Influence of Storage Humidity on the *in Vitro* Inhalation Properties of Salbutamol Sulfate Dry Powder with Surface Covered Lactose Carrier

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The influence of storage humidity on the *in vitro* inhalation properties of salbutamol sulfate dry powder with surface covered lactose carrier was investigated. In the present study, drug/carrier powder mixtures were prepared consisting of micronized salbutamol sulfate and lactose carriers with different particle surface conditions prepared by surface covering. Lactose carrier surfaces were covered with vegetable magnesium stearate (Mg-St-V) by a high-speed elliptical-rotor-type powder mixer (Theta-Composer®). These powder mixtures were aerosolized by a Jethaler®, and the *in vitro* inhalation properties of salbutamol sulfate were evaluated by a twin impinger. Compared with the powder mixed with uncovered lactose carrier, the *in vitro* inhalation properties of the powder mixture prepared using the surface covered lactose carrier were little decreased with increased in relative humidity (RH), showing that the *in vitro* inhalation properties of salbutamol sulfate were improved at high RH. Using this surface covering technique would thus be valuable for storage humidity of dry powder inhalation (DPI) with lactose carrier particles.

Key words dry powder inhalation; storage humidity; salbutamol sulfate; lactose carrier particle; vegetable magnesium stearate

Dry powder for inhalation (DPI) is generally formulated as a powder mixture of coarse carrier particles and micronized drug powders with aerodynamic particle diameters of 1—6 μm.[1—6] Carrier particles are used to improve the flow and dispersion of micronized drug powders. An optimal dry powder inhalation should possess the following characteristics, such as (1) a good flow properties, (2) a good dispersion properties, (3) good storage stability.[7] The influence of storage humidity is especially important for the DPI in Japan, as it is very humid in summer season. However, only a few studies reported on comparison of storage humidity on the *in vitro* inhalation properties of dry powder inhalations using carrier particles.[8—10] We previously reported effects of a lactose carrier surface-covered with magnesium stearate on *in vitro* inhalation properties of salbutamol sulfate.[11] We, therefore, carried out basic studies on the influence of storage humidity on the *in vitro* inhalation property of salbutamol sulfate dry powder with surface covered lactose carrier, and we investigated the possibility of designing a DPI using the surface covered lactose carrier particles. Our objective is the investigation of powder formulations showing a smaller decrease of inhalation property when exposed to high humidity conditions in order to design them less susceptible to moisture.

Experimental

Powder Samples As the carrier particle for dry powder inhalation, α-lactose monohydrate was used (Pharmatose® 200M, DMV, The Netherlands). Salbutamol sulfate was used as the drug, and was obtained from LEIRAS (Finland). Salbutamol sulfate was micronized by Spiral Jet Mill (100AS, HOSOKAWA MICRON, Japan). The cube-like fine crystals of the latter had a volume median diameter of 1.7 μm, as determined by laser diffraction (Lasermicronsizer, SEISHIN, Japan). Vegetable magnesium stearate (Mg-St-V) with mean particle diameter of 3.3 μm was obtained from TAIHEI CHEMICAL (Japan).

Physical Properties of Lactose Carrier Particles The mean particle diameter (Heywood diameter) of lactose particles was determined using an image analyzer (Luzex-FS, NIRECO, Japan) connected to a microscope (OPTIPHOT, Nikon, Japan). The specific surface area of lactose particles was measured by an air permeametry method (SS-100, Shimadzu, Japan).

The surface condition of lactose particle was observed by a scanning electron microscope (T-20, JEOL, Japan).

Surface-Covered of Lactose Carrier Particles Mg-St-V was added to lactose powder at 10.0 w/w%. The premixed powders were further mixed for 10 min with a high-speed elliptical-rotor-type powder mixer (Theta-Composer®, Tokuju, Japan).[12—17] The powder loading was 20 g. The clearance between the rotor and vessel wall was 0.5 mm. The rotor and vessel were rotated counter revolution at 3000 and 35 rpm, respectively. After mixing, Mg-St-V that did not deposit to the surface of the lactose particles and Mg-St-V that was easily detached from the surface of lactose particles were removed by suction at an airflow pressure of 4000 kPa using an Air Jet Sieve (HOSOKAWA MICRON, Japan) for 10 min.

Preparation of Powder Mixture Powder mixtures of 2.5 w/w% salbutamol sulfate were prepared by mixing 1.0 g of salbutamol sulfate and 39.0 g of lactose carrier particles in a glass bottle with a vortex mixer for 5 min.

Packing of Powder Mixture into a Capsule A total of 80 mg of powder mixtures were packed into a No. 2 HPMC hard capsule (Shionogi Qualicaps, Japan) and stored at a given relative humidity (RH) for 7 d in a desiccator at 25±1 °C. Saturated solutions of $MgCl_2 \cdot 6H_2O$, $Mg(NO_3)_2 \cdot 6H_2O$, NaCl, and KCl were used to obtain RH of 33, 53, 75, and 84%, respectively.

***In Vitro* Inhalation Property** The powder mixtures were aerosolized using a dry powder inhalation device (Jethaler®, Hitachi Unisia Automotive, Japan). The aerodynamic particle deposition was investigated using a twin impinger (Model TI-2, Copley) containing 7 and 30 ml of solvents (0.1 M hydrochloric acid) for stages 1 and 2, respectively. After the Jethaler® was connected to the mouthpiece of the twin impinger, a capsule was placed in the holder of the Jethaler®, which had a pin attached to pierce the capsule. An air-stream of 60 l/min was allowed to flow throughout the system by attaching the outlet of the twin impinger to a vacuum pump for 5 s. The drugs in stages 1 and 2, the capsule, and the device were collected by rinsing with fresh solvent. The rinsed solutions were diluted to appropriate volumes and the drug contents were determined by spectrophotometry (UV-160A, Shimadzu, Japan) at 224 nm.

In this study, since we focused on the detachment of drug particles from the surface of a carrier emitted from a capsule and a device, we employed the respirable particle percent (RP) of emitted particles from the inhalation system to represent the *in vitro* inhalation property. RP was proposed by Hino *et al.*[18] and Kawashima *et al.*[1] to evaluate inhalation behavior and expressed as:

$$RP=(ST2)/(EM)\times 100 \quad (1)$$

where EM is the amount (%) of particles emitted from the inhalation device and capsule, and ST2 is the amount (%) of drug deposited in stage 2 of the twin impinger.

Quantitative Analysis of Magnesium (Mg) Mg contained in Mg-St-V was quantified by the atomic absorption spectrophotometry (AA-625, Shimadzu, Japan) described in the Japanese Pharmacopoeia (JP XIV).[19,20]

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Table 1. Physical Properties of Lactose Carriers

Carrier	Percentage of Mg-St-V added (w/w%)	Mean particle diameter[a] (μm)	Percentage of surface covering[b] (w/w%)	Contact angle[c] θ_c (°)	Specific surface area[d] (m^2/g)
Lac-0	0	70.9±19.8	0	17±1.3	0.148±0.001
Lac-10	10.0	71.8±1.75	7.20±0.35	75±2.5	0.125±0.003*

a) Data are represented as mean±S.D. (n=100). *b*) Data are represented as mean±S.D. (n=3). *c*) Data are represented as mean±S.D. (n=5). *d*) Data are represented as mean±S.D. (n=3). * $p<0.01$, significant difference compared to Lac-0 by Student's unpaired t-test.

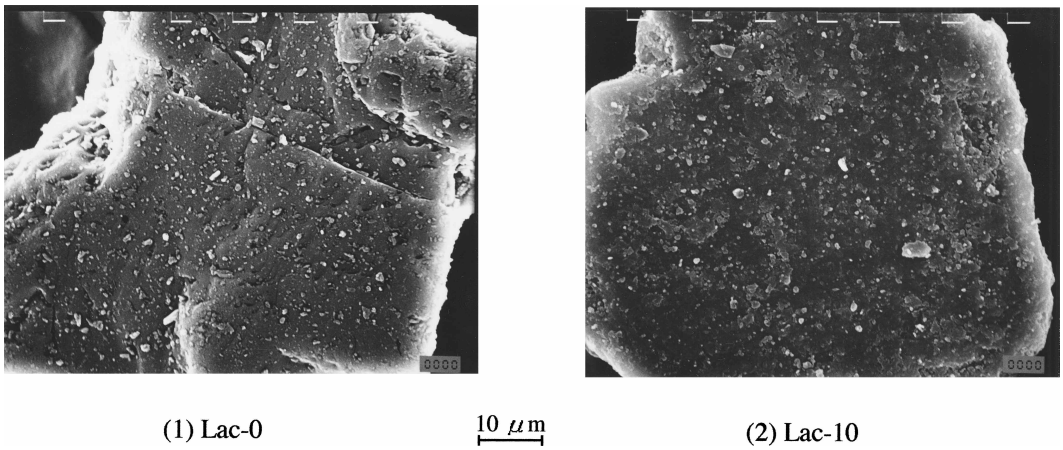

Fig. 1. Scanning Electron Microphotographs of Lactose Carrier Particles Used
Carrier: (1) Lac-0, (2) Lac-10.

Percentage of Surface Covering The percentage of the mass of Mg-St-V covered on the surface of lactose particles relative to the mass of surface covered lactose particles was calculated and expressed as the percentage of surface covering defined by Eq. 2.

$$\text{percentage of surface covering} = (\text{Mg-St-V covering})/(\text{surface covered carrier}) \times 100 \quad (2)$$

where Mg-St-V covering is the mass of Mg-St-V covered on the surface of lactose particles that was calculated by quantitative analysis of Mg contained in Mg-St-V. The surface covered carrier is the mass of lactose carrier particles covered with Mg-St-V.

Determination of Contact Angel The powder compacts compressed by Autograph (AG-5000G, Shimadzu, Japan) at 118 MN m^{-2}. A small droplet (25 μl) of water was placed on the powder compact using a microliter syringe. Contact angle (θ_c) was determined by photographic recording.[21]

Results and Discussion

Physical Properties of Lactose Carrier Particles Table 1 shows the mean particle diameter, percentage of surface covering, contact angle, and specific surface area. In the table, Lac-0 represents uncovered lactose particles, and Lac-10 represent lactose particles were added with 10.0 w/w% of Mg-St-V. The mean particle diameter was approximately same between two lactose carrier particles prepared. The specific surface area were smaller in covered lactose particles compared with uncovered lactose particles, because Mg-St-V covered to the surface of lactose particles would be made it smoother by covering depressions. Mg-St-V has been widely used as a lubricant in the pharmaceutical field because of its excellent lubricating effect. It has also been reported to have a marked tendency to deposit to the surface of larger particles and cover them.[22] We quantified Mg-St-V and calculated the percentage of Mg-St-V in the covered lactose particles as the percentage of surface covering (Eq. 2). Vegetable magnesium stearate (Mg-St-V) has extremely small affinity to water molecules because of its low HLB (*ca.* 2) and hydrophobicity.[22] The contact angle of lactose particles (Lac-0) was shown to be low at 17±1.3, which represented their surface hydrophilic. In contrast, the contact angle of lactose particles covered with Mg-St-V (Lac-10) was very high at 75±2.5, indicating that the surface of the particles became markedly hydrophobic (Table 1).

Figure 1 shows SEM photographs of the lactose carrier particles prepared. The surface of uncovered lactose particles was irregular, but the surface of covered lactose particles was smooth. In Lac-10 prepared with 10.0 w/w%, more Mg-St-V covered, filled depressions and gaps on the surface of lactose particles. These results were in agreement with the values of the percentage of surface covering shown in Table 1.

Influence of Storage Humidity on the *in Vitro* Inhalation Property Figure 2 shows the relationship between various RH and the RP of salbutamol sulfate using with the uncovered lactose carriers and the surface covered lactose carriers with Mg-St-V. When the uncovered lactose carriers were used, RP was significantly decreased with increased in RH. High RH results in an increase in adhesion forces between particles due to capillary action.[7] At 75% RH, capillary forces are the major contribution to the adhesion force between particles. Thus, the phenomenon can be explained by the formation of liquid bridges due to moisture condensation between particles.[10,23,24]

When the surface covered lactose carriers were used, little decrease in the RP with increases in RH was observed, and RP remained high. This was probably because the surface of lactose particles covered with Mg-St-V had a high contact angle (Table 1), and the strong hydrophobicity prevented the

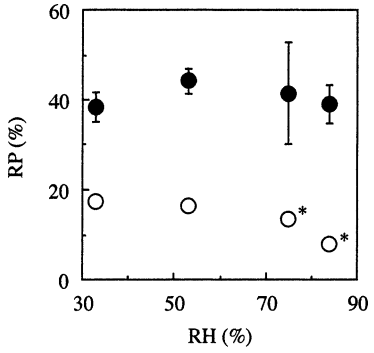

Fig. 2. Relationship between RP and RH

Carrier: ○, Lac-0; ●, Lac-10. Data are expressed as mean±S.D. (n=3—5). $*p<0.01$, significant difference compared to Lac-0, 33% RH by Student's unpaired t-test.

adsorption of water molecules on it, so that the adhesion between drug particles and surface covered carrier particles did not increase markedly, allowed facilitation of drug detachment from carrier surface in inhalation.

Arakawa *et al.* studied the effect of humidity on the adhesion of powder particles and reported that the adhesion between particles did not increase even after storage at a high relative humidity when the particles were made hydrophobic by coating with a fatty acid (capric acid).[25)]

In this study, we investigated the influence of storage humidity on the *in vitro* inhalation property of salbutamol sulfate dry powder with surface covered lactose carrier. When lactose was covered with Mg-St-V, which has a high contact angle and is markedly hydrophobic, under shear force applied in the Theta-Composer® and was used as carrier particles, little deterioration in the *in vitro* inhalation property was noted even after storage at a high RH; the *in vitro* inhalation property was stable against a high storage humidity. The preparation of covering the surface of lactose particles with Mg-St-V using Theta-Composer® is considered to be a useful pharmaceutical technology to ensure stable inhalation property of dry powder inhalations against changes in the storage humidity.

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