Effect of Surface Layering Time of Lactose Carrier Particles on Dry Powder Inhalation Properties of Salbutamol Sulfate

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The effect of the surface layering time of lactose carrier particles on the dry powder inhalation properties of salbutamol sulfate was investigated. Lactose carrier particles were layered with vegetable magnesium stearate by physical mixing. In the present study, drug/carrier powder mixtures were designed consisting of micronized salbutamol sulfate and lactose carriers with various particle surface conditions prepared by surface layering. These powder mixtures were aerosolized by a Jethaler®, and the *in vitro* deposition properties of salbutamol sulfate were evaluated by a twin impinger. Compared with the powder mixed with unlayered lactose carrier, the *in vitro* inhalation properties of the powder mixture prepared using the surface layering lactose carrier were significantly different, showing that the *in vitro* inhalation properties of the drug/carrier powder mixtures were improved. *In vitro* deposition properties (RP) increased with surface layering time. Using this surface layering system would thus be valuable for increasing the inhalation properties of dry powder inhalation.

Key words dry powder inhalation; lactose carrier particle; salbutamol sulfate; surface layering; vegetable magnesium stearate

In designing dry powder inhalation (DPI), a pharmaceutical technique utilizing inactive and coarse carrier particles such as lactose is applied to improve inhalation properties of micronized drug particles with aerodynamic particle diameters of 1—6 μm.[1—6] During inhalation, carrier particles help the emission of the drug from the device or capsules and improves its inhalation properties. Therefore, in designing a DPI using carrier particles, it is important for drug particles to be adequately separated from the surface of carrier particles for inhalation. The *in vitro* inhalation properties of DPI are reported to be related to the surface properties of the carrier particles.[2,7—9] We previously reported the effects of a lactose carrier prepared by dissolving the surface of lactose particles with a water solution of ethanol (70 v/v %) on *in vitro* inhalation properties.[7] In this study, we layered the surface of lactose particles with magnesium stearate in a dry condition. This technique is advantageous for the safety and environmental protection because of elimination of the possibility of residual solvent and the need to dispose of the waste fluid containing the solvent by the disuse of an organic solvent in the surface-layering process. We previously reported effects of a lactose carrier surface-layered with magnesium stearate on *in vitro* inhalation properties of salbutamol sulfate.[10] There has since been no other report on the use of lactose surface-layered with magnesium stearate as a carrier. Therefore, we layered the surface of coarse lactose particles, which have been used as an inhalation carrier, with vegetable magnesium stearate, which is widely used as a lubricant in the pharmaceutical field. Recently, in the field of pharmaceutics, there is a trend to limit the use of animal-derived excipients with preference to substitute them with vegetable excipients. We, therefore, carried out basic investigations on the effects of surface layering time of lactose carrier particles with vegetable magnesium stearate on DPI of salbutamol sulfate.

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 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 Layering of Lactose Carrier Particles Mg-St-V was added to lactose powder at 2 w/w%, and the mixing time were 10, 30, or 60 min, respectively. Lactose powder (9.8 g) and Mg-St-V (0.2 g) were placed in a glass bottle (diameter 3.5 cm, height 12 cm) and mixed physically by a Vortex-Mixer (Scientific Industries, U.S.A.) over a specified time. After mixing, Mg-St-V that did not adhere 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 in a desiccator at 22±2 °C for 24 h.

***In Vitro* Deposition 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 stage 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 airstream 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 separation 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* deposition property. RP was proposed by Hino *et al.*[11] and Kawashima *et al.*[2] to evaluate inhalation behavior and expressed as:

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

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Table 1. Physical Properties of Lactose Carriers

Carrier	Surface layering time (min)	Particle diameter[a] (μm)			Specific surface area[b] (m^2/g)	Percentage of surface layering[c] (w/w%)	Surface roughness[d] Ra (μm)
		D_{10}	D_{50}	D_{90}			
lac-0	0	50.7	63.3	90.5	0.150±0.003	0	0.95±0.12
lac-1	10	49.5	62.8	91.0	0.145±0.002*	0.618±0.003	0.45±0.05**
lac-2	30	50.5	62.0	89.5	0.142±0.002*	0.819±0.004	0.42±0.03**
lac-3	60	49.3	60.2	87.5	0.141±0.001**	1.05±0.03	0.26±0.10**

a) Data are represented as mean (n=100). *b*) Data are represented as mean±S.D. (n=3). *c*) Data are represented as mean±S.D. (n=3). *d*) Data are represented as mean±S.D. (n=3). $*p<0.05$, significant difference compared to lac-0 by Student's unpaired t-test. $**p<0.01$, significant difference compared to lac-0 by Student's unpaired t-test.

where EM is the amount (%) of drug particles emitted from the inhalation device and capsules, 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).[12,13]

The analytical procedure was the flame method, and the quantification method was the calibration curve method. Ten milligrams of Mg-St-V was mixed with 10 ml of HNO_3 (Wako Pure Chemical Industries, Japan) and heated at 100 °C for 30 min. It was then mixed with 2 ml of $HClO_4$ (60%, Wako Pure Chemical Industries, Japan) and heated at 100 °C for 20 min to decompose stearic acid. After cooling, distilled water was added until the total volume became 100 ml, and the resultant solution was used as the sample. Five Mg standard solutions with different concentrations were prepared from a standard Mg solution (Wako Pure Chemical Industries, 1000 mg/l Mg). The absorbance of Mg was measured in each standard solution, and a calibration line was prepared from the values obtained. The absorbance of Mg was measured in the sample solution, and its Mg concentration was determined according to the calibration line.

Percentage of Surface Layering The percentage of the mass of Mg-St-V layering the surface of lactose particles relative to the mass of surface layered lactose particles was calculated and expressed as the percentage of surface layering defined by Eq. 2.

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

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

Surface Roughness The surface roughness of single lactose particles was determined using a violet laser color 3D profile microscope (VK-9500, KEYENCE, Japan). The surface roughness parameter Ra (the arithmetic mean roughness) was evaluated according to JIS B0601 (1994).

Results and Discussion

Physical Properties of Lactose Carrier Particles Table 1 shows the particle diameter, specific surface area, percentage of surface layering, and surface roughness. In the table, lac-0 represents unlayered lactose particles, and lac-1, lac-2, and lac-3 represent lactose particles were layered with Mg-St-V by physical mixing for 10, 30, and 60 min, respectively. The particle diameter was approximately the same among all lactose carrier particles prepared. The specific surface area and the surface roughness were smaller in layered lactose particles compared with unlayered particles, because Mg-St-V layering the surface of lactose particles would be made them smoother by layering depressions. Magnesium stearate has been used widely as a lubricant because of its excellent lubricating effect. It has also been reported to have a marked tendency to adhere on the surface of larger particles and layer them.[14,15]

We quantified Mg in Mg-St-V and calculated the percentage of Mg-St-V in the layered lactose particles as the percentage of surface layering (Eq. 2). Mg content in Mg-St-V determined by atomic absorption analysis was 4.86%. As shown in Table 1, the percentage of surface layering is considered to have increased with surface layering time, because collision and friction between particles were repeated and more Mg-St-V pressed against and layered on the surface of lactose particles with surface layering time. The percentage of surface layering was 1.05±0.03 w/w% in lac-3 prepared with a long surface-layering time. Since the quantity of Mg-St-V added was 2.0 w/w%, 52.5% of the Mg-St-V added is considered to have contributed to surface layering of lactose. As for changes in the specific surface area by surface layering, the specific surface area of lac-3 was 0.141 m^2/g, which was 6% smaller than 0.150 m^2/g of lac-0. The specific surface area may have decreased with prolongation of the surface-layering time, as the frequency of collision and friction between Mg-St-V and lactose particles increased and more Mg-St-V adhered to the surface of lactose particles to fill depressions on it.

Figure 1 shows SEM photographs of the lactose carrier particles prepared. The surfaces of unlayered lactose particles were rough, but the surfaces of layered lactose particles were smooth. In lac-1 prepared with a short layering time, Mg-St-V layered over the surface of lactose particles. In lac-3 prepared with a long layering time, more Mg-St-V layered them, filling depressions and gaps on the surface of lactose particles. These results were in agreement with the values of the specific surface area, the surface roughness and the percentage of surface layering shown in Table 1.

Effects of Surface Layering Time of Carrier Particles on *in Vitro* Deposition Properties Table 2 shows *in vitro* deposition property of salbutamol sulfate with various lactose carriers. EM significantly decreased in layered carriers than in the unlayered carrier. The lac-0 particles were the most effective for emitting the drug particles from the inhalation device and capsules, probably because of their higher available surface area for drug adhering. Consequently, higher EM (%) was found with lac-0. However, ST2 values of drug deposited in stage 2 of the twin impinger was significantly higher for the lac-2 and the lac-3 compared to the lac-0. This was seen even though the amount of drug emitted by the lac-2 and the lac-3 was not as high as that compared to the lac-0. This finding indicated that the surface area of the carrier particles affect drug particle adhesion. RP was significantly greater in layered carriers than in the unlayered carrier, indicating the *in vitro* deposition properties of salbutamol sulfate were improved.

Figure 2 shows the effect of the surface layering time on the *in vitro* deposition properties (RP) of salbutamol sulfate and the specific surface area (Sw) of lactose carrier particles.

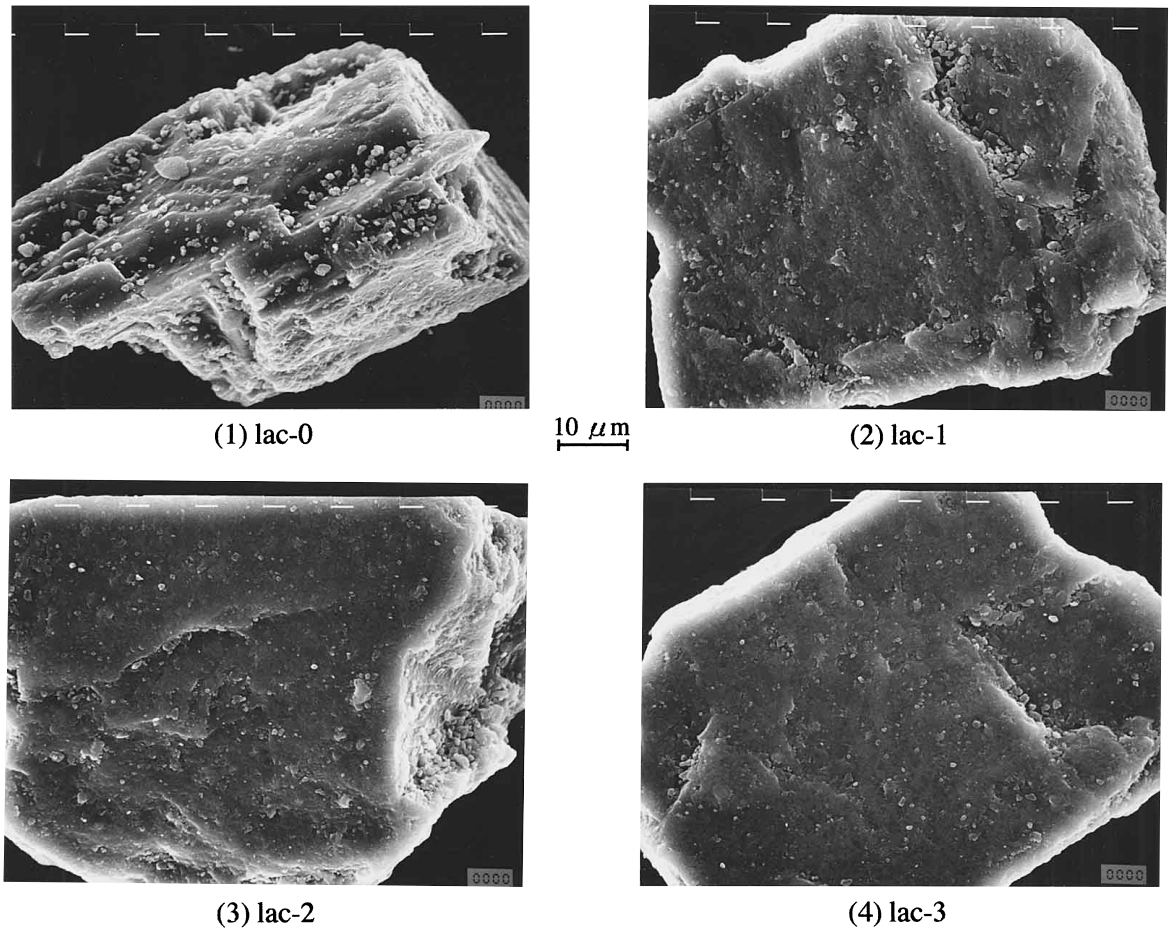

Fig. 1. Scanning Electron Microphotographs of Lactose Carrier Particles Used
Carrier: (1) lac-0, (2) lac-1, (3) lac-2, (4) lac-3.

Table 2. *In Vitro* Deposition Properties of Salbutamol Sulfate with Various Lactose Carriers

Carrier	EM (%)	ST2 (%)	RP (%)
lac-0	93.6±2.1	13.6±2.1	14.6±2.5
lac-1	82.0±1.2**	17.2±4.2	21.0±5.0*
lac-2	86.9±1.2**	22.6±4.6**	25.8±4.9**
lac-3	84.9±2.5**	21.9±2.7**	26.0±3.9**

Data are represented as mean±S.D. (n=3—5). * $p<0.05$, significant difference compared to lac-0 by Student's unpaired t-test. ** $p<0.01$, significant difference compared to lac-0 by Student's unpaired t-test.

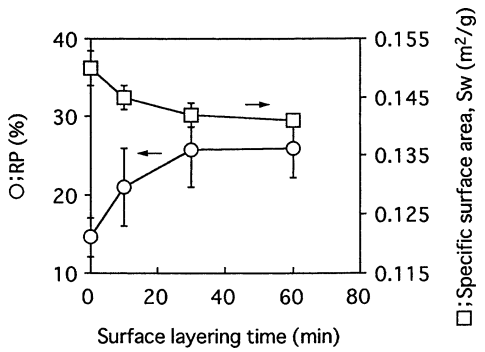

Fig. 2. Relationship between RP, Sw and Surface Layering Time
○, RP; □, Sw. Data are expressed as mean±S.D. (n=3—5).

When the surface layering time was compared with the RP and Sw, the RP values increased with surface layering time. On the other side, Sw decreased with increased in the surface layering time. The RP of the powder mixed with the surface layered lactose carrier was significantly higher than that of the powder mixed with the surface unlayered lactose carrier. Drug particles adhered in the concavity would become entrapped and relatively immobile in the depressions on the carrier surface.[1,3] The separation of drug particles from surface unlayerd lactose carriers would be lower, resulting in low RP values. With lac-3, which was lactose carrier surface layered for a long time, the amount of roughness on the lactose particle surface was smaller than that with unlayered lactose, and the carrier-particle specific surface area was smaller. This decreased the number of drug particles remaining in depressions and facilitated drug separation. This was in agreement with the results by Kawashima *et al.* that lactose particles with larger surface areas could carry higher amount of drug particles, whereas they held more firmly the drug particles in the inhaled air stream.[2]

In this study, the effects of surface layering time of lactose carrier particles on *in vitro* deposition properties of sulbutamol sulfate were investigated. RP obtained by surface layering with Mg-St-V were significantly better than that of unlayered lactose carrier. Surface layering of lactose carrier particles with Mg-St-V is an inexpensive and simple technique. Designing of carrier particles as well as drug particles is im-

portant for designing DPI. Surface layering of carrier particles may be an effective technique that may lead to improvements in inhalation properties of DPI. In discussing the effects of surface layering of carrier particles on *in vitro* inhalation properties of DPI, there are many factors to be investigated including the surface layering technique and differences in the surface-layering material. We will further study the effects of surface layering technique and the quantity of the surface layering material used on *in vitro* inhalation properties of DPI.

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