CHAPTER 6

DRY POWDER PREPARATION AND EVALUATION

Since proteins often are not very stable in aqueous solutions, freeze-drying or lyophilization, is commonly used in the manufacture or protein products to enhance their stability. Lyophilazation involves three process segments: Freezing transforms the protein-excipient solution in a vial into two or more phases, usually crystalline ice and an amorphous freeze-concentrate containing the protein, excipient, and water. Primary drying is the sublimation of ice from the frozen vial content under vacuum. Secondary drying, also under vacuum, involves the removal of water from the freeze-concentrate, reducing the residual moisture content to a level (e.g. < 3.0 wt %/wt) suitable for long-term storage [1].

Inhalation drug delivery has been used for many years for the delivery of pharmacologically active agents to the respiratory tract. The use of metered dose inhaler (MDI) delivery system is now under increasing threat because of the environmental concerns regarding chloroflurocarbon (CFC) propellants. A range of devices, such as DPI, which does not contain CFC propellants, has explored for the delivery of these drugs to the alveolar region [2]. 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 of 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 [3]. The forces are influenced by several fundamental physicochemical properties, including particle morphology, particle size distribution, density and moisture

content. 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 [4,5]. Strong adhesion forces result in lower amounts of drug detaching from carrier particles. The particle size of the carrier has to be controlled strictly, so that the drug particles can easily 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. Small carrier particles were shown to exert lesser adhesion forces on drug particles than larger particles [6]. The invitro respirable fractions of salbutamol from smaller lactose particles have been shown to be higher than those from larger lactose particles at different flow rates [7]. The choice of suitable flow rate was found to be a critical variable determining the emitted dose from dry powder inhalers [8]. [9] 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 %) in compared with deposition at 27.8 l/min (11.7 % ± 2.3%).

The present investigation focused on the deposition studies of insulin and calcitonin from the carrier lactose *in vitro*. Efforts are made to analyze the influence of particle size and other flow properties of carrier on drug deposition. Different size ranges of lactose carrier blended alone and in combination at different proportions with drug may cause different deposition pattern. Higher in deposition may help in efficient delivery and reduces the dose of drug and adverse effects.

Insulin and calcitonin, in the form of dry powder would make it easier for patients to take using various devices such as rotohaler, dischaler, etc. Formulation factors played major role in the development of DPI of these peptide drugs. The present studies demonstrated the preparation method of insulin and calcitonin DPI and its evaluation.

6.1 DRY POWDER PREPARATION

The relative pulmonary bioactivity of insulin and calcitonin was studied in solution with absorption promoters in chapter 4, the potent formulation with higher bioactivity was necessary to made into powder form. The dose of calcitonin is very less, necessary diluents were incorporated to increases the bulkiness. Also the drug powder was dispersed in suitable carrier for the final delivery to the lung by inhalation.

6.1.1 MATERIALS AND METHODS

Insulin porcine (25.5 IU/mg) was gifted by Sarabhai Chemicals (Vadodara, India). Salmon calcitonin (6123 IU/mg) was kindly gifted by Novartis Pharma AG, Basel (Switzerland). Citric acid anhydrous (extra pure), sodium tauroglycocholate was purchased from SD Fine-Chem Ltd (Boisar, India). Dimethyl β-cyclodextrin, dodecyl maltoside, oleic acid (cis 9-ocatadecanoic acid), sodium salt, bacitracin, bestatin and chymostatin, were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany), Borculodomo (Netherlands) gifted micronized lactose (Lactohale – LH 300). DMV International (Netherlands) donated Respitose (sv 0003).

6.1.2 METHOD OF PREPARATION

Insulin solution with absorption promoters 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 [10]. The solution was frozen at -40°C and freeze dried (Heto Drywinner model DW1 0-60E, Denmark) for 48 hrs cycle. The porous cake thus formed was size reduced by passing successively through 45μm and 25μm sieves (Hitco sieves, Hind Trading Company, Baroda, India) [11] followed by lab scale attrition method [12]. Respitose was separately passed through 45 μm and 25 μm sieves three times. The sieved Respitose and LactoHale were blended individually and in combinations with lyophilized insulin powder (Table 6.1). Capsules (size 3) were filled with

individually weighed 30 mg of the powder blend, equivalent to 76.5 IU (3.0 mg) of insulin. The capsules were packed under nitrogen atmosphere in HDPE bottles containing silica bags as dehumactant. The bottles were stored in a desiccator at refrigeration temperature (2-8°C) for further use.

Solution of calcitonin and lactose (1:50) with absorption promoters including sodium tauroglycocholate (0.2%), dimethyl β-cyclodextrin (0.3%) and protease inhibitors - chymostatin (0.04%), bacitracin (0.02%) was made in acetate buffer pH 3.9. The solution was frozen at -52°C and freeze dried (Heto Drywinner model DW1 0-60E, Denmark) for 52 hrs cycle. The porous cake thus formed was size reduced by passing successively through 45μm and 25μm sieves (Hitco sieves, Hind Trading Company, Baroda, India) [11] followed by lab scale attrition method [12]. Respitose was separately passed through 45 μm and 25 μm sieves three times. The sieved Respitose and LactoHale were blended individually and in combinations with lyophilized insulin powder (one part of calcitonin powder mixture with 9 parts of carrier)[Table 6.6]. Capsules (size 3) were filled with individually weighed 30 mg of the powder blend, equivalent to 360 IU (58.8 μg) of insulin. The capsules were packed under nitrogen atmosphere in HDPE bottles containing silica bags as dehumactant. The bottles were stored in a desiccator at refrigeration temperature (2-8°C) for further use.

6.2 EVALUATION

The prepared insulin and calcitonin dry powder formulations are evaluated by various methods to study their effective deposition in the alveolar absorptive surface of the lung.

6.2.1 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 regarded in Table 6.2 for insulin and in Table 6.7 for calcitonin.

6.2.2 IN VITRO DEPOSITION STYDY USING TWIN STAGE IMPINGER

The capsule placed in the rotahaler© (Cipla Ltd, India) device which had been fitted into molded rubber mouth piece attached to the glass throat piece of the impinger (Figure 6.1), which was operated at 30, 60 and 90 l/min (Rotameter, Gilmont, USA. GF-2500). The insulin/calcitonin capsule with dry powder 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 s, respectively. The effective cut off diameter of the upper stage was found to be 5.5, 6.3 and 7.4 μm, respectively [13,14]. Insulin deposition in the lower stage of the TSI was analyzed by HPLC method, an ODS (Octadecylsilyl silica gel) column at a 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 TSI was analyzed by HPLC using acetonitrile and water in the ratio 75:25 as a mobile phase, an amino column. The internal standard was glucose monohydrate. Calcitonin deposition in the lower stage of the TSI was analyzed by HPLC method.

6.2.3 IN VITRO DEPOSITION STUDY USING ANDERSON CASCADE IMPACTOR

Before starting the deposition study the parts of the impactor 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 before analyzing for the drug and lactose as described previously by HPLC method.

6.2.4 ANGLE OF REPOSE

For determining the angle of repose, a pile of the formulation samples was carefully build up by drooping the powder material through a funnel till the formed pile touches the tip of the funnel, 2 cm above the flat surface. The angle repose was calculated by inverting tangentially the ratio of height and radius of the formed pile (Table 6.5 and Table 6.8)

6.2.5 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. The obtained values are shown in Table 6.5 and Table 6.8.

6.2.6 CARR'S COMPRESSIBILITY INDEX

The Carr's compressibility index was calculated by following formula [15]:

$$\text{Carr's compressibility index} = \frac{\text{Tapped density} - \text{Fluff density}}{\text{Tapped density}} \times 100$$

The Carr's compressibility index values of the formulations were shown in Table 6.5 and Table 6.8.

6.2.7 DETERMINATION OF WATER CONTENT

The residual water content of the DPI formulation (1g) was determined by using automatic Karl-Fischer Titration (Chemito CL 48885, Mercury Labs, Baroda). Commercially available pyridine free reagent was standardized with known quantity of water (250mg) and used. The water content determination was carried out six times and the results are recorded in Table 6.5 and Table 6.8.

6.2.8 MEASUREMENT OF CONTENT UNIFORMITY

The content of single capsule was removed and spread over a butter paper and six samples were taken from six spots randomly. The content of insulin and calcitonin in each sample was analyzed by HPLC method, ~~as described earlier~~. The variation of the insulin content was found to be within a limit of ± 5%.

6.3 RESULTS AND DISCUSSION

In Table 6.1, 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 lactose of median particle size 39.3μm (IF1: Respitose®45.0μm), 20.4 μm (IF2: Respitose®25.0μm), and 7.2μm (IF3: LactoHale) respectively and the remaining formulations (IF4 & IF5) contain combination of LactoHale with sieved Respitose 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 6.2. Both the prepared Respitose®45.0 μm and Respitose®25.0 μm had multimodal particle size distribution. Respitose®45.0 μm contains four populations of particle size distribution including 5 μm, 9 μm, 20 μm, 45 μm median diameter and Respitose®25.0 μm had 7 μm, 10 μm, 20 μm, 40 μm. LactoHale grade contains unimodal particle size distribution possessing median diameter of 7.2 μm.

In Figure 6.2 the deposition of insulin at the lower stage of the TSI 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® 45μm, Respitose® 25μm and LactoHale (IF1, IF2 and IF3) was 24.9%, 28.9% and 35.1% respectively. As the size of the carrier decreases the deposition of drug increases. The deposition was further increases to 40.3% and 45.1% respectively of the formulations containing LactoHale in combination with Respitose®25μm and Respitose®45μm. When carriers of larger particle sizes with multimodal particle sizes distribution were used along with lesser size lactose carriers the

deaggregation of the drug particles were improved and the deposition increases proportionately. The same was observed when the formulations were aerosolized at other flow rates of 60 l/min and 90 l/min. At all the three flow rates the deposition of drug was found to be same in case of formulation with lactose of median size 39.3μm (Respitose®45 μm) and in formulation with Respitose®25μm the deposition was moderately increases as the flow rate increases. The difference in the deposition was not so much remarkable. But in case of LactoHale formulation (IF3) and the other two subsequent formulations (IF4 & IF5) where the lesser particle size lactose was used the deposition proportionately increases with the flow rates. This suggest that airflow rate of 30 l/min was sufficient to detach the respirable drug from the carriers of larger particle sizes (Respitose® 45μm and Respitose® 25μm). When carriers of smaller particle sizes were used then increase in airflow is necessary to detach the respirable fraction of drug. The lactose deposition from the formulations represented in Figure 6.3. As like drug deposition the amount of lactose at lower stage of impinger increases proportionately with the decrease in the particle size of the carrier. Flow rate of 30 l/min was sufficient to detach fine particles of lactose from the formulations with carriers of larger particle sizes. When LactoHale used alone and combination with Respitose then the lactose deposition in lower stage was higher and also shows greater change in the deposition at different flow rates. There was a direct relationship between the amount of lactose and insulin deposition at the lower stage of TSI. When the amount of carrier deposition was increased then the fine particle fraction of drug also increased. After aerosolization the drug and the carrier particles released from the surface of larger lactose particles and traveled alone and aggregate into the impactor. In deposition pattern both the drug particles as well as the carrier behave in a similar way at all the flow rates.

Anderson cascade impactor was employed to understand further the mechanism of drug and carrier delivery in the insulin formulations. Table 6.3 and 6.4 summarizes the drug as well as lactose deposition at a flow rate 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® 45μm (IF1) i.e. 32.1% and 14.1% respectively. When LactoHale and their combinations with Respitose

were used then the drug deposition decreases. The order of insulin and lactose deposited from the carrier in the throat and preseparator was as follows,

Respitose® 45µm > Respitose® 25µm > LactoHale > LactoHale + Respitose® 25µm > LactoHale + Respitose® 45µm

Similarly, the drug and lactose deposited in the stages 0-7 increases with the decrease in the particle size of the carrier. . The order of insulin and lactose deposited from the carrier in the 0-7 stages was as follows,

Respitose® 45µm < Respitose® 25µm < LactoHale < LactoHale + Respitose® 25µm < LactoHale + Respitose® 45µm

The fine particle fraction (FPF) for insulin at 30 l/min flow rate was 34.5% when LactoHale used as carrier and was only 23.7% when Respitose was used. Also FPF increases further to 45.7%, if LactoHale and Respitose were used in combination. The maximum deposition of 55.7% was obtained at 60 l/min in the formulation with carriers of combinations.

Drug particles aerosolized at 30 l/min and 60 l/min were found to deposit as for as stage 5 when Respitose was used as an excipient in the formulation and at this stage drug particles are apparently separated from carrier particles. LactoHale was employed as carrier, drug particles penetrate to stage 5 at 30 l/min airflow rate and when flow rate increased to 60 l/min drug particles penetrated to stage 6. When mixture of LactoHale and Respitose was used drug particles penetrate to stage 6 at low flow rate of 30 l/min and further penetrate deeper into stage 7 at higher flow rate of 60 l/min. The particle size and distribution of the carrier particles that influences the flow properties of the formulation plays an important role and discussed below.

The flow properties of insulin formulations were indicated in Table 6.5. The residual water content determined by Karl Fischer Titration method was found to be in a range 4.9% to 5.2% due to difference in the surface area of the particles.

The formulations prepared by using smaller size carrier system showing little higher water content than formulation prepared by larger size carrier system

The flow parameter angle of repose and compressibility index increases as the particle size of the carrier decreases that lead to decease in the flow of the powder. The LactoHale shows more decrease in the flow properties as compared to the other two grades (Respitose® 45 μm and 25 μm), indicating that as the particle size decreases the flow properties also decreases. Formulation with Respitose shows good flow properties compared to LactoHale, but the in vitro deposition of drug from Respitose was less. Even the Respitose having good flow but FPF (%) was less, hence the deposition was also poor. When LactoHale mixed with the Respitose then flow parameter decreases as compared to LactoHale and ultimately flow properties of the formulations improved (Table 6.5). The change in the flow properties was directly dependent on the percentage and particle size of micronized lactose with larger size Respitose.

In Table 6.6, the formulations of calcitonin prepared with lactose of different grades and the combinations of them as carriers were shown. Out of five formulations, three of them individually contain lactose of median particle size 40.9μm (CF1: Respitose®45.0μm), 21.7 μm (CF2: Respitose®25.0μm), and 7.2μm (CF3: LactoHale) respectively and the remaining formulations (CF4 & CF5) contain combination of LactoHale with sieved Respitose in the ratio of 1:1.

The particle size distributions of calcitonin powder mixture and the carriers used in the formulations were measured by particle size analyzer and summarized in Table 6.7. Both the prepared Respitose®45.0 μm and Respitose®25.0 μm had multimodal particle size distribution. LactoHale grade contains unimodal particle size distribution possessing median diameter of 7.2 μm.

In Figure 6.4 the deposition of calcitonin at the lower stage of the TSI 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® 45μm, Respitose® 25μm (CF1 and CF2) was 27.8% and 29.9% respectively. As the size of the carrier decreases further (CF3), the deposition of drug decreases and the

value obtained was 24.7 %. The deposition of calcitonin increases to 35.4% and 38.3% respectively of the formulations containing LactoHale in combination with Respitose®25µm and Respitose®45µm. When carriers of larger particle sizes with multimodal particle sizes distribution were used along with lesser size lactose carriers the deaggregation of the drug particles were improved and the deposition increases proportionately. The same was observed when the formulations were aerosolized at other flow rates of 60 l/min and 90 l/min. At all the three flow rates the deposition of drug was found to be increases as the flow rate increases. The difference in the deposition was remarkable. This suggest that airflow rate of 30 l/min was not sufficient to detach the respirable drug from the carriers. (Respitose® 45µm, Respitose® 25µm and LactoHale).

The flow properties of calcitonin dry powder preparations were indicated in Table 6.8. The residual water content determined by Karl Fischer Titration method was found to be in a range 5.1% to 5.3% due to difference in the surface area of the particles. The formulations prepared by using smaller size carrier system showing little higher water content than formulation prepared by larger size carrier system

The flow parameter angle of repose and compressibility index increases as the particle size of the carrier decreases that lead to decease in the flow of the powder. The LactoHale shows more decrease in the flow properties as compared to the other two grades (Respitose® 45 µm and 25 µm), indicating that as the particle size decreases the flow properties also decreases. Formulation with Respitose shows good flow properties compared to LactoHale. When LactoHale mixed with the Respitose then flow parameter decreases as compared to LactoHale and ultimately flow properties of the formulations improved (Table 6.8). The change in the flow properties was directly dependent on the percentage and particle size of micronized lactose with larger size Respitose.

In insulin dry powder preparation, 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. In Table 6.9 the comparison in FPF deposition studied by twin stage impinger and cascade impactor were shown. Lyophilization calcitonin

after mixing with diluent lactose increases the bulk of the drug to achieve the required dose. For higher deposition the size of carrier should be controlled and also flow has to be good enough to carry the drug particles deeper in the impactor. The lab scale sieving and attrition method of size reduction yields the required particle size for the drug deposition in the alveolar region of the lung with maximum stability. 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. The proportion of carriers and their particle size used have significant effect on deaggregation and deposition. When wide range of lactose carrier incorporated with the drug, then the carrying of the drug particles becomes deeper and the deposition in the lower stage of impactor also higher. There should be an optimum balance between the size of the carriers and their flow in the dry powder formulation for maximum deposition of the inhaled drug in the alveolar region.

The finding of this investigation suggest that insulin and calcitonin formulations containing lactose as carrier of suitable particle size in the form of lyophilized powder can be successfully prepared and able to delivered to the desired site for higher bioactivity.

6.4 COMPARISON OF INSULIN AND CALCITONIN DRY POWDER INHALER

For insulin dry powder preparation, during lyophilization the drug and enhancers in solution only freeze dried. But during calcitonin dry powder preparation lactose also used along with the drug and enhancer solution. The dose of calcitonin in compared to insulin is very less, so that lactose was used to increase the bulk of the calcitonin formulation. Both insulin and calcitonin, when a mixture of different sizes of carrier used then deposition was increased due to improvement in dispersion and deaggregation of the drug from carrier. The comparison between the FPF deposition of insulin and calcitonin DPI shown in Table 6.9. For insulin the FPF at a flow rate of 60 l/min was 55.3%, whereas for calcitonin it was only 43.5% at the same flow rate. The difference in the deposition may be due to the

inherent property of the drug, molecular weight, and physical interaction with the carrier lactose. In insulin DPI, the use of LactoHale increases FPF but in case of calcitonin DPI, it decreases the FPF. This is because, the lyophilized calcitonin powder mixture itself contains lactose of smaller particle sizes and when LactoHale incorporated, the flow property decreases and the deposition also decreases.

COMPOSITION OF INSULIN FORMULATIONS

Formulation	Composition
IF1	Lyophilized insulin + Respitose® (sieved # 45.0 μm)
IF2	Lyophilized insulin + Respitose® (sieved # 25.0 μm)
IF3	Lyophilized insulin + LactoHale®
IF4	Lyophilized insulin + 1:1 mixture of Respitose® (45.0 μm) and LactoHale®
IF5	Lyophilized insulin + 1:1 mixture of Respitose® (25.0 μm) and LactoHale®

TABLE 6.1

PARTICLE SIZE DISTRIBUTION OF INSULIN AND LACTOSE

		Size range (μm)				Median
		<6.4	6.4–10.0	10.0– 20.0	20.0-100.0	Size (μm)
Percentage by volume	Insulin	92.3	6.7	1.0	0.0	1.99
	Respitose® (sieved # 45.0 μm)	5.7	7.0	25.4	58.4	39.3
	Respitose® (sieved # 25.0 μm)	11.1	9.7	36.8	41.4	20.4
	LactoHale	43.1	28.3	24.4	4.2	7.2

TABLE 6.2

DEPOSITION OF INSULIN AND LACTOSE FROM DIFFERENT FORMULATIONS AT A FLOW RATE OF 30 l/min.

Part / Formulation	Insulin + Respitose® 45 µm (F1)		Insulin + Respitose® 25µm (F2)		Insulin + LactoHale (F3)		Insulin + Respitose® 25µm + LactoHale (F4)		Insulin + Respitose® 45µm + LactoHale (F5)	
	Drug	Lactose	Drug	Lactose	Drug	Lactose	Drug	Lactose	Drug	Lactose
Throat	32.1 ±0.8	35.3 ±1.1	29.4 ±1.4	34.1 ±1.2	18.0 ±0.9	32.9 ±1.3	16.2 ±0.8	30.4 ±1.5	15.2 ±0.7	29.4 ±1.4
Preseparator	14.1 ±0.8	22.4 ±1.2	12.5 ±0.5	22.0 ±1.1	7.4 ±0.4	19.4 ±1.0	7.1 ±0.7	16.1 ±1.4	6.4 ±0.4	14.4 ±0.8
Stage 0 (9.0-10.0 µm)	3.4 ±1.8	13.0 ±1.0	3.80 ±0.3	12.2 ±0.6	5.4 ±0.5	11.4 ±1.1	6.0 ±0.3	9.8 ±0.5	6.3 ±0.7	8.4 ±0.6
Stage 1 (5.8-9.0 µm)	4.3 ±1.0	3.4 ±0.2	5.0 ±0.8	3.9 ±0.2	10.0 ±0.9	4.4 ±0.4	10.2 ±1.1	4.8 ±0.5	10.6 ±1.3	5.4 ±0.8
Stage 2 (4.7-5.8 µm	5.9 ±0.6	4.0 ±0.8	6.8 ±0.3	4.4 ±0.3	8.4 ±1.2	4.8 ±0.4	9.2 ±0.7	5.0 ±0.6	9.4 ±0.8	5.4 ±0.2
Stage 3 (3.3-4.7 µm)	7.4 ±0.9	1.9 ±0.2	8.2 ±0.2	2.6 ±0.1	10.2 ±1.1	3.0 ±0.2	11.0 ±1.3	3.4 ±0.2	11.4 ±0.4	3.9 ±0.3
Stage 4 (2.1-3.3 µm)	7.0 ±0.9	1.7 ±0.2	8.8 ±0.3	1.8 ±0.1	9.7 ±0.3	2.0 ±0.2	10.9 ±1.4	2.2 ±0.5	12.1 ±0.9	3.1 ±0.3
Stage 5 (1.1-2.1 µm)	3.4 ±0.2	-	4.0 ±0.4	-	6.2 ±0.6	1.1 ±0.1	6.9 ±0.8	2.2 ±0.5	8.0 ±0.9	2.4 ±0.2
Stage 6 (0.7-1.1 µm)	-	-	-	-	-	-	2.2 ±0.4	1.0 ±0.1	4.8 ±0.9	2.0 ±0.2
Stage 7 (0.4-0.7 µm)	-	-	-	-	-	-	-	-	-	-
%Fine particle fraction (<5.8µm)	23.7 ±1.8	7.6 ±1.3	27.8 ±2.1	8.8 ±1.3	34.5 ±1.8	10.9 ±1.6	40.2 ±3.7	13.8 ±1.5	45.7± 2.9	16.8 ±1.4
Emitted dose	82.5 ±2.3	82.9 ±3.1	83.4 ±1.9	83.2 ±2.4	85.5 ±3.2	86.6 ±2.2	87.1 ±2.9	90.4 ±2.9	90.1± 3.5	92.1 ±1.9

TABLE 6.3

DEPOSITION OF INSULIN AND LACTOSE FROM DIFFERENT FORMULATIONS AT A FLOW RATE OF 60.0 l/min

Part / Formulation	Insulin + Respitose® 45µm (F1)		Insulin + Respitose® 25µm (F2)		Insulin + LactoHale (F3)		Insulin + Respitose® 25µm + LactoHale (F4)		Insulin + Respitose® 45µm + LactoHale (F5)	
	Drug	Lactose	Drug	Lactose	Drug	Lactose	Drug	Lactose	Drug	Lactose
Throat	30.3 ±1.5	34.1 ±1.3	27.4 ±1.7	32.4 ±1.9	15.3 ±1.1	27.4 ±1.5	12.2 ±0.9	26.5 ±0.8	10.2 ±0.6	27.7 ±1.3
Preseparator	16.4 ±1.1	23.4 ±2.2	13.2 ±1.0	23.2 ±1.4	7.2 ±0.8	17.5 ±1.2	6.9 ±0.5	15.3 ±0.9	5.3 ±0.4	12.9 ±1.0
Stage 0 (6.2-7.1 µm)	3.2 ±0.6	11.9 ±1.2	4.1 ±0.5	11.6 ±0.9	6.3 ±0.5	14.5 ±0.9	6.8 ±0.5	10.4 ±1.0	5.2 ±0.8	9.3 ±1.0
Stage 1 (4.0-6.2 µm)	6.4 ±0.2	2.3 ±0.3	4.5 ±0.5	2.1 ±0.2	5.0 ±0.8	2.3 ±0.3	5.2 ±0.8	3.0 ±0.3	7.2 ±0.6	3.0 ±0.2
Stage 2 (3.2-4.0 µm	5.4 ±0.4	2.2 ±0.3	3.5 ±0.4	2.7 ±0.5	4.2 ±0.6	2.0 ±0.2	4.9 ±0.4	2.1 ±0.2	4.8 ±0.8	3.3 ±0.2
Stage 3 (2.3-3.2 µm)	4.9 ±0.9	1.6 ±0.2	8.9 ±0.7	2.9 ±0.2	12.4 ±0.8	3.6 ±0.5	12.6 ±0.9	4.9 ±0.6	13.6 ±1.0	4.4 ±0.4
Stage 4 (1.4-2.3 µm)	4.2 ±0.3	1.9 ±0.2	9.4 ±0.9	2.8 ±0.2	11.9 ±1.0	2.7 ±0.5	12.8 ±1.1	2.4 ±0.3	14.3 ±1.2	3.9 ±0.8
Stage 5 (0.7-1.4 µm)	3.4 ±0.3	-	4.2 ±0.3	-	6.2 ±0.4	1.7 ±0.2	7.9 ±0.6	2.5 ±0.3	9.9 ±0.8	2.9 ±0.2
Stage 6 (0.5-0.8 µm)	-	-	-	-	1.1 ±0.1	-	1.9 ±0.2	1.3 ±0.2	3.8 ±0.5	2.8 ±0.3
Stage 7 (0.3-0.5 µm)	-	-	-	-	-	-	1.5 ±0.2	-	2.1 ±0.3	-
%Fine particle fraction (<6.2µm)	24.3 ±1.1	8.0 ±0.3	30.5 ±2.8	10.5 ±0.4	40.8 ±4.5	12.3 ±0.7	46.8 ±4.6	16.2 ±1.2	**55.7** ±4.8	20.3 ±0.6
Emitted dose	82.9 ±3.3	83.4 ±2.7	85.1 ±2.9	85.7 ±3.4	87.7 ±2.5	86.9 ±3.2	89.2 ±3.1	92.1 ±1.9	90.4 ±2.1	93.3 ±1.6

TABLE 6.4

FLOW PROPERTIES OF INSULIN WITH CARRIER

Formulation	Tap density	Angle of repose	Carr's compressibility index	Residual water content (%)
Insulin in Respitose® 45 µm	0.83±0.08	18.7±1.2	9.9±0.8	4.9±0.8
Insulin in Respitose® 25 µm	0.90±0.11	20.0±1.4	12.3±0.9	5.0±1.1
Insulin in LactoHale	0.97±0.12	21.9±1.4	17.5±1.0	5.2±0.7
Insulin in mixture of Respitose® 25µm + LactoHale	0.94±0.09	24.1±1.6	14.9±0.7	5.1±1.1
Insulin in mixture of Respitose® 45µm + LactoHale	0.89±0.07	20.7±1.0	13.4±0.8	5.0±1.0

TABLE 6.5

COMPOSITION OF CALCITONIN FORMULATIONS

Formulation	Composition
CF1	Lyophilized mixture of calcitonin and lactose + Respitose® (sieved # 45.0 μm)
CF2	Lyophilized mixture of calcitonin and lactose (sieved # 25.0 μm)
CF3	Lyophilized mixture of calcitonin and lactose + LactoHale®
CF4	Lyophilized mixture of calcitonin and lactose + 1:1 mixture of Respitose® (45.0 μm) and LactoHale®
CF5	Lyophilized mixture of calcitonin and lactose + 1:1 mixture of Respitose® (25.0 μm) and LactoHale®

TABLE 6.6

PARTICLE SIZE DISTRIBUTION OF CALCITONIN POWDER MIXTURE AND LACTOSE

		Size range (μm)				Median
		<6.4	6.4–10.0	10.0– 20.0	20.0-100.0	Size (μm)
Percentage by volume	Calcitonin powder mixture	89.9	6.3	2.7	0.0	2.1
	Respitose® (sieved # 45.0 μm)	5.5	6.9	26.3	59.3	40.9
	Respitose® (sieved # 25.0 μm)	10.8	9.1	37.9	42.1	21.7
	LactoHale	43.2	28.5	24.1	4.0	7.2

TABLE 6.7

FLOW PROPERTIES OF CALCITONIN WITH CARRIER

Formulation	Tap density	Angle of repose	Carr's Compressibi-lity index	Residual water content (%)
Lyophilized mixture of calcitonin and lactose + Respitose® (sieved # 45.0 µm)	0.88±0.08	19.4±1.0	10.3±1.0	5.0±0.9
Lyophilized mixture of calcitonin and lactose + Respitose® (sieved # 25.0 µm)	0.94±0.12	21.1±1.2	12.7±0.6	5.2±0.4
Lyophilized mixture of calcitonin and lactose + LactoHale®	0.99±0.09	23.4±0.7	18.6±0.5	5.3±1.1
Lyophilized mixture of calcitonin and lactose + 1:1 mixture of Respitose® (45.0 µm) and LactoHale®	0.97±0.10	22.1±1.2	14.6±0.9	5.1±0.7
Lyophilized mixture of calcitonin and lactose + 1:1 mixture of Respitose® (25.0 µm) and LactoHale®	0.92±0.07	20.9±0.8	13.1±1.1	5.1±0.8

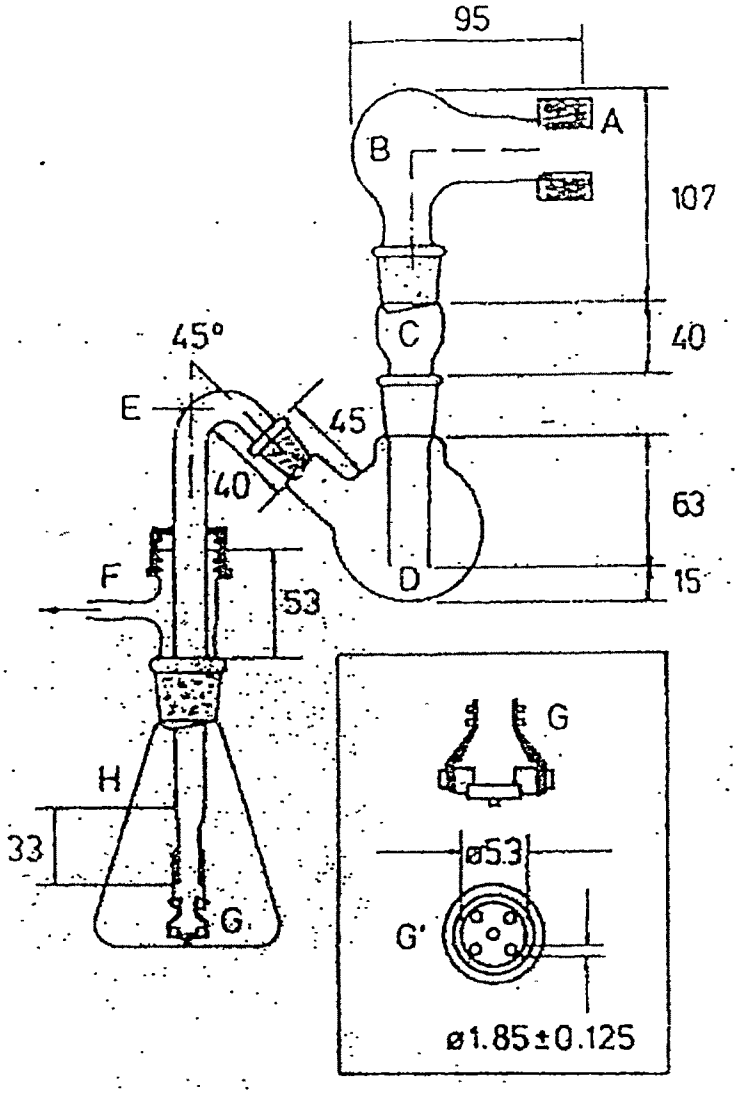
TABLE 6.8

COMPARISON OF INSULIN AND CALCITONIN DPI

	Formulation and composition	Deposition at different flow rates (%)				
		Twin Stage Impinger (Flow rate in l/min)			Cascade Impactor (Flow rate in l/min)	
		30	60	90	30	60
Insulin DPI	Insulin + Respitose® 45µm	24.9	25.4	25.6	23.7	24.3
	Insulin+Respitose®25µm	28.9	30.6	32.9	27.8	30.5
	Insulin + LactoHale	35.1	40.3	42.3	34.5	40.8
	Insulin + Respitose® 25µm + LactoHale	40.3	46.9	49.1	40.2	46.8
	Insulin + Respitose® 45µm + LactoHale	45.1	55.3	59.2	45.7	55.7
Calcitonin DPI	Calcitonin and lactose + Respitose® 45µm	27.8	34.1	39.6	-	-
	Calcitonin and lactose + Respitose® 25µm	29.9	36.5	44.5	-	-
	Calcitonin and lactose + Lactohale	24.7	29.6	35.3	-	-
	Calcitonin and lactose + Respitose® (25.0 µm) and LactoHale®	35.4	41.2	46.5	-	-
	Calcitonin and lactose + Respitose® (45.0 µm) and LactoHale®	38.3	43.5	49.2	-	-

TABLE 6.9

TWIN STAGE GLASS IMPINGER



A:MOUTH ADAPTER B:THROAT
C:NECK D:UPPER IMPINGEMENT CHAMBER
E:COUPLING TUBE F:SIDE ARM ADAPTER
G:LOWER JET ASSEMBLY
H:LOWER IMPINGEMENT CHAMBER

FIGURE 6.1

DEPOSITION OF INSULIN ON LOWER STAGE OF TWIN STAGE IMPINGER WITH DIFFERENT CARRIERS AT VARIOUS FLOW RATES

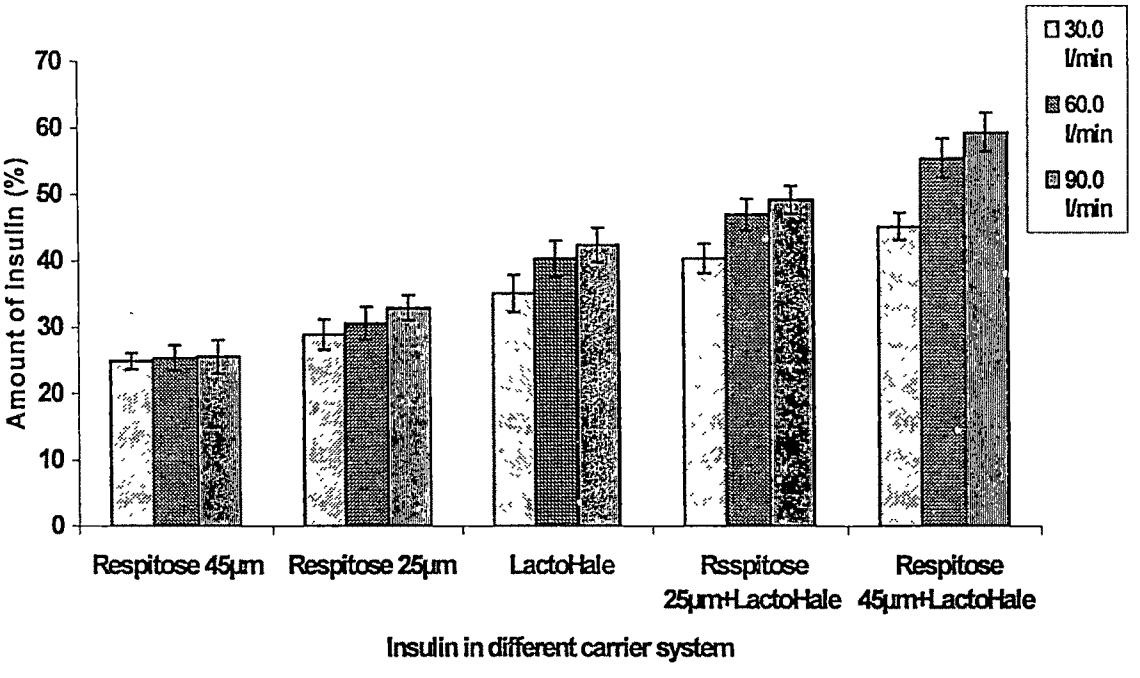


FIGURE 6.2

DEPOSITION OF LACTOSE ON LOWER STAGE OF TWIN STAGE IMPINGER WITH DIFFERENT CARRIERS AT VARIOUS FLOW-RATES

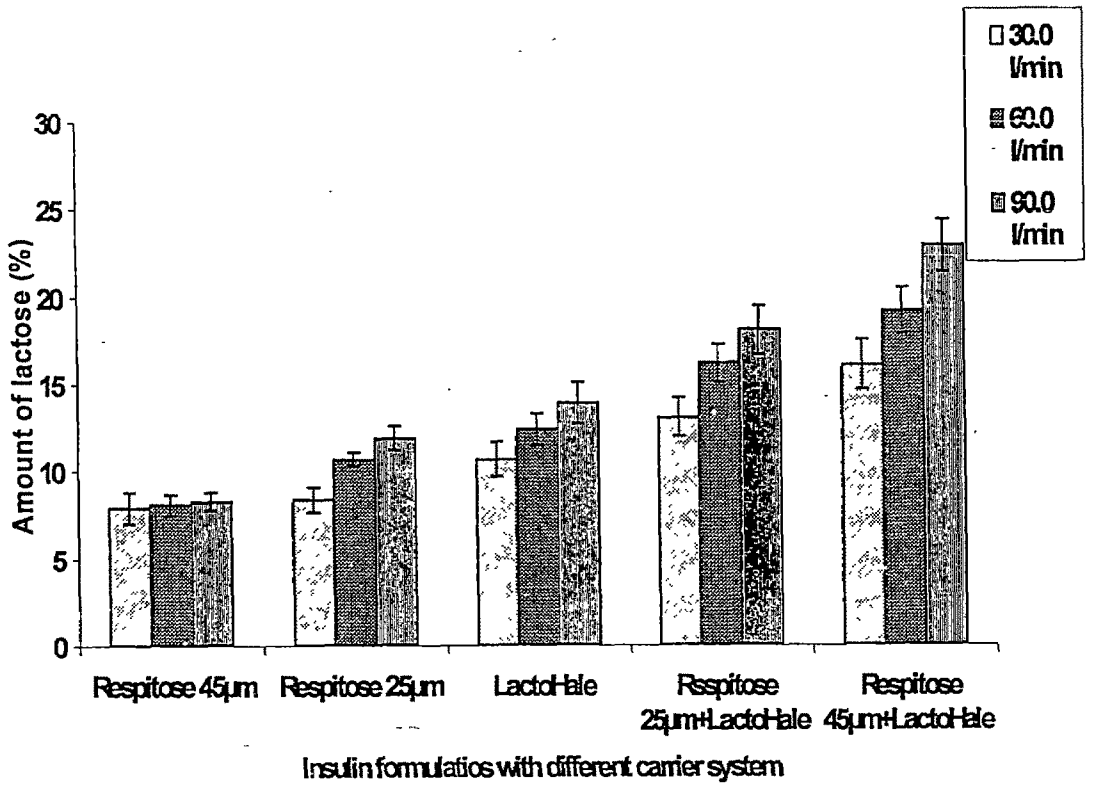


FIGURE 6.3

DEPOSITION OF CALCITONIN ON LOWER STAGE OF TWIN STAGE IMPINGER WITH DIFFERENT CARRIERS AT VARIOUS FLOW RATES

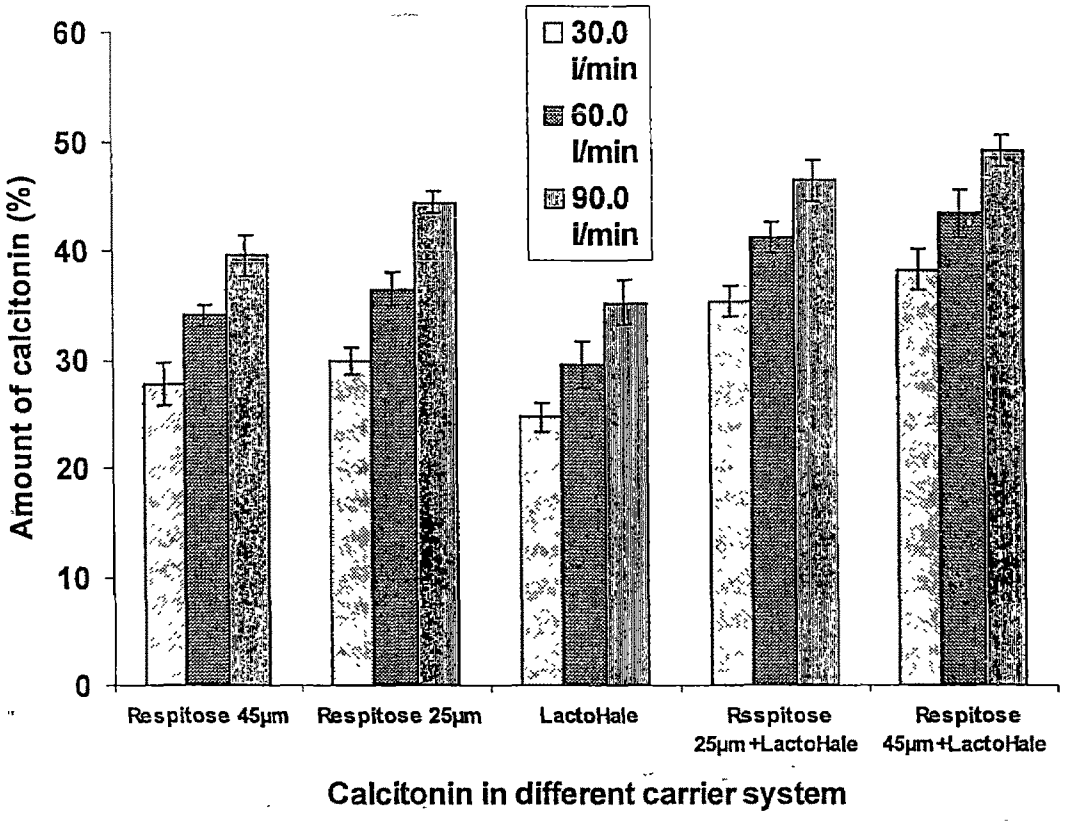


FIGURE 6.4

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