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Formulation and Evaluation of Insulin Dry Powder for Inhalation

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ABSTRACT **Purpose**. Dry powder formulation of insulin for pulmonary administration was prepared to obtain increased drug deposition in the alveolar absorptive region. The deposition was studied by investigating the dispersion and deaggregation of insulin from the carrier lactose using an Andersen cascade impactor and twin stage impinger. The subsequent absorption following the deposition was studied by in vivo method. **Methods**. Insulin in solution with absorption promoters was lyophilized. The powder was incorporated with lactose of different grades and their combinations as carriers to deliver using an inhaler device. Solid-state characteristics of the carrier as well as the drug powder were assessed by particle size and distribution measurement. The flow properties such as moisture content, powder density, angle of repose, and carr's compressibility index of the powder mixture were determined. The aerosol behavior of the powder was studied by dispersion using rotahaler© connected to a twin-stage impinger (TSI) and an eight-stage Andersen cascade impactor (ACI) operating at different flow rates of 30–90 l/min. The in vivo performance was studied by deliverance to the respiratory tract of guinea pigs. The intratracheal bioavailability with respective to intravenous route was calculated by measuring the blood glucose reduction. **Results**. The coarser particles of lactose in fractions of carrier containing a wide particle size distribution impacted in the preseperator of cascade impactor, and only the particle less than 10 μm size entered stage 0–stage 7. Formulation containing 1:1 mixture of Respitose ML006 (62%<50 μm) and Respitose ML003 (37.8%<50 μm) as carrier imparts well deaggregation of insulin, and higher deposition leads to 52.3% of fine particle fraction at 60 Lit/min and in vivo bioavailability of 82%. **Conclusions**. Insulin formulations containing 1:1 mixture of Respitose ML006 and Respitose ML003 as carrier can impart deeper deposition of drug particles and cause higher bioavailability. This suggests that carrier used in the formulation influenced the amount of insulin deposition in the alveolar region of the lung. Hence, it was concluded that the availability of insulin for systemic absorption depends on the particle size of the drug as well as the carrier lactose.

KEYWORDS Lyophilization, Carrier, Lactose, Particle size, Deposition, Impinger

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INTRODUCTION

Currently, the delivery of peptides and protein drugs by pulmonary route has attracted much attention due to the advantages the lung offers compared with conventional route of drug delivery (Wall, 1995; Smith, 1997). This approach of drug delivery having the advantages of rapid drug absorption and removal of the first pass metabolism is compared with conventional delivery alternatives (Patton et al., 1994; Niven, 1995; Stanley, 1999). Dry powder inhaler (DPI) devices that do not contain chloroflurocarbon (CFC) propellant were explored for the delivery of these drugs to the alveolar region (Prime et al., 1997). Powder inhalers are versatile delivery systems, which may require some degree of dexterity to operate, although one of the objectives of recent developments has been to simplify their operation. The degree of pulmonary deposition of inhaled drug is dependent not only upon the inhalation device used but also on properties of the drug and formulation. DPI formulation may contain drug alone, or drug blended with a suitable carrier material (which is usually lactose). After inhalation by the patients, the carrier should deposit in the upper airways and the micronized drug particles should be released into the inspired air with a view to such particles gaining access to the lower airways. Blending of the drug with carrier allows easier metering of small quantities of potent drugs and also to increase the bulk. The optimization and control of flow and deaggregation properties of the formulation is of critical importance in the development of powder inhalation products. These characteristics are a function of the principal adhesive forces between particles, including Van der Waals forces, electrostatic forces, and the surface tension of the absorbed liquid layers (Hinds, 1982). The forces are influenced by several fundamental physico-chemical properties including particle morphology, particle size distribution, density, and moisture content. Larger carrier particles were shown to exert stronger adhesion forces on drug particles than smaller particles (Staniforth et al., 1982) and the in vitro respirable fractions of salbutamol from smaller lactose particles have been shown to be higher than those from larger lactose particles at different flow rates (Ganderton & Kassem, 1992). If the particle size decreases then surface area increases and proportionally moisture content also, which reduces the flow property. The particle size of the carrier has to be controlled strictly, so that the drug particles can easily be detached from the carrier particles. Reproducible dose can be dispensed only if the cohesive forces are less, and flow should be uniform. When the forces imparted by inhalation exceed the interparticulate forces between drug and carrier particles, then the drug particles are detached from the carrier particles. The respirable fraction of the drug depends upon the strength of interaction between the drug and carrier particles, and the physical properties of both drug and carrier have been shown to influence these interactions (Hickey et al., 1990; Hickey et al., 1994). Strong adhesion forces result in lower amounts of drug detaching from carrier particles. The choice of suitable flow rate was found to be a critical variable determining the emitted dose from dry powder inhaler (Hindle & Byron, 1995). Pitcairn and associates (Pitcairn et al., 1994) examined the effect of inhalation flow rate on lung deposition of salbutamol sulfate. At an inhalation flow rate of 46 l/min, a significantly higher amount of drug deposited (14.1% ± 3.2%) compared with deposition at 27.8 l/min (11.7% ± 2.3%).

The present investigation focused on the deposition studies of insulin and the carrier lactose from different formulations in vitro and in vivo. Efforts were made with the view of analyzing the influence of particle size and other flow properties of carrier on insulin deposition. Different size ranges of lactose carrier blended alone and in combination with insulin may cause different deposition pattern. Higher in deposition may help in efficient delivery and reduce the dose of drug and adverse effects.

METHODS AND MATERIALS

Chemicals

Insulin porcine (25.5 IU/mg) was gifted by Sarabhai Chemicals (Vadodara, India). Citric acid anhydrous (extra pure), sodium tauroglycocholate was purchased from SD Fine-Chem Ltd (Boisar, India). Oleic acid (cis 9-ocatadecanoic acid), sodium salt, bestatin, and chymostatin were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). DMV International (Netherlands) gifted Respitose ML003, Respitose sv 003, and Respitose ML006.

Preparations of Insulin Dry Powder Formulations

Insulin solution with selected absorption promoters (Mahesh Kumar & Misra, 2003) including sodium tauroglycocholate (0.1%), oleic acid (cis 9-ocatadecanoic acid) sodium salt (0.2%), bestatin (0.02%) and chymostatin (0.04%) was made in citrate buffer pH 3.5. The solution was frozen at –45°C and freeze dried (Heto Drywinner model DW1 0–60E, Denmark) for 48 h cycle time. The porous cake thus formed was size reduced by passing successively through 45 μm and 25 μm sieves (Hitco sieves, Hind Trading Company, Baroda, India) (Joshi & Misra, 2000) followed by lab scale attrition method. Respitose ML003, Respitose sv 003, and Respitose ML006 were separately passed through 100-mesh sieve. These lactose are special grades mainly used for dry powder inhaler preparations (manufactured to claim Type 2 DMF by DMV International) and differ from each other by particle size (Table 2). The sieved respitose of three grades were blended individually and in combinations with lyophilized insulin powder (Table 1). Capsules (size 3) were filled with individually weighed 30 mg of the powder blend, equivalent to 24 IU (0.94 mg) of insulin. The capsules were packed under nitrogen atmosphere in HDPE (high-density polyethylene) bottles containing silica bags as dehumactant. The bottles were stored in a desiccator at refrigeration temperature (2–8°C) for further use.

TABLE 1 **Composition of Formulations**

Formulation	Composition
F1	Lyophilized insulin + Respitose ML003
F2	Lyophilized insulin + Respitose sv003
F3	Lyophilized insulin + Respitose ML006
F4	Lyophilized insulin + 1:1 mixture of Respitose sv003 and Respitose ML006
F5	Lyophilized insulin + 1:1 mixture of Respitose ML003 and Respitose ML006

Evaluation of Dry Powder Formulations

The prepared dry powder formulations of insulin were evaluated for particle size, in vitro deposition, flow properties, and in vivo bioavailability by using the following methods.

Laser Light-Scattering Measurement

Lactose, as well as the drug, was dispersed in 25 mL of chloroform and sonicated (Ralsonics probe sonicator-RP 1202, Bombay, India) for 2–3 min. The particle size was determined by employing light-scattering particle size analyzer (Hydro 2000 SM, Malvern Instruments Limited, UK) at obscuration of 10% ($n = 5$) and was recorded.

Twin Stage Impinger (TSI)

The capsule was placed in the rotahaler© (Cipla Ltd, India) device and fitted into molded rubber mouthpiece attached to the glass throat piece of the impinger, which was operated at 30, 60, and 90 l/min (Rotameter, Gilmont, USA. GF-2500). The capsule was broken by rotating the top and bottom of the device at two opposite direction. Air was drawn through the device for 20, 10, and 6.7 sec, respectively. The effective cut-off diameter of the upper stage was found to be 5.5, 6.3, and 7.4 μm, respectively (Srichana et al., 1986). Insulin deposition in the lower stage of the twin stage impinger (particles <5.5 μm) was analyzed by HPLC using a mobile phase comprising a mixture of acetonitrile and water, an ODS (Octadecylsilyl silica gel) column at temperature 40°C and UV detector set at a wavelength of 214 nm. The lower stage deposition was then calculated as a percentage of the nominal dose. Lactose deposition in the lower stage of the twin stage impinger was analyzed by HPLC using acetonitrile and water in the ratio 75:25

TABLE 2 **Particle Size Distribution of Insulin and Lactose Determined by Laser Light Scattering**

	Size range (μm)					
	<6.4	6.4–10.0	10.0–25.0	25.0–50.0	50.0–90.0	>90.0
Percentage by volume						
Insulin	92.3	6.7	1.0	0.0	0.0	0.0
Respitose ML003	–	0.8	8.6	28.4	51.2	11.0
Respitose sv003	–	1.2	13.1	36.5	43.4	5.8
Respitose ML006	–	1.8	19.2	41.0	34.3	3.7

as a mobile phase, an amino column. The internal standard was glucose monohydrate.

Anderson Cascade Impactor

The parts of the impacter including preseperator, eight stages, and collection plates were cleaned and rinsed with deionized water and were sonicated for 15–20 min to ensure that there was no blocking of any of the orifices. After cleaning, the parts were dried in a hot air oven. They were arranged in sequence and the deposition studies conducted at 30 l/min and 60 l/min for 21 sec and 10 sec. The insulin formulations were introduced to the impactor using a rotahaler© (Cipla Ltd, India) device. After actuating the dose into the cascade impactor, the glass throat, preseparator, and each stage were rinsed with the mobile phase. The drug and lactose were analyzed by HPLC method as described previously.

Angle of Repose

For determining the angle of repose, a pile of the samples was carefully built up by droping the powder material through a funnel until the formed pile touched the tip of the funnel, 2 cm above the flat surface. The angle of repose was calculated by inverting tangentially the ratio of height and radius of the formed pile, and it was recorded.

Tapped Density

Tapped density was determined by mechanically tapping a measuring cylinder containing 10 g of powder sample. After observing the initial volume, the cylinder was mechanically tapped, and volume reading was taken until little to no change in volume is observed. The plateau condition was obtained after 500 taps for all samples and was recorded.

Carr's Compressibility Index

The Carr's compressibility index (Srichana, 1998; Carr, 1965) was calculated by the following formula:

$$\text{Carr's compressibility index} = \frac{\text{Tapped density} - \text{Fluff density (Poured Bulk density)}}{\text{Tapped density}}$$

Determination of Residual Water Content

The residual water content of the DPI formulation (1.0 g) was determined by using an automatic Karl-Fischer Titration (Chemito CL 48885, Mercury Labs, Baroda). Commercially available pyridine free reagent was standardized with a known quantity of water (250 mg) and used. The water content determination was carried out six times.

Measurement of Content Uniformity of Insulin

The content of a single capsule was removed and spread over a butter paper, and six samples were taken from six spots randomly. The content of insulin in each sample was analyzed by HPLC method as described earlier. The variation of the insulin content was determined.

In Vivo Study

For assessing the in vivo performance of the developed formulation, guinea pigs weighing 750–800 gm were used. Animal experiments were approved by the Social Justice & Empowerment Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Government of India, New Delhi. Guinea pigs were housed in large polypropylene cages at air-conditioned temperatures (22°C–24°C), normal hygiene, and normal diet at a 12-h light/dark cycle, and water was given ad libitum. Guinea pigs were anaesthetized using intraperitoneal injection of urethane (120 mg/100 g). A small rigid tube of 1 mm OD was loosely inserted between two cartilaginous rings, into the lumen of the exposed trachea above the carina. This tube was then connected to a Harvard ventilator via inhalation and exhalation ports, while the animal continued to breathe spontaneously. The femoral vein was catheterized using silicone tubing (0.02-mm internal diameter and 0.05-mm outer diameter), and the patency of the catheter was confirmed by slowly flushing the cannula with 200 μL of heparinized saline. The absence of reflexes and the breathing rate were visually monitored throughout the experiment. A measure of 1.0 mg of the formulation (equivalent to 1.0 IU/Kg) was placed in the

inhalation port and insufflated into the lungs of the animals by forced ventilation. Immediately after the delivery, the tube was removed from the trachea. Blood samples (100 μL) were withdrawn at −30, 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, and 300 min through the lateral tail vein. After each sampling, an equal amount of saline was injected through the catheter tube inserted in the femoral vein. An insulin syringe was used for the withdrawal of blood samples from the tail vein and transferred to the micro centrifuge tube. At the end of the experiment, the guinea pigs were euthanized with an overdose of anesthesia and exsanguinated. After spontaneous clotting at room temperature, the blood samples were centrifuged at 3500–4000 rpm for about 5 min in cold centrifuge at 0°C (C-94, Remi Instruments, Mumbai). The serum was separated out using a micropipette and was transferred to a 1.0-mL eppendorf tube and kept in refrigerator at 0°C until completion of the study for subsequent glucose estimation. The glucose content was measured by the glucose oxidase–peroxidase method (Hyvärinen & Nike, 1962). The analysis is based on the enzyme-catalyzed reaction of glucose with molecular oxygen, followed by a second reaction that produces an intense red color. The color intensity is proportional to the amount of oxidized glucose in the sample. This analytical method yielded a serum glucose concentration in the range of 20 to 350 mg/dL with 2% precision. The mean percent blood glucose reduction (PBGR) was calculated from the amount (mg/dL) of blood glucose measured. The mean percent blood glucose reduction after intravenous administration also determined by the same method. The $AUC_{0-300\ min}$ (area under the blood glucose reduction–time curve) of both intravenously administered insulin and intratracheally instilled insulin formulations was calculated by the trapezoidal rule (Gibaldi & Perri, 1982).

The percent relative pulmonary bioactivity (F*) with respect to intravenously administered insulin was calculated as follows:

$$F^* = \frac{AUC_{0\text{-}300\ min}\ \text{Intratracheal Route} \times \text{Intravenous Dose}}{AUC_{0\text{-}300\ min}\ \text{Intravenous Route} \times \text{Intratracheal Dose}} \times 100 \quad (1)$$

RESULTS

In Table 1, the formulations of insulin prepared with lactose of different grades and the combinations of them as carriers were shown. Out of five formulations, three of them individually contain Respitose ML003, Respitose sv 003, and Respitose ML006, respectively (F1, F2, and F3). The remaining two fomulations contain combination of Respitose ML006 with Respitose sv 003 (F4) and Respitose ML003 (F5) in the ratio of 1:1. The particle size distributions of insulin and the carriers used in the formulations were measured by particle size analyzer and summarized in Table 2. Comparatively, the particle size distribution shows Respitose ML003 containing larger size range (37.8% of particles less than 50 μm), Respitose sv003 containing medium size range (50.8% of particles less than 50 μm), and Respitose ML006 containing smaller size range (62.0% of particles less than 50 μm).

In Fig. 1 the deposition of insulin at the lower stage of the twin stage impinger (particles <5.5 μm) that was operated at different flow rates of 30, 60, and 90 l/min are shown. At a flow rate of 30 l/min, the drug deposited from the formulations containing Respitose ML003, Respitose sv 003 and Respitose ML006 (F1, F2, and F3) was 21.5%, 25.6%, and 33.2%, respectively. As the size of the carrier decreases the deposition of drug increases. The deposition was further increased to 38.7% and 42.1%, respectively, of the formulations containing Respitose ML006 in combination with Respitose sv 003 and Respitose ML003. The same was observed when the formulations were aerosolized at other flow rates of 60 l/min and 90 l/min. At all three flow rates the deposition of drug was found to be the same in case of formulation with Respitose ML003 (F1). In the formulation with Respitose sv 003 (F2) the deposition was moderately increased as the flow rate increased but it was not remarkably different. But in case of Respitose ML006 (F3) and the other two subsequent formulations (F4 and F5) where the lesser particle size lactose was used, the deposition proportionately increased with the flow rates (up to 56.9% at a flow rate of 90 l/min). At lower stage of impinger no deposition of lactose was observed.

Anderson cascade impactor was employed to understand further the mechanism of drug and carrier delivery in the formulations. Tables 3 and 4 summarize the drug as well as lactose deposition at a flow rate

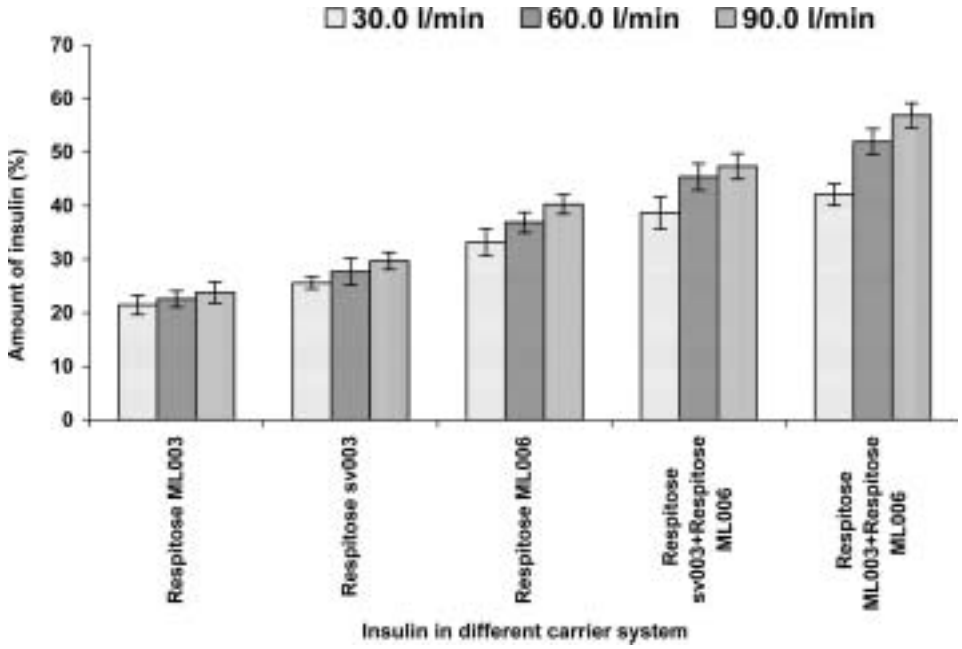

FIGURE 1 **Deposition of insulin on lower stage of twin stage impinger from formulations with different carriers at various flow rates.**

of 30 l/min and 60.0 l/min, respectively. At a flow rate of 30 l/min insulin deposited in the throat and the preseparator was found to be higher in case of Respitose ML003 (F1), i.e., 30.8% and 16.9%, respectively. When Respitose ML006 and their combinations with Respitose sv 003 (F2) and Respitose ML003 were used, then the drug deposition in the throat decreased.

In Anderson cascade impactor the order of drug deposited from the carrier in the throat and preseparator was as follows:

Respitose ML003 (37.8% lactose < 50 μm) >
Respitose sv 003 (50.8% lactose < 50 μm) >
Respitose ML006 (62.0% lactose < 50 μm) >
Respitose ML006 + Respitose sv 003 >
Respitose ML006 + Respitose ML003

Similarly, the drug deposited in the stages 0–7 increased with the decrease in the particle size of the carrier. The order of drug deposited from the carrier in the 0–7 stages was as follows:

Respitose ML003 < Respitose sv 003 <
Respitose ML006 < Respitose ML006 + Respitose sv 0003 < Respitose ML006 + Respitose ML003

The fine particle fraction (Particles <5.8 μm) for insulin at 30 l/min flow rate was 32.3% when Respitose ML006 was used as carrier. It was only 23.2% and 27.8%, respectively, when Respitose ML003 and Respitose sv 003 were used. Also FPF increased further to 38.3% and 42.2%, if Respitose ML006 was used with the other two grades in combination. The maximum fine particle fraction (particles <6.2 μm) of 52.3% was obtained at 60 l/min in the formulation containing a combination of Respitose ML006 and Respitose ML003.

The variation of the insulin content of the prepared formulations determined by assay was found to be within a limit of ± 5%. The flow properties were indicated in Table 5. The residual water content determined by the Karl Fischer Titration method was found to be in a range 4.6% to 5.1%. The flow parameter angle of repose and compressibility index increased as the particle size of the carrier decreased that led to decrease in the flow of the powder. All the lactose particles deposited only in preseperator and the throat region and nothing was observed in the 0–7 stage of the cascade impactor.

In Table 6, in vivo pharmacodynamics parameters of the intratracheally insufflated insulin formulations (F1–F5) in guinea pigs are shown. The $AUC_{0-300\ min}$ and the intratracheal bioavailability with respect to intravenous administration varies with respect to

TABLE 3 Deposition of Insulin and Lactose from Insulin Formulations with Different Carriers in Anderson Cascade Impactor at a Flow Rate of 30 l/min

	Formulation									
	Insulin + Respitose ML003 (F1)		Insulin + Respitose sv003 (F2)		Insulin + Respitose ML006 (F3)		Insulin + Respitose sv003 + Respitose ML006 (F4)		Insulin + Respitose ML003 + Respitose ML006 (F5)	
Part	Drug	Lactose	Drug	Lactose	Drug	Lactose	Drug	Lactose	Drug	Lactose
Throat	30.8 ± 0.9	45.3 ± 1.7	31.3 ± 1.6	45.1 ± 1.0	20.1 ± 1.2	47.3 ± 1.7	18.8 ± 1.1	47.5 ± 1.5	16.0 ± 1.1	47.4 ± 1.7
Preseparator	16.9 ± 0.6	32.4 ± 1.1	14.7 ± 1.3	32.9 ± 1.7	12.2 ± 0.8	33.3 ± 1.2	7.7 ± 0.8	36.9 ± 1.4	8.3 ± 0.7	38.6 ± 1.1
Stage 0 (9.0–10.0 μm)	5.8 ± 0.5	–	5.7 ± 0.8	–	6.3 ± 0.7	–	7.9 ± 0.3	–	7.9 ± 1.2	–
Stage 1 (5.8–9.0 μm)	6.7 ± 0.8	–	4.0 ± 0.9	–	11.1 ± 0.9	–	10.9 ± 1.4	–	12.6 ± 1.1	–
Stage 2 (4.7–5.8 μm)	5.2 ± 0.2	–	6.3 ± 0.4	–	7.9 ± 0.9	–	9.8 ± 1.0	–	8.9 ± 0.9	–
Stage 3 (3.3–4.7 μm)	6.8 ± 1.1	–	8.0 ± 0.3	–	10.0 ± 0.4	–	10.1 ± 1.0	–	10.9 ± 1.0	–
Stage 4 (2.1–3.3 μm)	6.0 ± 0.4	–	8.2 ± 0.5	–	8.4 ± 0.7	–	9.3 ± 0.8	–	11.4 ± 0.8	–
Stage 5 (1.1–2.1 μm)	3.1 ± 0.5	–	3.0 ± 0.6	–	6.0 ± 0.8	–	6.5 ± 0.4	–	6.1 ± 0.8	–
Stage 6 (0.7–1.1 μm)	–	–	–	–		–	2.6 ± 0.3	–	4.9 ± 0.5	–
Stage 7 (0.4–0.7 μm)	–	–	–	–	–	–	–	–	–	–
%Fine particle fraction (<5.8 μm)	21.1	–	25.5	–	32.3	–	38.3	–	42.2	–
Emitted dose (%)	79.3 ± 2.5	80.6 ± 3.5	82.7 ± 2.2	81.7 ± 2.7	84.0 ± 2.8	83.4 ± 2.9	85.6 ± 2.9	86.2 ± 2.7	88.4 ± 2.2	88.3 ± 3.2

TABLE 4 Deposition of Insulin and Lactose from Insulin Formulations with Different Carriers in Anderson Cascade Impactor at a Flow Rate of 60.0 l/min

	Formulation									
	Insulin + Respitose ML003 (F1)		Insulin + Respitose sv003 (F2)		Insulin + Respitose ML006 (F3)		Insulin + Respitose sv003 + Respitose ML006 (F4)		Insulin + Respitose ML003 + Respitose ML006 (F5)	
Part	Drug	Lactose	Drug	Lactose	Drug	Lactose	Drug	Lactose	Drug	Lactose
Throat	32.1 ± 1.2	46.3 ± 2.7	32.7 ± 2.2	47.1 ± 1.7	30.1 ± 1.5	48.9 ± 3.1	27.1 ± 1.9	50.1 ± 2.7	24.2 ± 2.2	51.4 ± 2.7
Preseparator	16.0 ± 0.8	33.4 ± 2.1	15.8 ± 1.7	33.9 ± 2.1	10.2 ± 1.1	34.7 ± 2.8	8.7 ± 1.0	36.8 ± 2.2	6.2 ± 1.4	37.6 ± 2.5
Stage 0 (6.2–7.1 μm)	6.0 ± 0.3	–	4.5 ± 1.0	–	6.8 ± 0.9	–	6.4 ± 0.8	–	6.0 ± 0.9	–
Stage 1 (4.0–6.2 μm)	6.5 ± 1.0	–	6.9 ± 0.8	–	9.2 ± 1.2	–	11.1 ± 1.0	–	12.1 ± 1.5	–
Stage 2 (3.2–4.0 μm)	5.7 ± 0.7	–	6.8 ± 0.7	–	8.2 ± 0.9	–	9.0 ± 1.2	–	11.2 ± 0.8	–
Stage 3 (2.3–3.2 μm)	4.1 ± 0.5	–	5.9 ± 0.4	–	6.9 ± 0.8	–	8.2 ± 0.5	–	9.0 ± 0.5	–
Stage 4 (1.4–2.3 μm)	3.8 ± 0.8	–	4.7 ± 0.7	–	5.2 ± 0.5	–	7.0 ± 0.6	–	8.4 ± 0.5	–
Stage 5 (0.7–1.4 μm)	3.1 ± 0.5	–	3.5 ± 0.5	–	3.9 ± 0.7	–	5.0 ± 0.6	–	6.1 ± 0.8	–
Stage 6 (0.5–0.8 μm)	–	–	–	–	2.5 ± 0.5	–	2.8 ± 0.3	–	3.0 ± 0.4	–
Stage 7 (0.3–0.5 μm)	–	–	–	–	–	–	–	–	2.5	–
% Fine Particle Fraction (<6.2 μm)	23.2	–	27.8	–	35.9	–	43.1	–	52.3	–
Emitted dose (%)	80.2 ± 1.7	81.3 ± 2.6	82.9 ± 3.2	83.0 ± 3.3	85.3 ± 2.2	85.7 ± 1.8	87.1 ± 3.1	88.9 ± 3.7	90.1 ± 2.6	90.7 ± 3.1

TABLE 5 **Flow Properties of Insulin with Carrier Lactose**

Formulation	Tap density	Angle of repose	Carr's compressibility index	Residual water content (%)
Insulin in Respitose ML003 (F1)	0.77 ± 0.04	16.8 ± 0.8	9.4 ± 0.7	4.6 ± 0.5
Insulin in Respitose sv003 (F2)	0.85 ± 0.09	17.8 ± 1.0	11.9 ± 0.8	4.8 ± 0.5
Insulin in Respitose ML006 (F3)	0.90 ± 0.11	20.1 ± 0.9	16.5 ± 0.6	5.1 ± 0.4
Insulin in mixture of Respitose sv003 and Respitose ML006 (F4)	0.87 ± 0.07	19.1 ± 1.1	14.0 ± 0.4	4.9 ± 0.8
Insulin in mixture of Respitose ML003 and Respitose ML006 (F5)	0.80 ± 0.11	18.1 ± 1.2	12.4 ± 0.7	4.7 ± 0.9

TABLE 6 **Pharmacodynamic Parameters after Intratracheal Insufflation of Insulin Formulations**

Formulation	$AUC_{0–300\ min}$ (% min)	F*
Insulin in Respitose ML003 (F1)	6590 ± 410	53 ± 1.27
Insulin in Respitose sv003 (F2)	7087 ± 308	57 ± 1.51
Insulin in Respitose ML006 (F3)	8082 ± 317	65 ± 1.28
Insulin in mixture of Respitose sv003 and Respitose ML006 (F4)	9077 ± 357	73 ± 2.48
Insulin in mixture of Respitose ML003 and Respitose ML006 (F5)	10196 ± 396	82 ± 2.70

$AUC_{0–300\ min}$ indicates area under the blood glucose reduction–time curve of intratracheal insufflated insulin over 300 min; F, percent pulmonary bioactivity.

lactose grade used in the formulation. Formulation containing combination of Respitose ML006 with Respitose sv003 (F4) and Respitose ML003 (F5) shows higher bioavailability of 73% and 82%, respectively, in comparison to other formulation with single carrier (53%–65%).

DISCUSSION

The study with twin stage impinger suggests that lactose of larger particle sizes used along with lesser size as carrier for the insulin (formulation with both Respitose ML003 and Respitose ML006:F5) then deaggregation of the drug particles was improved and the deposition increased proportionately. After aerosolization, the drug and the carrier particles released from the surface of larger lactose particles and traveled alone and aggregated into the impactor. In twin stage impinger the air flow rate of 30 l/min was sufficient to detach the respirable drug from the carriers of larger particle sizes (Respitose ML003 and Respitose sv 003). When carriers of smaller particle sizes (Respitose ML006) were used then increase in air flow was necessary to detach the respirable fraction of drug. At all the three flow rates (30 l/min, 60 l/min, and 90 l/min) the carrier in the formulation does not enter into the lower stage of the impinger and only deposited in the upper stage.

In Anderson cascade impactor drug particles aerosolized at 30 l/min and 60 l/min were found to deposit as for as stage 5 when Respitose ML003 and Respitose sv 003 were used as an excipient in the formulation, and at this stage drug particles are apparently separated from carrier particles. Respitose ML006 was employed as a carrier, drug particles penetrate to stage 5 at 30 l/min air flow rate, and when flow rate increased to 60 l/min drug particles penetrated to stage 6. When mixture of Respitose ML006 and Respitose ML003 was used drug particles penetrated to stage 6 at low flow rate of 30 l/min and further penetrated deeper into stage 7 at higher flow rate of 60 l/min. This may be due to saturation of active sites in the carrier surfaces which leads to well deaggregation of drug particles from lactose carrier. In twin stage impinger, formulation containing Respitose ML006 with Respitose ML003 shows maximum fine particle fraction of 42.1% and 52.0%, respectively, at 30 l/min and 60 l/min. Similarly, the same formulation had fine particle fraction of 42.2% and 52.3% using Anderson cascade impactor at same flow rate. Hence, the results of the twin impinger and the eight-stage impinger were in good agreement and some discrepancies due to the effective cut-off diameter of the stages in the two impactors vary and also alter as a function of flow rate. The three grades of lactose used in the formulations

travel up to throat only due to 98% of the particles having particle size more than 10 μm.

The residual water content of the formulations determined by Karl Fischer titration method was found to be in a range 4.6% to 5.1% due to difference in the surface area of the particles. The formulations prepared by using smaller size carrier system showed little higher water content than formulation prepared by larger-size carrier system. The flow parameter angle of repose and compressibility index increased as the particle size of the carrier decreased, which led to decease in the flow of the powder. The Respitose ML006 shows more decrease in the flow properties as compared to the other two grades (Respitose ML003 and Respitose sv 003), indicating that as the particle size decreases the flow properties also decrease. Formulation with Respitose ML003 shows good flow properties compared to Respitose ML006, but the in vitro deposition of drug from Respitose ML003 was less. Even the Respitose ML003 having good flow but FPF (%) was less, hence the deposition was also poor. When Respitose ML006 mixed with the Respitose ML003 then flow parameter decreased as compared to Respitose ML006 used alone and ultimately flow properties of the formulations improved. The change in the flow properties was directly dependent on the percentage and particle size of micronized lactose with larger size respitose.

The formulation showing higher in vitro deposition imparts better bioavailability during in vivo study. This proved the reliability of Anderson cascade impactor and twin stage impinger for studying the dry powder formulations in vitro.

It may be concluded that for higher deposition of drug particles the size of carrier should be controlled and also flow has to be good enough to carry them deeper to the impactor. Formulation containing combinations of two grades of lactose (Respitose ML003 and Respitose ML006:F5) imparts deeper penetration of drug particles which leads to higher systemic absorption.

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