



# The Study of Spray-Freeze-Drying Technique for Development of Novel Combination pMDIs, Part I: Study on the Preparation Method

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## Abstract

Clinically available pressurized metered-dose inhalers (pMDIs) mainly directly use micronized drugs as inhalable microparticles. Although technology for preparing pMDIs has proven to obtain clinically appropriate aerosol performance, the fine particle fraction and delivered dose content uniformity (DDCU) of pMDIs still need to be improved. DDCU problem is usually exacerbated by patients' handling errors prior to taking a dose. In this study, novel phospholipid microparticle inhalation pMDIs were prepared by a spray-freeze-drying process using mometasone furoate and formoterol fumarate dihydrate as model drugs and distearoylphosphatidylcholine as an excipient. Combined with the material composition, the atomization and freeze-drying processes were also studied. Our data showed that both atomization parameters of gas-liquid ratio and freeze-drying curve settings met the requirements of drug design. According to aerodynamic performance *in vitro* and DDCU evaluation, the performance of the phospholipid microparticle inhalation pMDI was better than that of the micronized drug microparticle pMDI. In conclusion, preparing pMDIs with particle engineering has the potential to ensure accuracy of quantification and to improve the efficiency of drug deposition in lungs in clinical practice.

## Keywords

- ▶ spray-freeze-drying
- ▶ inhalable microparticles
- ▶ pMDIs
- ▶ phospholipids

## Introduction

Chronic respiratory disease, commonly represented by chronic obstructive pulmonary disease (COPD) and asthma, has been a challenging global health problem. It is estimated that chronic respiratory disease will be the third leading cause of death worldwide by 2030.<sup>1</sup> Currently,  $\beta$ -receptor agonists, bronchodilator agents, and inhaled corticosteroids are the most effective APIs (active pharmaceutical ingre-

dients) for the treatment of COPD and asthma, yet, only significantly effective when administered deeply into the lungs. However, when the drugs were systemically distributed, adverse effects occur.<sup>2</sup> Thus, administering via inhalation may be the best way to deliver these APIs into the respiratory system for maximal efficacy. Evidence suggested that dosage form design combines physiological respiratory characteristics with aerosol properties, and the concentration of a drug at the target site is closely related to the

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aerodynamic properties of the drug microparticles.<sup>3,4</sup> Due to factors such as bronchospasm, inflammation, and mucus hypersecretion, airways of patients with COPD and asthma are much narrower than those of healthy people, leading to the exacerbated collision of drug particles in the airways, which influences the deposition of drugs at the target site of airways and the subsequent therapeutic effect of the drugs.<sup>5,6</sup>

A pressurized metered-dose inhaler (pMDI) is a dosage form with the largest annual output, the largest number of users, and the widest range of inhalation preparations. Maximizing delivery of the drug to the lungs is one of the key technical requirements during its development.<sup>7</sup> A next-generation pharmaceutical impactor (NGI) has been developed for evaluating aerosol performance, with fine particle fraction (FPF) as the key index. In general, the higher the FPF, the greater the amount of drug deposited in the lungs at the time of clinical application.<sup>8</sup> Suspension pMDIs tend to maintain dispersed for a short time, resulting in the problem of delivered dose content uniformity (DDCU). The propellant used for these pMDIs is hydrofluoroalkane (HFA), which is a continuous phase with low polarity. Unfortunately, many dispersing excipients used commonly have very low solubility or even do not dissolve in HFA. Given above, it is necessary to develop a novel technology to increase the delivery efficiency of pMDI in the lung and to improve the DDCU of suspension pMDIs per actuation.

Asthma guidelines advised against the use of LABA (long-acting  $\beta$  agonist) as monotherapy for long-term control, and recommend that ICS (inhaled corticosteroid) therapy should not be discontinued while taking LABA. Therefore, an ICS/LABA inhaler can be used as a reliever and a scheduled maintenance therapy with better symptom control than monotherapy. Inspired, drug combinations may be delivered via a single inhaler, with advantages of enhancing therapeutic effect, improving adherence, and possibly reducing cost. In addition, the combinations of LABAs and ICSs are available via pMDI (e.g., formoterol/budesonide, Symbicort, AstraZeneca; formoterol/Mometasone, Dulera, Merck) and DPI (e.g., salmeterol/fluticasone, Advair, GSK).<sup>9,10</sup> For pMDIs, the properties of different APIs are not the same, and the physicochemical environment, required to maintain well-dispersed microparticles, is different. Therefore, the development of pMDI will be more challenging.

In this study, mometasone furoate (MF), a water-insoluble drug, and formoterol fumarate dihydrate (FF), a water-soluble drug, were selected as model drugs, and inhalable microparticles were prepared by spray-freeze-drying (SFD). This process directly forms solid inhalable microparticles from the solution/suspension. Compared with the traditional micronized APIs, the microparticles may have an advantageous morphological structure. The microparticles can reduce the aerodynamic particle size by reducing the density, and then enhance the FPF of the pMDI. Moreover, the process makes it possible to form drug-containing microparticles via the incorporation of excipients that cannot be dissolved in HFA, leading to the improvement of the dispersion state of inhalable microparticles in the pMDI, which is expected to

achieve better DDCU.<sup>11–13</sup> FPF and DDCU were used as key indicators to compare the SFD microparticle pMDI with Dulera. A comparative study was also performed to further reveal the effects of the two preparation methods on the performance of the pMDIs *in vitro*.

## Materials and Methods

### Materials

The micronized drug FF was purchased from Guangzhou Greensyn Co., Ltd. with a purity of 99.6% (batch NO. FF/101/17–18). The micronized drug MF was purchased from Hunan YuXin Pharmaceutical Co., Ltd. with a purity of 99.6% (batch NO. KS-200801). Dulera<sup>®</sup> (Merck, batch NO. U006804) distearoylphosphatidylcholine (DSPC) was purchased from Shanghai LangXu Biotechnology Co., Ltd. with a purity of 98% (batch NO. 20210501). Soy lecithin (SPC) was purchased from Shengyang Tianfeng Biological Pharmaceutical Co., Ltd. (batch NO. SY-SO-211003). Hydrogenated SPC (HSPC) was purchased from Nippon Fine Chemical Co., Ltd. (batch NO. C00257). 1,1,1,2-Tetrafluoroethane (HFA134a) was purchased from XueHui Refrigeration Equipment Co., Ltd. Dioxane (Sinopharm Reagent, China) has a purity of no less than 99.5% (batch NO. 20210527). Anhydrous calcium chloride was purchased from Shanghai TiTan Technology Co., Ltd. with a purity of no less than 96.0% (batch NO. P1902809). Methanol of high-performance liquid chromatography (HPLC) grade was used, and all other reagents were of analytical grade.

### Methods

#### Preparation of Feedstock for Spray

A mixture solution was prepared by mixing MF with aqueous dioxane to obtain a 30 mg/mL MF solution. Then, FF was added to the solution at a concentration of 1.43 mg/mL ( $W_{(MF)}:W_{(FF)} = 115:5.5$ ).

Aqueous solutions of anhydrous calcium chloride ( $\text{CaCl}_2$ ) and FF were prepared in parallel and in triplicate by adding the chemicals to water and dissolving thoroughly, followed by the addition of three phospholipids, namely, SPC, HSPC, and DSPC, so that the phospholipids were well dispersed in the water. Subsequently, the three sample groups were sheared at 10,000 rpm for 2 minutes, and MF was added during the high-shear dispersion. Finally, model drug phospholipid suspensions were obtained by homogenizing five times at high pressure (170 MPa).

#### Feedstock Atomization

The SFD process can directly freeze atomized droplets, and the diameter of the droplets largely determines the size of the final solid inhalable microparticle.<sup>14</sup> Herein, the atomization process was conducted with a double-fluid nozzle, in which the kinetic energy of high-pressure gas was used to cut the feedstock into small droplets. The larger the increase in the surface area of a particle, the higher its surface energy, the smaller the resulting droplet diameter, and the greater the kinetic energy of the gas required. For a particular spray

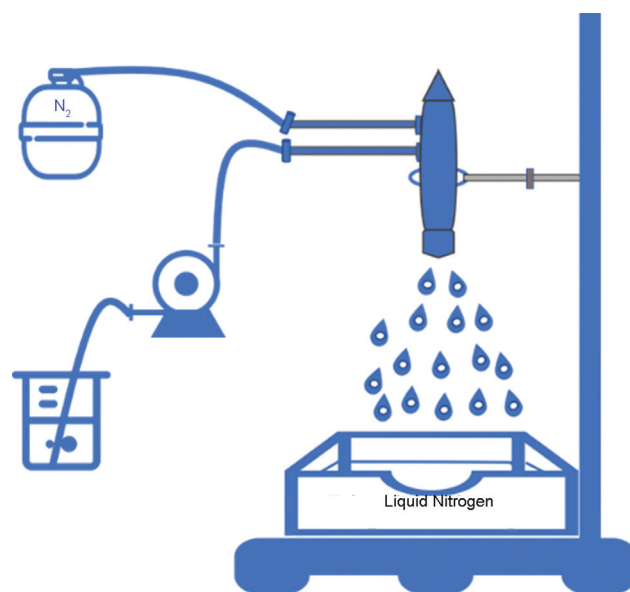
nozzle, the ratio of gas pressure to liquid flow rate (gas–liquid ratio) is the main factor affecting the atomization effect. In general, the droplet diameter will gradually decrease with the increase of gas–liquid ratio, and this trend had a marginal effect. When gas–liquid ratio increases to a specific interval, the droplet size does not decrease. It is important to note that different liquids have different surface tensions, which may influence the atomization effect. In this study, the effect of the gas–liquid ratio on the atomization efficiency was investigated by using the composition of the fixed drug solutions and the geometric particle size distribution of the solid inhalable microparticles as indicators. In addition, the gas–liquid ratio was fixed to investigate the effect of different pharmaceutical liquid compositions on the atomization efficiency.

The feedstock was delivered to the double-fluid nozzle (the diameter of gas cap and nozzle is 5 and 0.5 mm, respectively) via a YZ2515X-A peristaltic pump, while the high-pressure nitrogen valve was opened. The pressure valve was adjusted to control the atomization pressure, and the flow rate of the drug solution was controlled by the peristaltic pump for spray-freeze operation (►Fig. 1).

Typical formulations are shown in ►Table 1. F1 and F2 are micronized APIs. F3–F5 were used to screen the process parameters of SFD by preparing MF solution with a concentration of 30 mg/mL. After confirming the process, F6 microparticles (MF:FF = 115:5.5) were prepared to meet the clinical dose. pMDI made of F6 had poor aerosol performance, so phospholipid excipients were screened to improve the performance of inhalable microparticles. After screening different phospholipids (F7, F8), phospholipid DSPC was optimized to prepare F9 microparticles as a matrix excipient. The formulation ratios of F6 and F9 were identical and the formulation ratio of F9 is DSPC:MF:FF = 63:21:1.

### Freeze Drying

The microparticles prepared by spraying and freezing were transferred to a Virtis Advantage 2.0 el-85 freeze dryer (AIT, United States). The eutectic point of a typical representative formulation was measured at the prefreeze stage. The eutectic points were  $-17.7^{\circ}\text{C}$  for F4,  $-11.8^{\circ}\text{C}$  for F7,  $-17.0^{\circ}\text{C}$  for F8, and  $-15.9^{\circ}\text{C}$  for F9. The prefreeze stage temperature was set



**Fig. 1** Schematic representation of the spray-freeze process of the drug liquid.

at 10 to  $20^{\circ}\text{C}$  below the eutectic point of each formulation, ►Fig. 2 shows the freeze-drying curve program we used. After freeze drying, the obtained samples were sealed and stored in a moisture-proof cabinet protected from light.

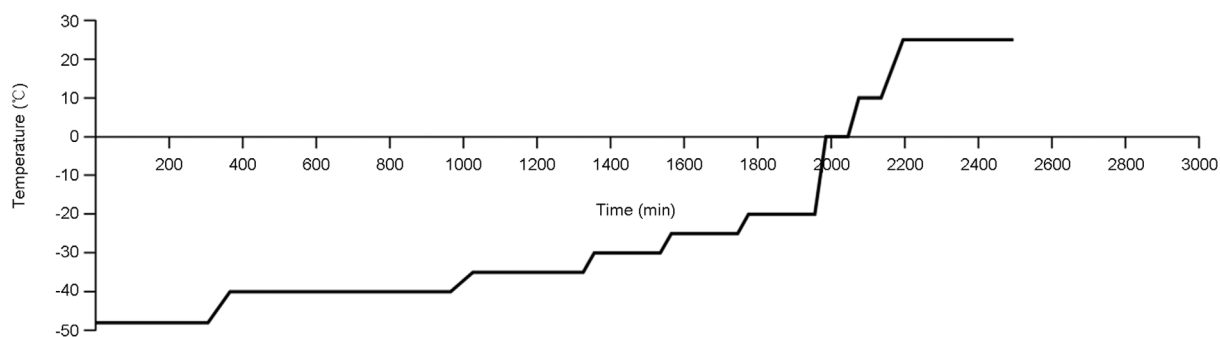
### Preparation of pMDIs

The micronized MF and FF ( $W_{(\text{MF})}:W_{(\text{FF})} = 115:5.5$ , with polyvinylpyrrolidone addition for the improvement of drug dispersion in HFA), the model drug particles prepared by the SFD method, and the model drug phospholipid microparticles prepared by SFD were placed into 14-mL aluminum cans (Anomatic, China) according to their different groups, and crimped after addition of DF 316 valves (Aptar, China). Each formulation pMDI was prepared by filling with HFA134a. The crimper (Shenghua, China) parameters were as follows: a primary pressure of 8 to 9 bar, an operating pressure of 3.5 to 4.5 bar, and a closure height of 5.55 mm. The filler machine (Shenghua, China) pressure was 8.5 to 9.5 bar, and the working pressure was 7.5 to 8.5 bar. The

**Table 1** Characteristics of inhalable microparticles

Formulation	Compound	Solvent	Gas–liquid ratio (MPa·min/mL)
F1	Micronized MF	N/A	N/A
F2	Micronized FF	N/A	N/A
F3	MF	80% (v/v) aqueous dioxane	0.033
F4	MF	80% (v/v) aqueous dioxane	0.010
F5	MF	80% (v/v) aqueous dioxane	0.005
F6	MF:FF = 115:5.5	80% (v/v) aqueous dioxane	0.010
F7	SPC:MF = 1:1	Water	0.010
F8	HSPC:FF = 47:1	Water	0.010
F9	DSPC:MF:FF = 63:21:1	Water	0.010

Abbreviations: FF, formoterol fumarate dihydrate; HSPC, hydrogenated soy lecithin; MF, mometasone furoate; SPC, soy lecithin.



**Fig. 2** Freeze-drying curve program.

theoretical filling volumes were 2.42, 2.42, and 9.79 mg/mL, respectively.

### Moisture Determination

A sample of microparticles prepared by SFD was weighed at 50 mg and injected into a titration cup. Each sample was measured three times in parallel. The moisture content was calculated using the following Eqn. (1):

$$\text{Moisture} = \frac{T \times EP}{m} \times 100\% \quad (1)$$

where  $T$  is the average of three determinations of the titer of Karnofsky's reagent (mg/mL);  $EP$  is the volume consumed by Karnofsky's reagent (mL); and  $m$  is the amount of sample to be tested (mg).

### Measurement of Size Distribution

A HELOS (H3838) & RODOS/T4 laser particle sizer (Sympatec, Germany) and a R2 lens (0.25/0.45–87.5  $\mu\text{m}$ ) were used for assay. Size distributions of the samples were determined under a 107-mbar vacuum using 3 bar of dispersion pressure. Each sample was analyzed three times in parallel.

### Evaluation of Aerosol Performance

Chromatography conditions were tested and methodological validation was performed. In this determination, an Ultimate 3000 HPLC (Thermo, United States) and an Ultimate XB-C18 column (4.0 mm  $\times$  300 mm, 5  $\mu\text{m}$ ) were used. The mobile phase was a mixture of methanol and aqueous phosphoric acid (pH 3.5), the column temperature was 40°C, the detection wavelength was 225 nm, the flow rate was 1.5 mL/min, and the injection volume was 20  $\mu\text{L}$ . The peak area  $A$  exhibited a linear relationship with the concentration of FF ( $C_{\text{FF}}$ ) over the range of 0.02 to 0.4  $\mu\text{g/mL}$ , with a linear equation of  $A = 0.5078C + 0.0014$  and a correlation coefficient ( $R^2$ ) = 0.9991. The concentration of MF ( $C_{\text{MF}}$ ) in the range of 0.05 to 20  $\mu\text{g/mL}$  also showed good linearity with peak area  $A$ , with a linear equation of  $A = 0.3162C + 0.0047$ , and  $R^2 = 0.9997$ . Given above, the method was exclusive and has satisfactory accuracy and stability.

Then, aerodynamic characterization tests were performed using the method described in general by Chinese Pharmacopoeia Part IV general rules 0951. A NGI (Copley

Scientific, United Kingdom) was operated at an air flow rate of 30 L/min ( $\pm 5\%$ ), and the actuator, adapter, throat, collection discs, and micropore filter membranes were cleaned with dispensed solvents. The sample solutions were analyzed by HPLC mentioned above. The schematic of NGI is shown in **Fig. 3**.<sup>15</sup>

After HPLC determination of the amount of drug deposited at each plate in the NGI, the PPF was calculated by CITDAS V3.10 (Copley Inhaler Testing Data Analysis Software, Copley Scientific Ltd., Nottingham, UK).

### Examination of DDCU under Various Shaking Modes

A picture of DDCU is shown in **Fig. 4**. Three manual shaking strategies were used for evaluation: mode 1, immediate actuation after vigorous shaking (20 times with 5-second shaking); mode 2, delayed actuation after vigorous shaking (rest for 30 seconds after shaking); and mode 3, immediate actuation after mild shaking (shaking with vertical inversion, 5-second flip for 5 times). The percentage of the injected dose per press was calculated using the theoretical value of drug content per actuation in the pMDI as a benchmark.

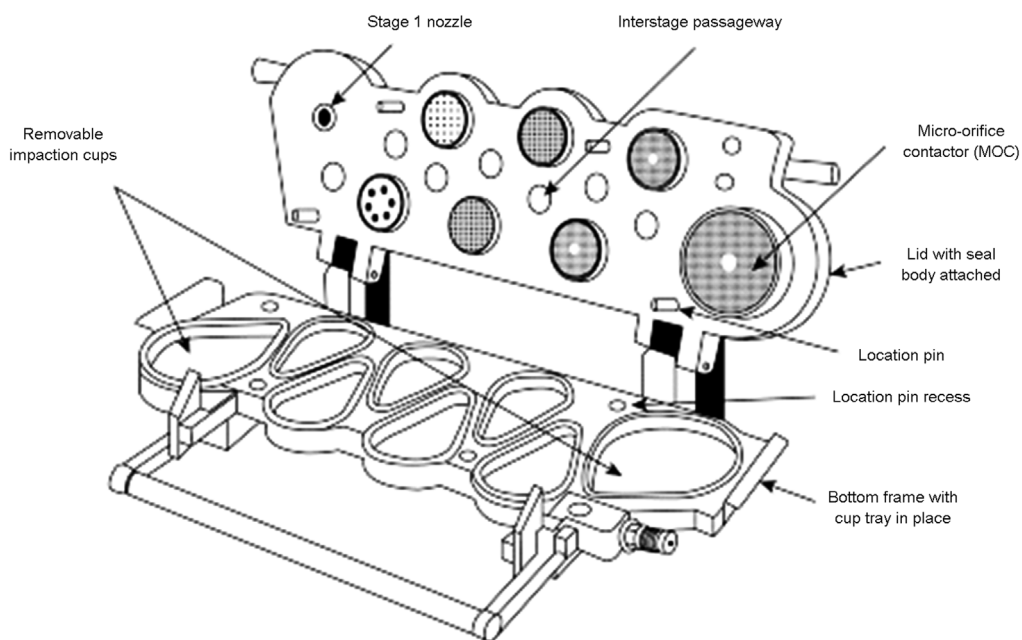
## Results

### Observations of Appearance and Morphology of Different Inhalable Microparticles

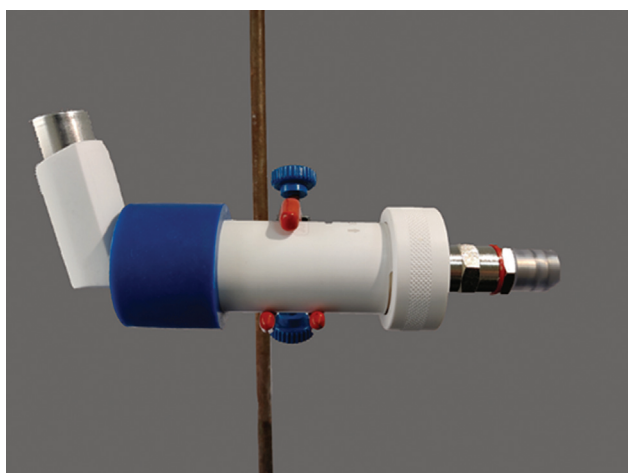
As shown in **Table 2** and **Fig. 5**. Compared with the F1 and F2 micronized API, F3, F4, F5, F6, and F9 microparticles obtained by SFD became loose and easy to disperse, while F7 and F8 failed to form dispersible microparticles through SFD.

### Microparticle Moisture Content

F3, F4, and F5 formulations were only different in their atomization process parameters; their freeze-drying process was the same. Therefore, F4 was selected as a typical formulation of the three. F6 was inhalable microparticles of the SFD drugs, F7 and F8 could not obtain inhalable microparticles, and F9 was inhalable phospholipid microparticles of the SFD drugs. The moisture content of the F4, F6, and F9 inhalable microparticles was lower than 3% (**Table 3**), indicating that the present drying process could remove the moisture in the frozen microparticles.



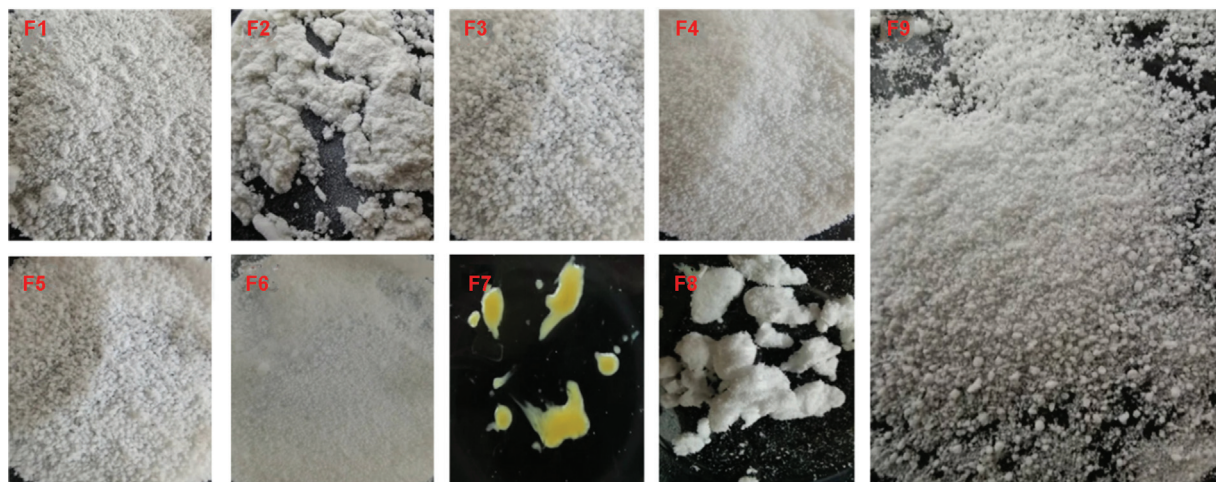
**Fig. 3** Schematic representation of next-generation impactor (NGI).<sup>15</sup>



**Fig. 4** Picture of representative DDCU. DDCU, delivered dose content uniformity.

**Table 2** Description of properties of freeze-dried samples

Prescription	Appearance
F1	White powder
F2	White powder
F3	White loose powder, no adhesion
F4	White loose powder, no adhesion
F5	White loose powder, no adhesion
F6	White loose powder, no adhesion
F7	Light-yellow, gel-like, adhesion
F8	White cotton-like or agglomerate-like powder, a little adhesion
F9	White loose powder, no adhesion



**Fig. 5** Appearance of microparticles.

**Table 3** Residual moisture of freeze-dried samples

Prescription	Residual moisture (%)
F4	1.92 ± 0.14
F6	0.80 ± 0.13
F9	2.44 ± 0.24

**Table 4** Results of particle size distribution for partial prescriptions ( $n = 3$ )

Prescription	D <sub>10</sub> (μm)	D <sub>50</sub> (μm)	D <sub>90</sub> (μm)
F1	0.42 ± 0.01	1.38 ± 0.03	4.19 ± 0.01
F2	0.55 ± 0.04	1.44 ± 0.01	3.00 ± 0.05
F3	0.90 ± 0.27	2.31 ± 0.08	5.24 ± 0.13
F4	1.11 ± 0.04	2.66 ± 0.06	5.78 ± 0.11
F5	1.10 ± 0.03	2.58 ± 0.10	6.07 ± 0.02
F6	1.11 ± 0.02	2.64 ± 0.01	5.64 ± 0.02
F9	0.55 ± 0.01	2.08 ± 0.01	7.36 ± 0.03

### Geometric Particle Size Distributions

F3, F4, F5, F6, and F9 were prepared by SFD, and their geometric particle size distributions were measured by laser diffraction. As shown in ▶Table 4, the particle sizes D<sub>50</sub> of F3, F4, and F5 were all smaller than 3 μm, slightly larger than those of F1 and F2. D<sub>10</sub> and D<sub>90</sub> of F1–F5 show the same trend. Although the gas–liquid ratio used in these three formulations decreased gradually (▶Table 1), the particle size did not show a trend of gradual increase, indicating that for the aqueous dioxane solvent system, a gas–liquid ratio in the range of 0.033 to 0.005 MPa·min/mL could achieve a good atomization effect.<sup>16</sup> The continuous phase of F9 was water, and the particle size distribution of the obtained inhalable microparticles was similar to F3, F4, F5, and F6 with a continuous phase of aqueous dioxane, indicating that the two solvent systems had no significant effect on the particle size of the inhalable microparticles. A D<sub>50</sub> of inhalable microparticles less than 3 μm could be obtained for all formulations and their atomization parameters, and it was possible to gain better aerodynamic performance.

### Evaluation of Aerodynamic Performance *In Vitro*

The most important purpose of the development of inhalation preparation is to increase the dose delivered to the lungs. Despite various limitations, the amount of drug microparticles deposited in the lungs still shows a close correlation with the aerodynamic particle size distribution. The aerodynamic performance of our samples was evaluated by the impactor method and characterized by FPF, as there is a positive correlation between FPF and lung bioavailability.<sup>17</sup> Different inhalable microparticles (F1–F2, F6, and F9) were filled with HFA 134A to obtain pMDIs. Aerodynamic evaluation was performed using the NGI, and the results are described below.

**Table 5** FPF of different pMDIs

Sample	FF (%)	MF (%)
F1–F2 pMDI	37.77 ± 2.39	35.76 ± 2.41
F6 pMDI	30.38 ± 5.38	6.70 ± 1.67
Dulera	42.53 ± 3.53	41.47 ± 1.35
F9 pMDI	47.13 ± 2.88	48.24 ± 0.91

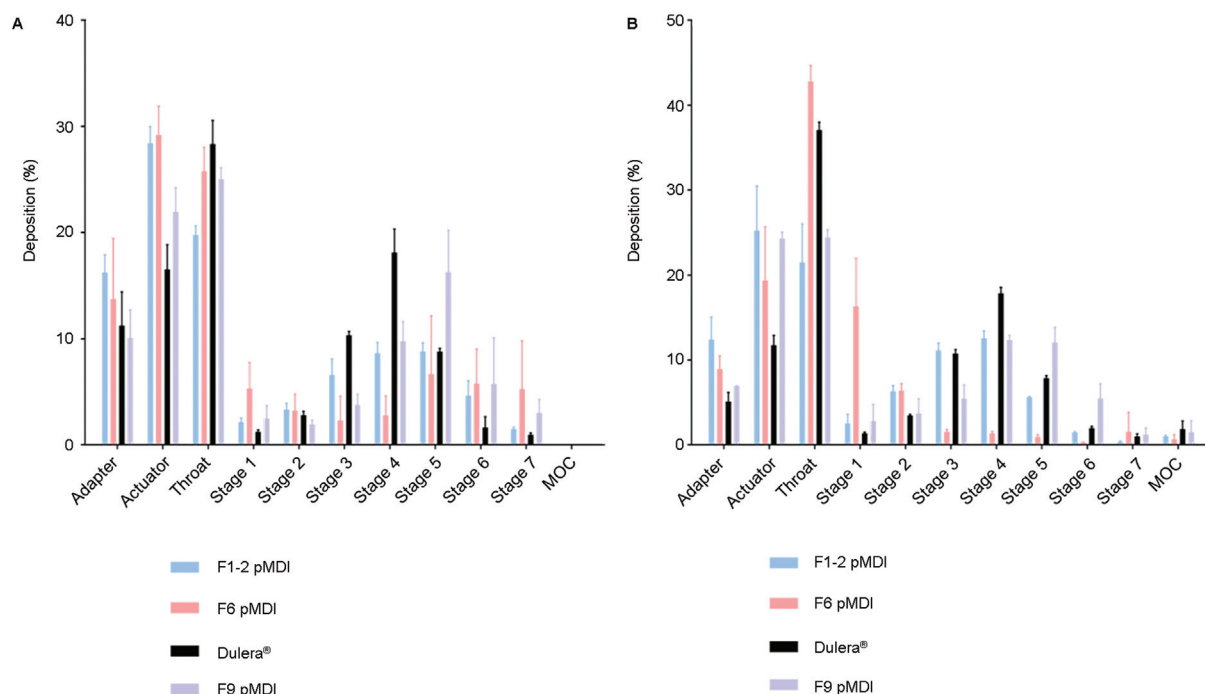
Abbreviations: FF, formoterol fumarate dihydrate; FPF, fine particle fraction; MF, mometasone furoate.

As shown in ▶Table 5 and ▶Fig. 6, the FPF of the F1–F2 pMDI without the SFD process was approximately 35%. However, the FPF of the F6 pMDI with the SFD process decreased, especially the FPF of MF, which was less than 10%. Compared with F1–F2 pMDI, F6 pMDI and Dulera, F9 pMDI had more drug microparticles deposited in Stage 5 and Stage 6 of NGI (▶Fig. 6). Interestingly, the FPF of the F9 pMDI, produced by filling inhalable microparticles after SFD, was significantly improved, which was not only higher than the F1–F2 pMDI, but also higher than the commercial brand-name drug Dulera.

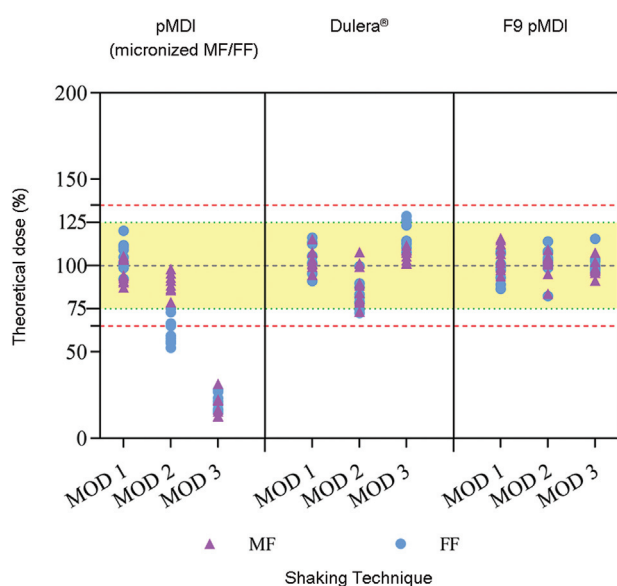
### DDCU under Different Shaking Modes

The physical dispersion stability of HFA pMDIs prepared by micronized drug crystals is generally poor, and they generally suffer from aggregation and sedimentation, which leads to changes in the drug delivered dose. Although the pMDI products have instructions for use, due to a variety of factors including the complexity of the operation, improper shaking intensity before use, and improper timing of inhalation, this may lead to uneven delivery of the dose and affect clinical outcomes.<sup>18–21</sup> Common operating errors in the process of using the drug are that patients do not sufficiently shake the pMDI before taking the medicine and do not use the pMDI as soon as possible after shaking.<sup>18</sup> In practice, there may be a long or short interval between shaking and actuating, which may result in a drug delivery above or below the expected dose, and that depends on the rate of aggregation and sedimentation of the suspension system.<sup>22</sup> To reduce the risk of operating error in the two types of clinical use, three shaking modes (mode 1, 2, and 3) were designed to evaluate the DDCU of different types of microparticle inhalation pMDIs.

The results in ▶Fig. 7 showed that the delivered dose of the three pMDIs was close to the theoretical dose in mode 1 shaking, and the uniformity was good. In mode 2 shaking, the delivered dose of the F1–F2 pMDI decreased. Although the delivered dose of Dulera did not decrease significantly, DDCU became worse, and some doses even exceeded the acceptable range stated on the drug label. The DDCU and accuracy of the F9 pMDI were both good. In mode 3 shaking, the delivered dose of the F1–F2 pMDI decreased more obviously, and the DDCU of Dulera also became worse, with some doses exceeded the limit. Given above, only F9 pMDI still maintained excellent performance in both DDCU and accuracy.



**Fig. 6** Aerodynamic particle size distributions for (A) FF and (B) MF. FF, formoterol fumarate dihydrate; MF, mometasone furoate.



**Fig. 7** DDCU results under different shaking modes. DDCU, delivered dose content uniformity.

## Discussion

As we all know, the formula for calculating the aerodynamic particle size is Eqn. (2):

$$d_{ae} = d_{geo} \sqrt{\frac{\rho_p}{\rho_0 X}} \quad (2)$$

where  $d_{ae}$  is the aerodynamic diameter of a particle,  $d_{geo}$  is the geometric diameter,  $\rho_p$  is the density of the drug par-

ticles,  $\rho_0$  is the unit density, and  $X$  is the shape factor. According to the above formula, the aerodynamic particle size is proportional to geometric particle size. Therefore, the existing research and development of the inhalation products focus on reducing the geometric particle size of the drug microparticles, so as to obtain higher PPF and increase the amount of drug deposited in the lung.<sup>23,24</sup>

F1–F2 pMDI was directly prepared by the micronized MF and FF particles, and its good aerodynamic performance was confirmed in this study, with a PPF slightly higher than 35% (Table 5). Our data suggested that smaller geometric particle sizes ( $D_{50}$  less than 2  $\mu\text{m}$ ,  $D_{90}$  less than 5  $\mu\text{m}$ ) could effectively reduce the aerodynamic particle size.

For F6, the inhalable microparticles of MF and FF were prepared by the SFD process without adding matrix excipients. After SFD treatment, the PPFs of the two drugs in pMDI were not improved significantly, and the difference in PPF between the two drugs was still large, reaching approximately 20%. Thus, the aerodynamic performance was not as expected. At the same time, the particle size distribution measured by laser diffraction showed that the  $D_{50}$  of the F6 pMDI was less than 3  $\mu\text{m}$ , and  $D_{90}$  was less than 6  $\mu\text{m}$ . Thus, the geometric particle size difference between F6 and F1–F2 was not large, which meets the particle size requirements for inhalation administration. Inspired, it is not sufficient to only consider the geometric particle size distribution and density of the drug particles for development of inhalation product. Unlike dry powder inhalers, the dispersion and suspension of drug microparticles in HFA must be considered in pMDI studies.<sup>25,26</sup>

F9 inhalable microparticle was obtained by the SFD process, with MF and FF used as model drugs, and DSPC used as an excipient. Our data showed that the PPF of the

pMDI made of F9 was significantly increased to 48% (►Table 5), which was higher than that of Dulera, and the FPF (MF) and FPF (FF) were similar, with a difference of less than 2%. The geometric particle size of the F9 inhalable microparticles was close to that of micronized API particles (F1 and F2), whose  $D_{50}$  values were both less than 3  $\mu\text{m}$ . However, after the SFD treatment, the density of the microparticles was decreased, which further reduced the aerodynamic particle size and increased the FPF. Thus, the addition of DSPC may improve the aggregation of inhalable microparticles<sup>27</sup> and yield a higher FPF.

The uniformity of each delivery dose is also very important when evaluating the delivered dose from pMDI. To simulate the handling errors that may occur during patients shaking inhalation prior to administration, in addition to investigating the mode of actuation after shaking immediately, the effects of shaking intensity and actuation delay after shaking on the DDCU were also investigated.

The DDCU results of micronized API pMDI, Dulera, and F9 pMDIs were different (►Fig. 7). All three pMDIs performed well upon mode 1 shaking strategy. However, excellent dose accuracy and uniformity were only maintained with F9 pMDIs upon mode 2 and 3 shaking strategies. For micronized API pMDI, after mild shaking or delays imparted, from shake to actuation, not only the uniformity was poor, but also the mean of delivery dose was decreased. Although Dulera was of poor uniformity, the mean delivered dose was still close to the theoretical dose, and this may be related to the different types of HFA used in the two pMDIs. The self-made sample was filled with HFA134a, while Dulera was filled with HFA227, and the densities of the two were 1.21 and 1.41 g/mL, respectively.<sup>28</sup> The two micronized drugs were in crystal form, with higher densities and faster sedimentation in HFA134a. Similarly, in HFA134a, because of the low density and improved suspension state of inhalable phospholipid microparticles, F9 pMDI maintained consistency in drug delivery when simulated handling errors are applied.

Good performance can be achieved with microparticle inhalation using the SFD process. However, in the SFD process, attention should be paid to the types of excipients and the characteristics of the solvent. Suitable process parameters should be selected according to the characteristics of the materials.

In the atomization stage, the solvent systems with different compositions may have different surface tensions, which will affect the setting of the atomization gas-liquid ratio parameter. In the freeze-drying stage, the primary problem to be solved in process optimization is to ensure the frozen state of the material during solvent removal. If the temperature rises too fast, there will be an overall or partial melting phenomenon, the material cannot only fail to maintain the original structure, but also collapse, and the eutectic point for most of the drugs and excipients mixed with water will drop below 0°C.<sup>29,30</sup> This study involves a variety of APIs, excipients, and solvents, which have different effects on the eutectic point. Therefore, a reasonable freeze-drying curve should be set according to different eutectic points.

In this work, SPC, HSPC, and DSPC were used as matrix excipients for inhalable microparticles. By observing the appearance of the SFD samples, it was found that the presence of SPC could not form a suitable shape of inhalable microparticles when used as a matrix excipient. The presence of HSPC could form microparticles, but there was a significant adhesion between the microparticles. Only the DSPC microparticles had a suitable shape and were easy to separate. According to the investigation, the phase transition temperatures of SPC, HSPC, and DSPC were -20, 50, and 55°C, respectively,<sup>31</sup> and their appearance and properties were consistent with the phase transition temperature trend of the three phospholipids: the higher the phase transition temperature, the better the inhalable microparticles formed and dispersed. Therefore, our study suggested that phase transition temperature can be taken as one of the indicators to choose matrix excipients as more suitable for inhalable microparticles, such as dipalmitoylphosphatidylcholine.<sup>32</sup>

## Conclusion

In summary, using DSPC as an excipient, a novel phospholipid microparticle pMDI was prepared by SFD with excellent aerosol performance. The FPFs were not only higher than that of the self-made micronized API pMDI, but also higher than that of the brand name Dulera. In DDCU evaluation, the phospholipid microparticle pMDIs could maintain consistency in drug delivery in comparison to the micronized API pMDI and brand name Dulera under the conditions of mild shaking and a delay actuation after shaking. The inhalable microparticles were obtained by the SFD of the drugs and excipients, which provides a novel method for inhalation preparation.

## Conflict of Interest

The authors declare no conflict of interest.

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