, (**B**) micronized MF, (**C**) DSPC, (**D**) SFD APIs, and (**E**) SFD Lip-APIs. API, active pharmaceutical ingredient; DSC, differential scanning calorimetry; DSPC, distearoylphosphatidylcholine; FF, formoterol fumarate dihydrate; MF, mometasone furoate; SFD, spray-freeze-drying.

DSC

As shown in the ►**Fig. 3**, DSC thermogram of micronized MF showed an endothermic peak at 229°C, which represented a melting peak. The exothermic peak appeared at 239°C, indicative of that there is a chemical degradation reaction. The DSC thermogram of FF showed a melting endothermic peak at 134°C. However, the DSC thermogram of SFD APIs showed (1) a gentle endothermic peak occurring at approximately 50°C in comparison with micronized MF/FF, indicating the transition from a glassy to a viscoelastic state; (2) an exothermic peak at 170°C that may be caused by amorphous crystallization; and (3) a melting endothermic peak at 224°C, which was close to the melting endothermic peak of micronized MF, but without the melting endothermic peak of FF. The DSC thermogram of SFD Lip-API showed an endothermic peak at 89°C, which was possibly due to the melting of the DSPC skeleton. In addition, a relatively gentle endothermic peak at 239°C was observed and this may relate to the melting of MF. The endothermic peak at this position was approximately 10°C higher than micronized MF, and this is possibly due to the interactions between MF and DSPC.

Bulk-Density Determination

As shown in ►**Table 3**, the bulk density of the micronized MF and FF ranged between 170 and 300 mg/cm^3; however, after SFD treatment, the bulk density of the microparticles was obviously decreased, by approximately an order of magnitude, suggesting a significant reduction effect of SFD on the density of drug particles.

Table 3 Bulk density of different inhalable microparticles

Particles	Bulk density (mg/cm^3)
Micronized MF	188.62 ± 2.76
Micronized FF	274.97 ± 3.67
SFD APIs	21.19 ± 0.86
SFD Lip-APIs	15.46 ± 1.04

Abbreviations: API, active pharmaceutical ingredient; FF, formoterol fumarate dihydrate; MF, mometasone furoate; SFD, spray-freeze-drying.

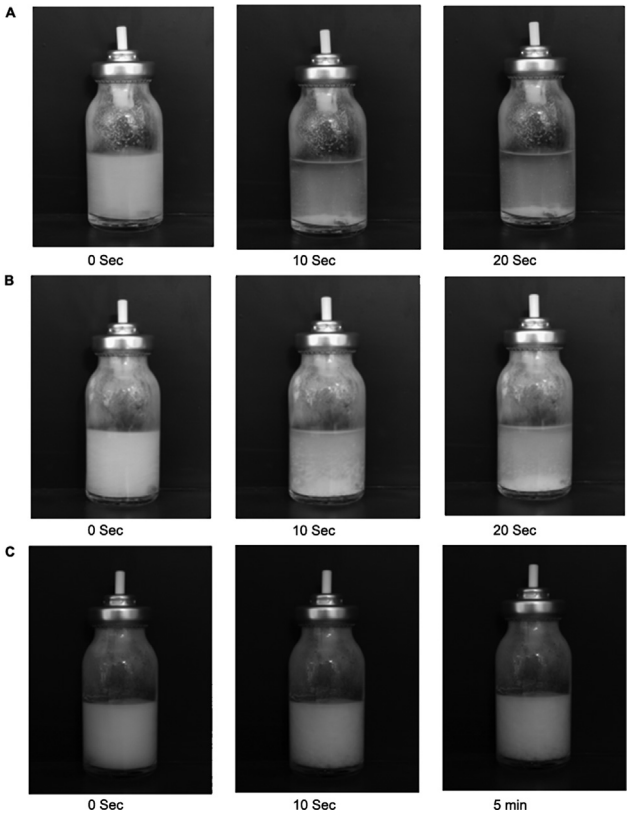

Fig. 4 Suspension and dispersion state diagrams of (**A**) micronized API pMDI, (**B**) SFD API pMDI, and (**C**) SFD Lip-API pMDI in HFA134a. API, active pharmaceutical ingredient; pMDI, pressurized metered dose inhaler; SFD, spray-freeze-drying.

Suspension and Dispersion of the Inhalable Microparticles

Many types of inhalable microparticles exhibit different dispersion and suspension performances in an HFA continuous phase environment. ►**Fig. 4** demonstrates the suspension and dispersion states of different inhalable microparticles in HFA134a. ►**Fig. 4A** shows that crystal aggregation and sedimentation of micronized drugs quickly occurred within 10 seconds, while ►**Fig. 4B** shows that the SFD APIs did not completely sediment at 10 or even 20 seconds, and no accumulation of inhalable microparticles at the bottom of the bottle. However, HFA134a was relatively turbid, indicating that some of the microparticles were still suspended, and the microparticles had a cloud shape in HFA134a, indicating a serious phenomenon of particle aggregation. ►**Fig. 4C** shows that SFD Lip-APIs had neither sedimentation nor obvious agglomeration, indicating a good uniformity. After shaking and keeping it for 5 minutes, the SFD Lip-API pMDI was still consistent with the initial state of shaking, and the suspension and dispersion were also excellent.

Drug Concentration–Time Curve of Inhalable Microparticles in Airway Administration

Beagle dogs were intratracheally administered with mixed solution of drug and inhalable microparticles, respectively. At indicated time points, a plasma sample was obtained, and the concentration–time curves of MF and FF were determined using a HPLC-MS/MS method. The results are shown in ►**Fig. 5**. Our data suggested that the C_{max} and $AUC_{(0-\infty)}$ of the two groups had no significant differences ($p > 0.05$).

PK Parameters

As shown in ►**Tables 4** and **5**, C_{max} reflects the rate of absorption and $AUC_{(0-\infty)}$ reflects the extent of absorption. Our data showed that Group 1 and Group 2 exhibit similar rates and degrees of absorption, indicating that the amount of drug distributed in lung tissue after the administration of inhalable microparticles and the drug solution was close. It can be further speculated that inhalable microparticles can dissolve rapidly in the mucus layer to release drug molecules after depositing in the inner surface of lung organs. Meanwhile, the T_{max} (Group 1) of MF was 0.71 ± 0.66 hours and T_{max} (Group 2) was 0.69 ± 0.31 hours (►**Table 4**). With T_{max} (Group 1) of 0.25 ± 0 hours and T_{max} (Group 2) of 0.33 ± 0.21 hours for FF after administration (►**Table 5**), the peak times of both active ingredients of the two groups were close, and it reflected that the drug dissolution time after deposition of the inhalable microparticles was very short, exhibiting a

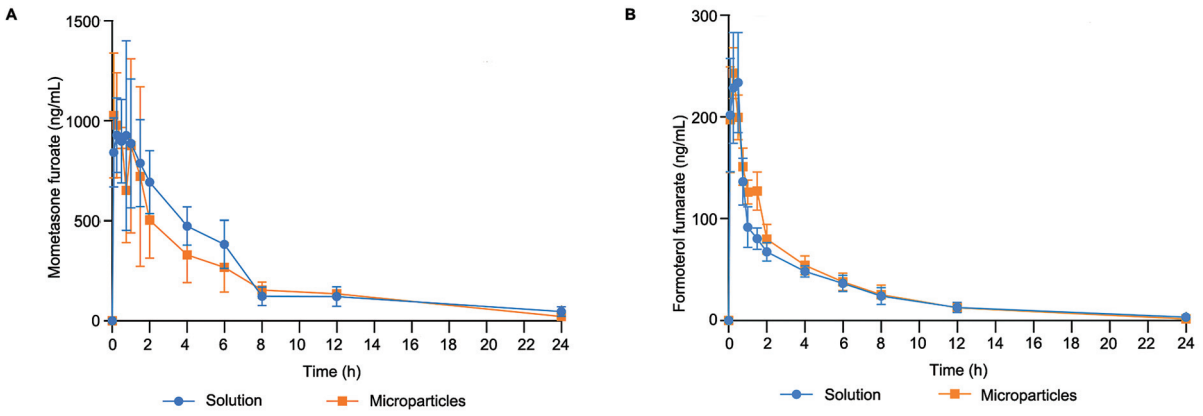

Fig. 5 Concentration–time curve of (**A**) MF and (**B**) FF in plasma samples of beagle dogs. Data were presented as mean of at least three trials, and value was recorded as mean ± standard deviation. Comparison between two groups was conducted using *t*-test with statistical significance at $p < 0.05$. FF, formoterol fumarate dihydrate; MF, mometasone furoate.

Table 4 Pharmacokinetic behaviors of MF in the plasma after airway administration in beagle dogs

Parameter	Group 1					Group 2				
	M01 (♂)	M02 (♂)	F01 (♀)	F02 (♀)	Mean ± SD	M01 (♂)	M02 (♂)	F01 (♀)	F02 (♀)	Mean ± SD
$T_{1/2}$ (h)	5.80	5.18	3.78	4.49	4.81 ± 0.87	10.93	14.34	5.61	4.12	8.75 ± 4.74
T_{max} (h)	0.08	1.00	1.50	0.25	0.71 ± 0.66	0.75	0.75	1.00	0.25	0.69 ± 0.31
C_{max} (ng/mL)	1,290	1,400	1,130	1,360	1,295 ± 119	1,390	1,220	833	1,110	1,138 ± 234
$AUC_{(0-24\ h)}$ (h × ng/mL)	4,529.76	5,964.45	5,346.41	3,807.53	4,912.04 ± 942.04	7,187.31	6,417.55	5,273.54	3,867.17	5,686.39 ± 1,445.38
$AUC_{(0-\infty)}$ (h × ng/mL)	4,805.91	6,584.89	5,405.88	3,890.43	5,171.78 ± 1,129.51	8,259.88	7,724.90	5,608.47	3,955.22	6,387.12 ± 1,984.72

Abbreviations: AUC, area under the curve; C_{max}, maximum concentration; MF, mometasone furoate; SD, standard deviation; $T_{1/2}$, elimination half-life; T_{max}, time to reach maximum concentration.
Note: Group 1: inhalable microparticles. Group 2: a mix solution of MF and FF.

Table 5 Pharmacokinetic behaviors of FF in plasma after airway administration in beagle dogs

Parameter	Group 1					Group 2				
	M01 (♂)	M02 (♂)	F01 (♀)	F02 (♀)	Mean ± SD	M01 (♂)	M02 (♂)	F01 (♀)	F02 (♀)	Mean ± SD
$T_{1/2}$ (h)	4.18	3.90	3.85	3.81	3.94 ± 0.17	5.04	5.77	5.03	4.33	5.04 ± 0.59
T_{max} (h)	0.25	0.25	0.25	0.25	0.25 ± 0	0.50	0.08	0.25	0.50	0.33 ± 0.21
C_{max} (ng/mL)	249	265	207	251	243 ± 25	284	270	229	183	242 ± 45
$AUC_{(0-24\ h)}$ (h × ng/mL)	828.78	794.45	633.86	727.97	746.27 ± 85.83	834.32	749.33	621.81	554.67	690.03 ± 125.58
$AUC_{(0-\infty)}$ (h × ng/mL)	842.24	805.21	639.27	733.68	755.1 ± 89.41	862.08	799.22	639.54	561.91	715.69 ± 138.87

Abbreviations: AUC, area under the curve; C_{max}, maximum concentration; FF, formoterol fumarate dihydrate; SD, standard deviation; $T_{1/2}$, elimination half-life; T_{max}, time to reach maximum concentration.
Note: Group 1: inhalable microparticles. Group 2: a mix solution of MF and FF.

similar absorption behavior to that of the drug solution without the dissolution process.

Discussion

The aerodynamic particle size of inhalable microparticles is one of the key indicators in the development of inhalable formulations. When the geometric particle size is reduced to the range of 1 to 5 μm, the aerodynamic particle size can be further reduced by reducing the density, thereby increasing the fine particle fraction (FPF). Our data showed that bulk densities of both the SFD APIs and SFD Lip-APIs decreased significantly when compared with micronized MF (►**Table 3**). However, the FPF value of SFD APIs pMDI was not high accordingly, especially for MF, which was only approximately 7%.[1]

SEM images of SFD APIs revealed the formation of solid bridges between the microparticles (►**Fig. 1**). Although the individual microparticle's diameter is smaller than 1 μm, several microparticles bonded to each other through solid bridges, resulting in an enlarged geometric particle size. Solid bridges are difficult to disperse in the HFA environment through shaking, presenting a cloud shape when the dispersed suspension state was observed in a glass bottle. Therefore, when SFD reduces microparticle density, FPF may not be necessarily enhanced if solid bridges appear among the microparticles.

Interestingly, the FPF value of SFD Lip-APIs was increased to 48%, suggesting a better FPF performance of the particles.[1] Compared with the SFD API solution, which was used as atomized feedstock, the feedstock of the SFD Lip-APIs is suspension, in which FF was in a solution form, but the proportion was only approximately 1%, which did not easily form solid bridges during freeze-drying. Thus, the SFD Lip-APIs had a low density and failed to form solid bridges, leading to the effective enhancement of the FPF value.

Unlike dry powder inhalers, the dispersibility of inhalable microparticles in the propellent in pMDI must be considered. pMDI valves had a quantitative volume of tens of μL, specifically 50 and 63 μL. For suspension pMDIs, the concentration of inhalable microparticles in suspension of several tens of μL of the actuation can be uniform and accurate in delivery, only when the inhalable microparticles can be maintained in HFA for a certain period of time (usually tens of seconds) for uniform dispersion. However, a stronger drug microparticle cohesion often led to an aggregation phenomenon; thus, how to reduce aggregation of microparticles has become a key technical difficulty for suspension pMDIs.

Phospholipid excipients such as DSPC have relatively small molecular polarity, low cohesion in the environment of HFA propellant, and easily disperse.[11] Thus, DSPC was chosen as an excipient in SFD Lip-APIs. Evidence suggested that increasing the roughness of the surface is beneficial to reduce the aggregation strength of microparticles, such as bumpiness or ripples that can significantly reduce interparticle bonding and aggregation phenomenon.[1] Our SEM results showed that the matrix of the SFD Lip-APIs was composed of DSPC, and the drug was embedded in the DSPC (►**Fig. 1**). The structure avoids contact between the drug microparticles and the surface of the microparticles, and was bumpy and porous due to the sublimation of the solvent during freeze-drying. This improved not only the dispersion state of the inhalable microparticles in the HFA, but also their suspension by decreasing the density of the particles. Therefore, SFD Lip-APIs could maintain a state of dispersed uniformity for more than 5 minutes, which was superior to conventional micronized APIs and SFD APIs (►**Fig. 4**).

The solvent removal process during spray drying is very rapid, and the solutes that dissolve in the drug solution tend to form an amorphous form, resulting in thermodynamically unstable states with the possibility of crystal form transformation.[12] If a transformation from amorphous to crystallization occurs, the density of the microparticles will increase and the geometric particle size may also change, which in turn affects the aerodynamic particle size.[13] DSC results of SFD APIs showed that the microparticles had an exothermic peak for MF crystallization (►**Fig. 3**); however, the PXRD results showed no characteristic diffraction peaks (►**Fig. 2**), confirming that its state was amorphous and there was a risk of transferring amorphous MF into crystal MF. For SFD Lip-APIs, although no characteristic diffraction peaks appeared in its PXRD results, an MF melting peak was observed in DSC assay, and the exothermic peak did not appear. This may be attributed to SFD Lip-APIs, as the drug crystals were embedded in the phospholipids, which reduced the risk of transferring the amorphous state into a crystal state, resulting in better physical stability than amorphous drugs.

After airway administration, the *in vivo* behavior of the drug in inhalable microparticles or droplets mainly includes the following stages. Stage 1: the inhalable microparticles or droplets deposit on the inner surface of the lung. Different from inhalable droplets, microparticles deposited in the lung must be dissolved before absorbed. Stage 2: the dissolved drug molecules are absorbed from the airway into the pulmonary capillaries. Stage 3: the dense capillary network enables the absorbed drug to distribute rapidly to the systemic circulation. Stage 4: the drug in systemic circulation is distributed to other organs and eliminated gradually. In this stage, the *PK* behaviors depend on the drug properties rather than on the pulmonary route. Therefore, the concentrations of MF and FF in the systemic circulation may reflect the absorption in the pulmonary route and the amount of drug distributed in lung tissues. As shown in ►**Tables 4** and **5**, T_{max} values of MF in Group 1 and Group 2 were 0.71 ± 0.66 and 0.69 ± 0.31 hours, respectively. T_{max} values of FF in Group 1 and Group 2 were 0.25 ± 0 and 0.33 ± 0.21 hours, respectively. However, C_{max} of both APIs displayed no significant difference between inhalable microparticles and solution, reflecting that the drug amount of inhalable microparticles distributed in lung tissues was close to that of the drug solution, and the drug dissolution time after deposition of inhalable microparticles was very short, indicating that inhalable microparticles could dissolve rapidly in the mucus layer to release drug molecules after depositing in the inner surface of lung organs.

Conclusion

Inhalable phospholipid microparticles prepared by SFD were characterized by their low density, less adhesion to each other, and excellent dispersed suspension properties in HFA, which were important for pMDIs to achieve good aerodynamic performance and delivered dose content uniformly. Meanwhile, our *PK* results demonstrated that the DSPC, as a matrix excipient in the microparticles, achieved excellent aerosol performance without hindering the release and absorption of the drug.

Conflict of Interest

None declared.

Ethics Statement

The usage of beagle dogs in the present study was approved by the Animal Ethics Committee of Zhejiang University Laboratory Animal Center.

Reference

1 Xi Q, Miao JY, Cao Z, Wang H. The study of spray-freeze-drying technique for development of novel combination pMDIs, part I: study on the preparation method. Pharmaceutical Fronts 2022;4 (03):e179–e187

2 Chow AHL, Tong HHY, Chattopadhyay P, Shekunov BY. Particle engineering for pulmonary drug delivery. Pharm Res 2007;24 (03):411–437

3 Weers J, Tarara T. The PulmoSphere™ platform for pulmonary drug delivery. Ther Deliv 2014;5(03):277–295

4 Weers JG, Miller DP. Formulation design of dry powders for inhalation. J Pharm Sci 2015;104(10):3259–3288

5 Vandevanter DR, Geller DE. Tobramycin administered by the TOBI Podhaler for persons with cystic fibrosis: a review. Med Devices (Auckl) 2011;4:179–188

6 Vishali DA, Monisha J, Sivakamasundari SK, Moses JA, Anandharamakrishnan C. Spray freeze drying: emerging applications in drug delivery. J Control Release 2019;300:93–101

7 Cun D, Zhang C, Bera H, Yang M. Particle engineering principles and technologies for pharmaceutical biologics. Adv Drug Deliv Rev 2021;174:140–167

8 Zhao J, Yang J, Xie Y. Improvement strategies for the oral bioavailability of poorly water-soluble flavonoids: An overview. Int J Pharm 2019;570:118642

9 Dolovich MA. Influence of inspiratory flow rate, particle size, and airway caliber on aerosolized drug delivery to the lung. Respir Care 2000;45(06):597–608

10 Mitchell JP, Nagel MW. Cascade impactors for the size characterization of aerosols from medical inhalers: their uses and limitations. J Aerosol Med 2003;16(04):341–377

11 Swarbrick J. Encyclopedia of Pharmaceutical Science and Technology. 4th ed. Boca Raton, FL: CRC Press; 2013

12 Weiler C, Egen M, Trunk M, Langguth P. Force control and powder dispersibility of spray dried particles for inhalation. J Pharm Sci 2010;99(01):303–316

13 Ma Y, Gao J, Jia W, et al. A comparison of spray-drying and freeze-drying for the production of stable silybin nanosuspensions. J Nanosci Nanotechnol 2020;20(06):3598–3603