Development of Dry Salbutamol Sulfate Powder with High Inhalation Performance Independent of Inhalation Patterns

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While dry powder inhalations are commonly used to treat pulmonary diseases, their clinical performance depends on patient inspiratory flow patterns. The purpose of this study was to develop a new powder with high and stable therapeutic performance for various patients. We applied the supercritical antisolvent (SCF) method to salbutamol sulfate (SS) to prepare a bulky SS particle (SS-SCF). Tests of in vitro inhalation performance with a human inspiratory flow simulator revealed SS-SCF to be less susceptible to inspiratory flow patterns than milled SS. When inspired, the unique structure seemed to be broken resulting in small fragments that could be delivered to the lungs. However, stability tests under physical stress showed tolerance for transportation and handling. In addition, optimization of the concentration of the SS solution applied to SCF method improved the in vitro inhalation performance of SS-SCF. These results indicated that a unique bulky SS powder prepared by the SCF method was useful for dry powder inhalation.

Key words  dry powder inhalation; inspiratory flow pattern; supercritical fluid drying; twin stage liquid impinger; inhalation performance

The most common treatment for pulmonary diseases like asthma and chronic obstructive pulmonary disease (COPD) is inhalation therapy. Dry powder inhalants (DPIs) have become common in the field of inhalation therapy because they are free from environmentally damaging propellants and easy to use with small portable devices. With passive DPIs, because the aerosolization is activated by inhalation, handling is easier than that with pressurized metered dose inhalers (pMDIs). Many studies have investigated the therapeutic performance of dry powders influenced by particle size, morphology, surface roughness, crystallinity, and so on. In addition, inspiratory flow patterns and inhalation devices have a significant impact on particle dispersion. The clinical performance of inhalable drugs is susceptible to inspiratory patterns. Numerous studies have shown the influence of inhalation performance of inspiratory patterns. For stable therapeutic performance, not only drug administration guidance for patients but also the development of new formulations whose inhalation performance is independent of inspiratory flow patterns is needed.

We previously constructed a human inspiratory flow simulator, and evaluated the influence of human inspiratory flow patterns and inhalation device type on the in vitro inhalation performance of dry salbutamol sulfate (SS) powders physically mixed with coarse lactose monohydrate powders, revealing that the effect of inspiratory flow patterns depended on the particle diameter of the active pharmaceutical ingredient (API). Peak flow rate (PFR) most affected in vitro inhalation performance among three critical parameters selected for the characterization of human inspiratory flow patterns; flow increase rate (FIR), inspiratory flow volume (area under the curve corresponding to inspiratory flow volume, AUC) and PFR. For the formulation consisting of micronized API particles and lactose monohydrate carriers, the higher PFR resulted in better deposition in lung. These results suggested that inhalation performance independent of inspiratory flow patterns could be improved by a lower adhesion force between API and carrier particles, that is to say, good dispersibility, because the detachment of the micronized SS particles from the carrier particle is a bottleneck for increasing pulmonary delivery of the SS particles.

The powder dispersibility of DPIs is influenced by particle size, shape, surface characteristics and so on. From the standpoint of particle shape, the shape with a smaller surface contact area generally results in a higher dispersibility. The particles prepared by the supercritical fluid antisolvent method presented various shapes as a function of materials and preparation conditions. As described previously, we successfully prepared unique sea-urchin-like lactose monohydrate particles by the supercritical carbon dioxide antisolvent (SCF) method with a V-shaped nozzle designed by us. Although the sea-urchin-like lactose monohydrate particles were effective for gene delivery to the lungs in mice, their in vitro inhalation performance has not yet been investigated. We have not examined if the SCF method is applicable to other drug substances to prepare sea-urchin-like particles with high in vitro inhalation performance. In this study, we applied the SCF method to salbutamol sulfate (SS) to prepare a bulky SS particle (SS-SCF). We selected SS as a model drug in this study because SS inhalants are widely used clinically. To ascertain the advantages of SS-SCF for inhalation compared with milled SS particles, the influence of inhalation devices and inspiratory flow patterns was investigated.

Experimental

Materials  As a model API for DPIs, salbutamol sulfate (SS) (DOLDER LTD., Switzerland) micronized by a Spiral Jet Mill (100AS, HOSOKAWA MICRON, Japan) (SS-milled, 2.42 μm) was used. The other reagents and solvents used were of analytical grade and HPLC grade, respectively.

Preparation of the Sea-Urchin-Like Powder by the SCF Method  We previously prepared a sea-urchin-like dry gene powder with lactose as a filler by using supercritical carbon dioxide as an antisolvent. In the present study, we employed the same system to prepare bulky dry SS powders. The system was composed of two CO₂ pumps (PU-2086 Plus) connected to a cooling system (CCA-111, TOKYO RIKAKIKAI Co., Ltd., Tokyo, Japan) in order to send the carbon dioxide in a liquid state, a sample solution pump (PU-2085 Plus), a modi-
fier pump (PU-1580), an oven (GC353B, GL Sciences Inc., Tokyo, Japan) and a back pressure regulator (SCF-Bpg, pressure regulation range was 1—50 MPa), which were manufactured and assembled by JASCO Co., Tokyo, Japan, except for the cooling system and the oven. The dry powder preparation vessel (2.0 cm i.d. and 14 cm height) had a unique V-shaped nozzle designed by us. The pressure and temperature in the vessel were regulated at 25 MPa and 35°C, respectively. The CO₂ (14 mL/min) and ethanol (3.5 mL/min), a modifier, were mixed in a mixing column, and flowed into the preparation vessel from one end of the V-shape nozzle. Water (0.035 mL/min) was introduced from the other end. The 10% SS aqueous solution (2.0 mL) was injected into the water stream through a manual injector. One hour after injection, the flow of water and ethanol was stopped. CO₂ was flowed for an additional half an hour to remove the residual solvent from the vessel. Following depressurization, the powder was collected from the vessel.

We also prepared SS-SCF powders from 5% and 20% SS solutions to examine the effect of the SS concentration on in vitro inhalation performance.

Morphological Analysis by Scanning Electron Microscopy The morphology of the dry powder was examined using scanning electron microscopy (SEM; JSM-6060, JEOL, Tokyo, Japan). Before the observation, powder samples were manually dispersed on a specimen mount with double faced tape, and coated with platinum by a sputter coater (JFC-1600, JEOL, Tokyo, Japan).

Analysis of Crystallinity by X-Ray Powder Diffraction and Differential Scanning Calorimetry Powder crystallinity was determined by X-ray powder diffraction (XRPD; Smart Lab, Rigaku Corporation, Japan) and differential scanning calorimetry (DSC; DSC-8230, Rigaku Corporation, Japan). The operating conditions for XRPD were as follows: a parallel beam at room temperature using CuKα radiation at 30 mA and 40 kV with an angular increment of 5°/min over a 20 range of 5—45°. In DSC, 5 mg of sample was loaded and crimped in an aluminum pan, and scanned from 50—250°C at 5°C/min.

Measurement of Size Distribution of Particles Dispersed by Inhalation Device Using Lazar Micron Sizer Particle size distribution was measured with a laser diffraction scattering method using a diffractometer with a dry dispersing unit (LSM-30; Seishin Enterprise Co., Ltd., Japan). In order to determine the influence of the inhalation devices, we used three types of Jethaler® (Single-type, Dual-type, and Reverse-type, Hitachi Automotive Systems, Ltd., Japan) connected to a dry dispersing unit. These devices differed in structure and resistance, which resulted in various drops in pressure, and were classified as Single, Dual, and Reverse in ascending order of inhalation resistance. As a comparison, particles dispersed by the dry dispersing unit without an inhalation device were measured. Aliquots (5 mg) of the dry powder were packed in No. 2 HPMC capsules (Shionogi Qualicaps, Japan) and placed in the holder with a pin to pierce them. Then, the dry dispersing unit was operated at a constant air pressure of 0.4 MPa to disperse the particles into the laser beam. The volume median diameter was used as the mean particle size of the samples.

In Vitro Testing of the Powder with Different Inhalation Devices Aerodynamic particle deposition was determined using a twin-stage liquid impinger (TSLI; European Pharmacopeia Apparatus A, Copley Scientific Ltd., U.K.) with three different inhalation devices. Stages 1 and 2 in the TSLI contained 7 and 30 mL of purified water, respectively. Aliquots (5 mg) of the SS-SCF powder or SS-milled powder were packed in No. 2 HPMC capsules (Shionogi Qualicaps, Japan). After the device was connected to the mouth piece of the TSLI, the capsule containing the powders was placed in the holder with a pin to pierce it. Then, the pump was operated at 30 L/min for 5 s to disperse the powder in the capsule. While the operating flow rate of TSLI is set at 60 L/min in European Pharmacopeia,24 we set it at 30 L/min to maintain a uniform flow rate in three inhalation devices because it seemed difficult for patients to achieve higher PFR with a highly resistant device. After dispersion, the dry powders were rinsed from the capsule, the device, the throat, stage 1, and stage 2 with purified water. The collected samples were diluted to 100 mL, and the concentration of SS was measured by high performance liquid chromatography (HPLC). The tests were performed in triplicate. The in vitro inhalation performance of the prepared powders was characterized by output efficiency (OE) and stage 2 deposition (St2). OE stands for the amount ratio of drug particles emitted from a capsule and an inhalation device (Eq. 1), while St2 represents the amount ratio of the drug deposited on stage 2 of the TSLI (Eq. 2).

\[
OE = \frac{\text{mass recovered from TSLI}}{\text{mass balance}} \times 100 \quad (1)
\]

\[
\text{St2} = \frac{\text{mass recovered from stage 2}}{\text{mass balance}} \times 100 \quad (2)
\]

where the mass balance is the mass of SS recovered from all parts of the apparatus (capsule, device, and TSLI).

Morphological Analysis of Dispersed Sea-Urchin-Like Powders To ascertain visually the state of the SS-SCF powders following inspiration, a specimen mount for SEM with double-sided tape on its top was set on the bottom of stages 1 and 2 in the TSLI instead of purified water. The system was operated in the conventional method, with a PFR of 30 L/min and inspiration time of 5 s in three different inhalation devices. The morphology of SS-SCF powders was observed by SEM.

In Vitro Testing of the Powder with Inspiratory Flow Pattern Simulator To ascertain the inter-individual variation of inhalation performance of SS-SCF powders in clinical usage, we applied the inspiratory flow simulator described in our previous paper. We previously determined that peak flow rate (PFR) was the most influential on the in vitro inhalation performance of physically mixed dry powders among three typical parameters of inspiratory flow patterns, that is, PFR, FIR, and AUC.15 In this study, we changed PFR from 13 to 53 L/min to determine the influence of inspiratory flow patterns on in vitro inhalation performance. After dispersion of the powder into the TSLI by a flow simulator, the powders were collected and assayed as described in the previous section.

Stability of SS-SCF against Physical Stress Because the SS-SCF prepared in this paper had a unique bulky shape, it was likely that the particle broke during transportation or handling. To confirm the stability of the particles against physical stress, TSLI assessments and SEM observations were applied to the powders in capsules shaken or dropped. In the shaking test, the sample-loaded capsules were placed in a container of normal PTP (Press Through Packages) size (5×15 mm), and shaken by a vortex mixer (SCIENTIFIC INDUSTRIES,
U.S.A.) operated with 3/10 grade intensity. In the dropping test, the sample-loaded capsules were dropped from a height of one-meter on the top of a laboratory bench. Then the powder in the capsules were observed by SEM and the capsules were tested using TSLI with a Reverse type Jethaler® and PFR=30 L/min. The tests were performed in triplicate.

**Determination of SS by HPLC** The SS concentration was determined by a HPLC system (Agilent Technologies, Inc., U.S.A.) consisting of a quaternary pump (G1311A), a degasser (G1322A), a UV-vis detector (G1314B), a column oven (G1316A), and an auto sampler (G1329A). The mobile phase was composed of 0.025 M phosphate buffer (pH 2.8), acetonitrile, and methanol (90:9:1, volume ratio). The flow rate was set at 1.0 mL/min. The column (ZORBAX Eclipse XDB-C18, 5 micron, 4.6×150 mm; Agilent Technologies, Inc., U.S.A.) was heated at 35°C. The injection volume was 100 μL. The UV (UV) absorbance of each sample was measured at 224 nm. It was confirmed that the UV absorbance correlated linearly with the concentration of SS between 1.0 and 150 μg/mL.

**Results and Discussion**

**Morphological Analysis of the Sea-Urchin-Like SS Powder by SEM** Figure 1 shows the morphology of the particles prepared by the SCF method. The diameter of the particles was nearly 50 μm. Compared to the sea-urchin-like lactose particle, the SS-SCF had fewer needles per particle. In general, such bulky particles are with lower density and have advantages for inhalation, because of their small aerodynamic diameter compared with their geometric diameter.

\[ D_{\text{aero}} = D_{\text{geo}} \times \left( \frac{\rho}{\chi \rho_0} \right)^{1/2} \]  

where \( D_{\text{aero}} \) is the aerodynamic diameter, \( D_{\text{geo}} \) is the geometric diameter, \( \rho_0 \) is the unit density of calibration spheres, \( \rho \) is particle density and \( \chi \) is the dynamic shape factor.

A small aerodynamic diameter of around 1 to 5 μm is desirable for inhalation, while a larger geometric diameter is preferable to minimize aggregation.

**Analysis of Crystallinity of the Sea-Urchin-Like Powder by XRPD and DSC** The crystallinity of the SS-SCF powder
was examined using XRPD and DSC. The SS-SCF powder showed the same XRPD pattern as SS-bulk powder, although the peak intensity was lowered. In other studies, spray dried SS powder showed a halo XRPD pattern suggesting amorphous state.\textsuperscript{27} The SCF-treated powder also had smaller endothermic peak on the DSC diagram (Fig. 2b), suggesting a lower crystallinity.

**Powder Dispersibility Analysis of SS-SCF by Inhalation Devices**

The particle size distribution and $D_{50}$ of SS-SCF dispersed with three different inhalation devices are shown in Fig. 3. In the lower resistant device (Single), the particle distribution was similar to that without a device. As the inhalation resistance of the device increased, the size distribution peak around 2$\mu$m increased. The smaller particles of around 2$\mu$m might be generated by breaking the structure of SS-SCF. Thus, the higher the inhalation resistance, the smaller the $D_{50}$ value (Fig. 3b).

**Morphological Analysis by SEM after Dispersion into TSLI**

To back up the results described above, a morphological analysis of the SS-SCF dispersed into TSLI with different inhalation devices was conducted. The dispersed powder recovered on stage 2 of TSLI was the needle shape which is less than 10$\mu$m in longer diameter derived by breaking the structure of SS-SCF, while that on stage 1 was the sea-urchin-like shape the same as intact particle (Fig. 4). The needles on stage 2 did not differ among the three different devices. These results suggested that the breaking of the particle was needed in order to deliver SS-SCF to the lung.

**In Vitro Testing of the Powder with Different Inhalation Devices**

Figure 5 shows the influence of inhalation devices on \textit{in vitro} inhalation performance. In the case of Reverse, the deposition on stage 1 was smaller, and that on stage 2 was greater than the other two devices. These results were well correlated with the most effective particle size reduction by Reverse as shown in Fig. 3. From these results, breaking of the structure of SS-SCF was needed in order to improve the \textit{in vitro} inhalation performance of the powder. Compared with SS-milled, the SS-SCF resulted in the greater stage 2 deposition in all three devices. In the case of SS-milled, the dispersion of the powder required high shear stress by a highly resistant device to achieve good \textit{in vitro} inhalation performance, because particles with a smaller geometric diameter such as SS-milled tend to make tight agglomerates. On the other hand, SS-SCF tends not to make agglomerates because of its unique
structure, resulting in higher dispersibility than SS-milled.

**In Vitro Testing of the Powder with Reproduced Inspiratory Flow Patterns** To assess the influence of inspiratory flow patterns, the human inspiratory flow simulator described in our previous paper was adapted. The stage 2 deposition of SS-SCF varied from 11.9 to 22.7% at a PFR of between 13 and 53 L/min. On the other hand, that of SS-milled increased dramatically from 2.8 to 48.8% in the same range of PFR (Fig. 6). These results suggested that the in vitro inhalation performance of the SS-SCF powder was less susceptible to inspiratory patterns than SS-milled. The greater stability of in vitro inhalation performance of SS-SCF was attributed to higher dispersibility. Many other studies reported that in vitro inhalation performance such as stage 2 deposition of physically mixed dry powder was influenced by PFR and shear stress, and the value was saturated at a high shear stress. The formulation with a lower PFR value for saturated in vitro inhalation performance would lead to stable in vitro inhalation performance regardless of the human inspiratory flow pattern.

As for SS-SCF, the saturated tendency appeared from a lower PFR, which is a desirable property for clinical usage. Although SS-SCF seemed to make inhalation therapy less sensitive to inspiration patterns, the maximum St2 value was half of that observed for SS-milled. To improve the in vitro inhalation performance of SS-SCF under various human inspiratory flow patterns, we prepared several SS-SCF powders with 5 and 20% SS solutions. The stage 2 deposition depended on the SS concentration and relatively high and stable (15 to 30%) deposition was achieved with a PFR of 13—53 L/min with SS-SCF prepared from the 20% solution (Fig. 7). The mechanism of the improved St2 is under investigation.

**Stability Analysis of the Powder under Physical Stress** To confirm the stability of SS-SCF particles during handling and transportation, SEM and TSLI were applied to powders subjected to physical stress (Fig. 8). Shaking and dropping had almost no effect on the structure and St2 value. These results suggested that SS-SCF has tolerance for physical stress, and the breaking of SS-SCF will occur by inhalation. We hypothesized that the movement of whole capsule by shaking or
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hitting may not generate micron-level fluctuation to collapse the structure of the particle. On the other hand, stimulus by inspiratory flow influenced needles of SS-SCF in a capsule to collapse the structure of the particle.

Conclusions

Unique bulky SS particles consisting only of API were obtained by the SCF-method. The particles had good handling ability because of their bulky shape, and when inspired, the unique structure seemed to be broken resulting in small fragments that could be delivered to the lungs. On the other hand, SS-SCF was robust against physical stress. From in vitro testing with a human inspiratory flow simulator, SS-SCF has more stable inhalation performance regardless of human inspiratory flow patterns compared with SS-milled. The inhalation performance of SS-SCF could be improved by using a higher concentration of SS solution applied to SCF method. Those results indicated that the unique bulky SS powder prepared by the SCF method was useful for dry powder inhalation system with stable inhalation performance for various patients.

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References


Fig. 8. Influence of Physical Stress on *In Vitro* Inhalation Performance (a) of SS-SCF and SEM Images of SS-SCF Particles with No Stress (b), after Shaking Stress (c), and after Dropping Stress (d)


