JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 927–934

Received January 31, 2007
Accepted March 28, 2007
DOI 10.1211/jpp.59.7.0003
ISSN 0022-3573

Physical characteristics and aerosolization performance of insulin dry powders for inhalation prepared by a spray drying method

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Abstract

The objective of this study was to investigate the influence of formulation excipients on the physical characteristics and aerosolization performance of insulin dry powders for inhalation. Insulin dry powders were prepared by a spray drying technique using excipients such as sugars (trehalose, lactose and dextran), mannitol and amino acids (L-leucine, glycine and threonine). High performance liquid chromatography and the mouse blood glucose method were used for determination of the insulin content. The powder properties were determined and compared by scanning electron microscopy, thermo-gravimetric analysis and size distribution analysis by a time-of-flight technique. The in-vitro aerosolization behaviour of the powders was assessed with an Aerolizer inhaler using a twin-stage impinger. Powder yield and moisture absorption were also determined. Results showed that there was no noticeable change in insulin content in any of the formulations by both assay methods. All powders were highly wrinkled, with median aerodynamic diameters of 2–4 μm, and consequently suitable for pulmonary administration. The tapped density was reduced dramatically when glycine was added. The powders containing mannitol, with or without L-leucine, were less sensitive to moisture. The highest respirable fraction of $67.3 \pm 1.3\%$ was obtained with the formulation containing L-leucine, in contrast to formulations containing glycine and threonine, which had a respirable fraction of $11.2 \pm 3.9\%$ and $23.5 \pm 2.5\%$, respectively. In addition, powders with good physical properties were achieved by the combination of insulin and trehalose. This study suggests that L-leucine could be used to enhance the aerosolization behaviour of the insulin dry powders for inhalation, and trehalose could potentially be used as an excipient in the formulations.

Introduction

As an administration route, pulmonary delivery of drugs is a promising alternative to parenteral injection and oral administration, especially for proteins and peptides (Irngartinger et al 2004). Pulmonary delivery also avoids the discomfort and poor compliance associated with injection. Certain drugs such as insulin are readily absorbed through the alveolar tract into blood circulation as soon as they reach the lung (Rave et al 2005).

Therapeutic proteins and peptides can be spray-dried from their solutions to give powders with an aerodynamic diameter of approximately 1–5 μm (Timsina et al 1994) or less (Leach 1996), which is the ideal particle size to reach deep into the lung following inhalation. In addition, it has been proved that the fine particle dose, defined as the dose of drug in particles with a diameter of <5 μm, is linearly correlated with the degree of lung deposition for a range of dry-powder inhalers (Olsson et al 1996). However, their activities can be altered during the process because of the high temperature needed for evaporation of the solvent, or because of exposure to the liquid–air interface (Tzannis & Prestrelski 1999). Usually, such dispersible pharmaceutical-based dry powders can be combined with pharmaceutical excipients that are suitable for pulmonary administration and generally recognized as safe. The excipients may serve simply as bulking agents for reducing the concentration of drug in the powder, or serve to enhance the stability of the formulations, especially in the case of proteins and peptides. It has been found that excipients such as sugars and polyol can protect macromolecules during spray drying and during subsequent storage (Labrude et al 1989; Broadhead et al 1994). Two stabilizing mechanisms are thought to explain this:

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one is water replacement and the other is glassy immobilization (Arakawa et al 1993). Some excipients such as amino acids can improve the aerosolization properties of dry powders used for inhalation (Li et al 2003; Najafabadi et al 2004; Chew et al 2005; Li et al 2005; Rabbani & Seville 2005). Some reports have also described the application of excipients (e.g. mannitol, leucine, trehalose, lactose) in the preparation of protein and peptide dry powders for inhalation (Costantion et al 1998; Chan et al 2004; Jiang et al 2005). Pharmaceutical excipients also have an effect on the physical properties of powders, including their morphology, particle size and distribution, moisture absorption, density and electrostatic charge. These properties can directly influence the degree of pulmonary deposition of drug. So, for optimal, functional and therapeutic performance of dry powders for inhalation, the selection of appropriate excipients and the control of formulation properties are of critical importance.

The effect of spray drying on the degradation and physical properties of insulin with mannitol has been investigated (Stahl et al 2002). Exubera, recently approved by the Food and Drug Administration to control blood glucose, is manufactured by spray drying with mannitol and glycine as inactive ingredients. The results showed that the spray drying method for preparing insulin powders for inhalation was viable, with negligible degradation. Particle density and size need to be considered to obtain an optimized formulation capable of deep lung deposition after pulmonary delivery.

So far there have been few reports concerning the effects of diluents and excipients on the properties of inhalation dry powders or solid peptide formulations, especially as far as insulin is concerned. When formulated with an appropriate composition to produce adequate physical characteristics, the powders exhibited excellent aerosolization properties. The present investigation focused on the physical characteristics and aerosolization performance of insulin dry powders for inhalation with different excipients. Powders were prepared by spray drying using excipients that are approved by the Food and Drug Administration for inhalation, such as lactose and mannitol (Sham et al 2004), materials such as trehalose and dextran that appear suitable for inhalation dry powders (Patton et al 1995; Bosquillon et al 2001), and amino acids that may improve the dispersibility of the powders, such as L-leucine, glycine and threonine. The physical characteristics of the powders (i.e. particle size, tapped density, morphology, moisture content and moisture absorption) were determined. In-vitro aerosolization properties were assessed with an Aerolizer inhaler device in a twin-stage impinger (TSI).

Materials and Methods

Materials

Porcine insulin (28.0 U mg^{-1}; Lot no 0512A04) was purchased from Jiangshu Wanbang Bio-pharm Co., Ltd., China. Trehalose was a product of Nanning Zhongnuo Bioengineering Ltd., China, Lactose and mannitol were supplied by Tianjin Chemical Reagent Co., China, L-leucine, glycine and threonine were supplied by Shanghai Kangda Amino acids Factory, China. Dextran was supplied by Sinopharm Chemical Reagent Co., Ltd., China. Citric acid was obtained from Shenyang Zhengxin High-technology Institute, China. All other reagents used were of analytical grade.

Formulation and preparation of the dry powders

Insulin was dissolved in an aqueous solution that was adjusted to pH 3.0 with citric acid, and then the excipients given in Table 1 were added. The pH of the aqueous solution was then adjusted to 6.7 ± 0.3 with a 0.1 mol L^{-1} NaOH solution. The solution was then passed through a 0.22-μm filter and spray dried using an SD-1000 spray-drier (EYELA, Japan). It was operated at an inlet temperature of 110°C, a liquid feed rate of 5.4 mL min^{-1}, a drying air flow rate of 0.65 m^3 min^{-1}, and an atomizing air pressure of 110 kPa, resulting in an outlet temperature of 71–75°C. Once the aqueous mixture was consumed, the outlet temperature was maintained at <75°C for about 10 min by slowly reducing the inlet temperature to provide secondary drying. The insulin dry powder was collected from the collection vessel and under the lid. All the samples were stored at 4°C in tightly closed glass containers. The yield was calculated as the weight of powder obtained (including moisture) after spray drying to the total solid content in the liquid feed.

Assay of insulin

Two assay methods were used to evaluate the insulin content using the raw insulin powder as the standard (28.0 U mg^{-1}). One was the high performance liquid chromatography (HPLC) method according to the European Pharmacopoeia (2005) and the other was the mouse blood glucose method according to the Pharmacopoeia of the People's Republic of China (2005). The insulin content of the powders was expressed as a percentage of the nominal dose.

The HPLC system comprised an L-7100 pump, an L-7400 UV detector and an L-7200 autosampler (Hitachi Company, Japan), with a HiQ sil C18W column (250×4.6 mm, 5 μm; Kya Tech Co, Japan). Mobile phase A consisted of 0.2 mol L^{-1} anhydrous sodium sulfate and 0.27% (v/v) phosphoric acid, adjusted to pH 2.3 with ethanolamine. Mobile phase B consisted of acetonitrile/mobile phase A (45:55, v/v). Mobile phase was composed of 38 vols of mobile phase A and 62 vols of mobile phase B. The column temperature was maintained at 40°C in an oven (AT-130, JZH; Tianjing Xinzhou Technology Ltd., China). A detection wavelength of

Table 1 Formulation of insulin dry powders

Formulation	Insulin (%)	Sugar (%)	Amino acid (%)	Sample solution (mL)
Insulin/trehalose	0.3	1.0	0	100
Insulin/lactose	0.3	1.0	0	100
Insulin/dextran	0.3	1.0	0	100
Insulin/mannitol	0.3	1.0	0	100
Insulin/mannitol/L-leucine	0.3	1.0	0.3	100
Insulin/mannitol/glycine	0.3	1.0	0.3	100
Insulin/mannitol/threonine	0.3	1.0	0.3	100

214 nm was chosen, and a 20-μL injection volume was used. Solutions were prepared by dissolving an accurately weighed sample in 0.01 mol L^{-1} HCl to obtain a solution with a known concentration of approximately 1 mg mL^{-1}.

Male mice (Shenyang Pharmaceutical University, China), 18–22 g, were used to determine the biopotency of the insulin powders as described in the Chinese Pharmacopoeia (2005). The samples were dissolved in 0.9% NaCl (pH 2.5) and diluted to the appropriate concentrations (25 mU mL^{-1} and 50 mU mL^{-1}, respectively). Each mouse received a subcutaneous injection of 0.2 mL insulin solution. At 40 min after injection, blood samples were withdrawn from the fossa orbitalis. About 100 μL of blood was collected in heparinized microcentrifuge tubes, and centrifuged at 4400 $\times g$ for 15 min to separate the plasma. The blood glucose level was measured using a glucose assay kit (Baoding Great Wall Co., China).

Powder characterization

Scanning electron microscopy

The morphology of the powders was examined with an SSX-550 scanning electron microscope (Shimadzu, Japan) at 15 kV after coating with gold using a JFC-1200 Fine Coater (Jeol, Japan) with a current of 20 mA for 200 s.

Density and particle size

The powder density evaluated by the tapped density was measured in a PT-R powder characteristics tester (Hosokawa Micron Corp, Japan).

The particle size distribution of the sample powders was determined using a PSD 3603 analyser (TSI inc., USA) using an aerodynamic time-of-flight technique. Two types of diameters can be calculated. The geometric diameter is the equivalent spherical diameter of a particle with the same density as the measured particles; the aerodynamic diameter is the equivalent spherical diameter of a particle with the same unit density as the measured particle, which is processed by computer in order to convert time-of-flight data into a particle size. The latter is more suitable for evaluating the aerosolization behaviour of powders. The main parameters, D_{10}, D_{50} (median diameter) and D_{90}, represent the aerodynamic diameter at a particle size distribution of 10, 50 and 90%, respectively. The span of the volume distribution that can be used to evaluate the width of the volume distribution corresponding to the median diameter (D_{50}) was calculated by the equation $(D_{90} - D_{10})/D_{50}$. A large span is indicative of a more heterogeneous size distribution.

Moisture content

The moisture content of the powder was measured by thermogravimetric analysis using a Shimadzu TGA-50 apparatus (Japan). Approximately 10 mg of the powders weighed by thermo-gravimetric analysis were crimped in an aluminium pan and heated from 25°C to 105°C at a rate of 10°C min^{-1} with a nitrogen gas purge. The sample was kept at 105°C for 15 min. The percentage of the initial weight that was lost during the heating process from 25°C to 105°C was ascribed to the moisture content of the powder.

Moisture absorption

An experiment was conducted at varying relative humidity (RH; between 45 and 85%) for 24 h at 25°C in the climatic cabinet (MMM Medcenter Einrichtungen GmbH, Germany). After 24 h, the samples that had been spread uniformly in open Petri dishes and weighed in advance were taken out and weighed again. The moisture absorption profile was expressed as a percentage by the increasing weight at different RH.

In-vitro aerosol deposition

The pulmonary deposition of the dry powders in-vitro was investigated using a TSI. As the collection fluid, 7 mL of 0.01 mol L^{-1} HCl was used in stage 1 and 30 mL in stage 2. A hard gelatin capsule (size 3) was filled with the powders, containing approximately 3 ± 0.3 mg insulin, and transferred to an Aerolizer dry powder inhaler (Schering, Co., Switzerland) connected to the TSI. When the capsule was pierced, the air pump was turned on to make the powder pass through the TSI at 60 L min^{-1} for a period of 10 s. This procedure was repeated until 10 actuations had been conducted. The powders deposited at each stage were washed out with 0.01 mol L^{-1} HCl and recovered. The powders deposited in the inhaler and capsules were also collected. After being diluted to a suitable concentration, each insulin solution was assayed by HPLC. The respirable fraction of the insulin powders is defined as the powder mass recovered in stage 2. The results are expressed as the percentage of drug dose transferred to the capsules (loaded dose).

Statistical analysis

Statistical analysis of the comparison of the yield, tapped density, moisture content, sorbed moisture weight at each relative humidity and fraction at each stage of the samples was performed using the Kruskal–Wallis test. In all cases, post-hoc comparisons of the individual differences between the various formulations were performed using Nemenyi's test after the Kruskal–Wallis test. Results of the HPLC method and the mouse blood glucose method were analysed using the Student's *t*-test. A value of $P < 0.05$ was accepted as statistically significant in all cases. All values were expressed as mean ± s.d. and every independent experiment was performed in triplicate.

Results and Discussion

Preparation of insulin dry powders

Seven types of insulin dry powders with different excipients were prepared by a spray drying method. The yields, ranging from 13 to 55% (Table 2) were not very high; in the literature the yields usually range from 20 to 50% (Maa et al 1998a; Stahl et al 2002). The insulin/mannitol/L-leucine and insulin/mannitol/glycine formulations gave the highest yield (54.6 ± 1.6% and 53.6 ± 2.1%, respectively). The yield (13.0 ± 1.9%) was significantly affected by the addition of lactose ($P < 0.05$) as compared with other excipients, since many particles adhering to the inside wall of the spray dryer

Table 2 Yield and insulin content of dry powders

Formulation	Yield (%)	Content (%) (by the HPLC method)	Content (%) (by the mouse blood glucose method)
Insulin/trehalose	42.5 ± 2.0	95.1 ± 0.2	97.3 ± 0.6
Insulin/lactose	13.0 ± 2.1	96.0 ± 0.3	96.6 ± 0.8
Insulin/dextran	46.0 ± 2.4	96.6 ± 0.1	94.3 ± 0.9
Insulin/mannitol	44.9 ± 1.5	96.3 ± 0.2	97.1 ± 0.9
Insulin/mannitol/L-leucine	54.6 ± 1.0	96.9 ± 0.2	95.2 ± 0.6
Insulin/mannitol/glycine	53.6 ± 1.8	94.1 ± 0.1	96.5 ± 1.0
Insulin/mannitol/threonine	46.1 ± 1.7	95.2 ± 0.2	94.0 ± 0.8

during the drying process were not recovered. The increased spray drying yields seen with the addition of amino acids suggest some changes in the powders, such as particle size, morphology and cohesive force of the particles, which might contribute to the aerosolization behaviour of the powders. The insulin content is given in Table 2. The HPLC assay gave an estimate of the insulin content that was in good agreement with that obtained by the mouse blood glucose method ($P > 0.1$), proving that insulin was stable in all the formulations. This suggests that the excipients have no influence on the insulin during the spray drying process and they might even be able to provide protection.

Physical characteristics

Morphology of the powders

The scanning electron micrographs of the spray dried powders are shown in Figure 1A–G. The surfaces of all the particles were highly wrinkled and irregular. The wrinkled structure prevents particles from adhering tightly to each other, which means that the particles have less chance to aggregate and so less energy is required to disperse them. It can be predicted that these powders will have good dispersing and aerosolization properties. Bosquillon et al (2001) have reported that the type of sugars incorporated did not affect the powder morphology. This was also the case in this study in that alteration of the type of excipients caused no substantial change in particle shape. So, it can be concluded that the main factors influencing the morphology may be the insulin itself or the preparation conditions. According to Maury (2005), one possible reason for the wrinkled surface might be attributed to the insulin molecules present at the water/air interface of the atomized liquid feed, as a result of which the ratio of viscous to surface forces there increases, thereby preventing smoothing of the Marangoni fluctuations. Since wrinkled particles contain a high void space, the particles become lighter, as confirmed by density measurements.

Density and particle size

Particle size and density play important roles in determining how much of a drug is delivered to the lungs and how fast it is absorbed. This is why these factors have attracted so much attention. The tapped density and particle size distribution analysis of the spray dried powders are shown in Table 3.

The tapped density of the powders ranged from 0.11 to 0.22 $g cm^{-3}$. Compared with three sugars, mannitol produced a lower density and smaller particle size. The insulin/lactose powders exhibited the highest tapped density value of 0.221 $g cm^{-3}$, while the powders incorporated with glycine produced the lowest tapped density of 0.112 $g cm^{-3}$, which was significantly lower ($P < 0.05$) than others. This indicates that glycine is a good low-density additive. It was interesting that the reduction in tapped density gave rise to the yields of the powders shown in Figure 2. The lighter particles may be more easily captured through centrifugal force in the cyclone.

Due to the low tapped density, the aerodynamic diameters (D_{50}) of all powders were much lower than their physical size, which ranged from 2.0 to 4.0 μm, indicating that the powders are suitable for deposition in the deep lung. Meanwhile, the larger geometric diameter of the powders may increase bioavailability by reducing phagocytosis in the lung (Rudt & Müller 1992). The geometric volume median diameter of all powders, in decreasing order, was shown to be insulin/mannitol/threonine > insulin/mannitol/glycine > insulin/trehalose > insulin/lactose > insulin/dextran > insulin/mannitol > insulin/mannitol/L-leucine, while the order of the median aerodynamic diameter was insulin/mannitol/threonine > insulin/lactose > insulin/trehalose > insulin/mannitol/glycine > insulin/dextran > insulin/mannitol > insulin/mannitol/L-leucine. The difference was because of the difference in the tapped density. The particle diameters seen from the scanning electron micrographs appear to be in good agreement with the results of the particle size determination, with the exception of insulin/mannitol/glycine and insulin/mannitol/threonine (geometrical volume median diameters were 8.10 and 8.99 μm, respectively). This suggests that the larger size obtained in these two formulations was owing to cohesive agglomerates that failed to de-aggregate even under high shear conditions during the size determination. Table 3 showed that the presence of L-leucine seemed to reduce the median aerodynamic diameter, but not very remarkably. As far as the particle size distribution of the insulin/dextran, insulin/mannitol and insulin/mannitol/L-leucine powders was concerned, 90% of the particulates exhibited an aerodynamic diameter less than 5.0 μm, and 50% less than 3.0 μm. This suggests that these powders are more suitable for inhalation than other powders.

Moisture content and moisture adsorption profiles

The moisture content of the various powders was found to be over the range 3.5–12.2% (Figure 3). These results show that quite low moisture content can be obtained with formulations containing mannitol. The moisture content is often thought to be responsible for the physicochemical instability of protein and peptide dry powders. Previous studies have demonstrated that the moisture content of insulin spray dried powders has an effect on the glass transition temperature (T_g) and stability of the powders. Water acts as a plasticizer and lowers the T_g of amorphous solids. Increasing the moisture content of the formulation reduces the T_g and increases the degradation rate of insulin (White et al 2005). In general, dry powder proteins with a lower moisture content are more stable (Forbes et al 1998; Maa et al 1998b). At a lower moisture content level, the Maillard reaction takes place more slowly because of the

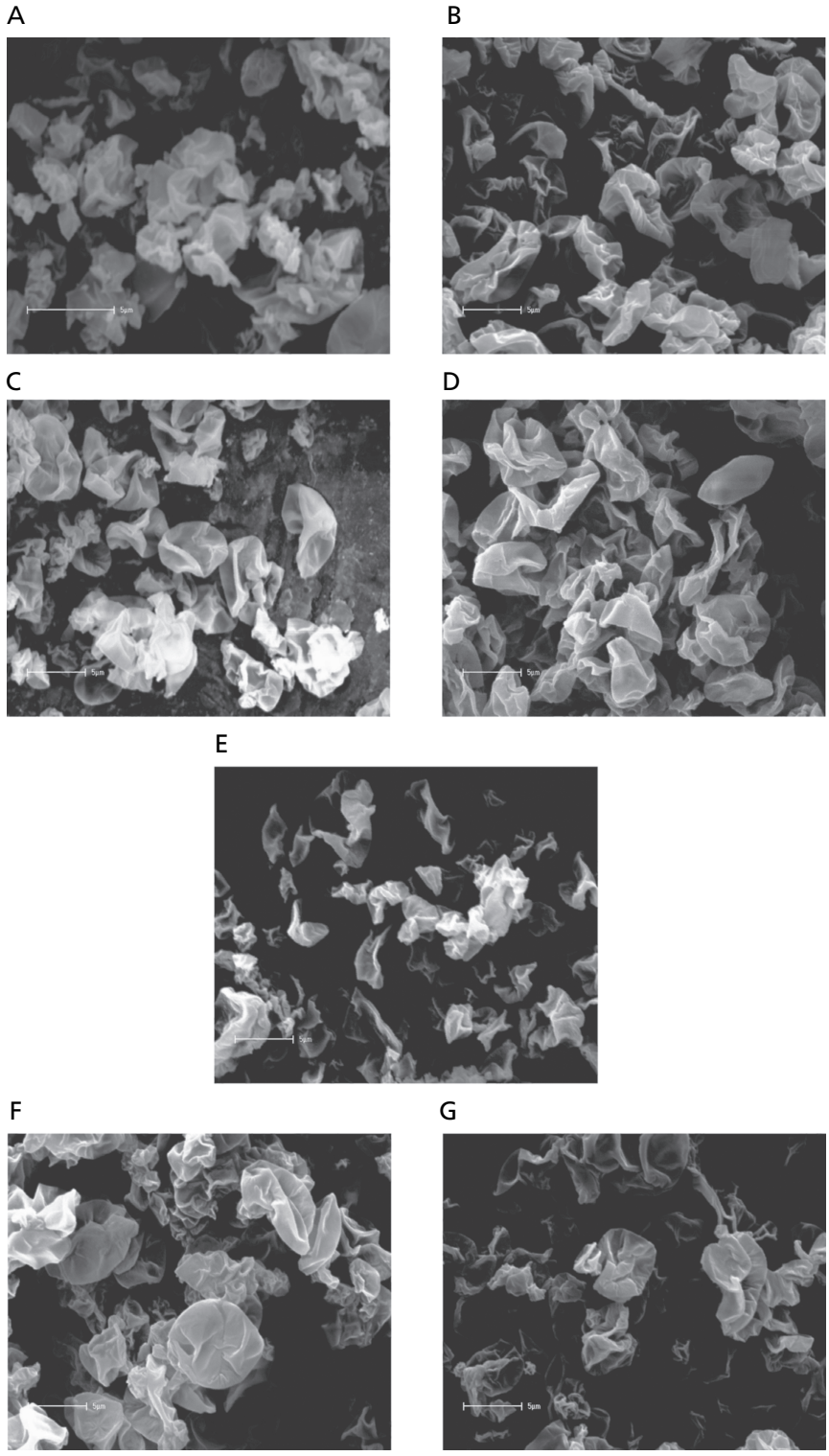

Figure 1 Scanning electron micrographs of spray dried powders containing insulin and excipients: A. insulin/trehalose; B. insulin/lactose; C. insulin/dextran; D. insulin/mannitol; E. insulin/mannitol/L-leucine; F. insulin/mannitol/glycine; G. insulin/mannitol/threonine.

restriction of diffusion and mobility of the reactants. As the moisture content increases, molecular mobility increases, leading to an enhanced reactant mobility, which should facilitate the reaction (Todo et al 2003). Therefore, reducing the moisture content is a very critical step in the selection of a formulation and preparation process.

Table 3 Tapped density and particle size and distribution of the powders

Formulation	Tapped density ($g\ cm^{-3}$)	d[a] (μm)	Aerodynamic diameter			Span
			D_{10} (μm)	D_{50}[b] (μm)	D_{90} (μm)	
Insulin/trehalose	0.150 ± 0.002	7.22 ± 0.36	1.97 ± 0.31	3.14 ± 0.35	5.03 ± 0.53	0.97
Insulin/lactose	0.221 ± 0.002	7.10 ± 0.39	2.60 ± 0.23	3.74 ± 0.39	5.74 ± 0.37	0.84
Insulin/dextran	0.161 ± 0.003	6.19 ± 0.63	1.82 ± 0.17	2.78 ± 0.64	4.25 ± 0.69	0.87
Insulin/mannitol	0.139 ± 0.004	5.96 ± 0.18	2.13 ± 0.14	2.50 ± 0.18	4.86 ± 0.26	1.09
Insulin/mannitol/L-leucine	0.141 ± 0.003	5.35 ± 0.36	1.77 ± 0.31	2.23 ± 0.36	4.68 ± 0.17	1.30
Insulin/mannitol/glycine	0.112 ± 0.001	8.10 ± 0.70	1.95 ± 0.29	3.02 ± 0.70	5.07 ± 0.64	1.03
Insulin/mannitol/threonine	0.141 ± 0.002	8.99 ± 0.90	2.53 ± 0.31	3.77 ± 0.89	5.81 ± 0.95	0.87

[a]Geometrical volume median diameter; [b]median aerodynamic diameter.

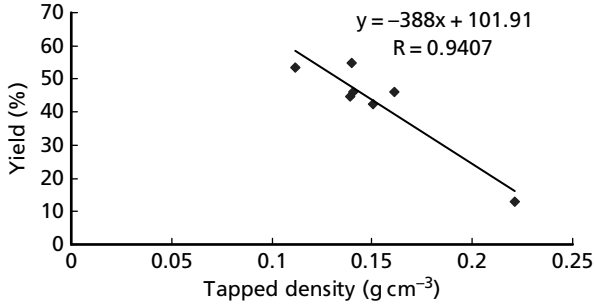

Figure 2 Correlation between the yield and tapped density.

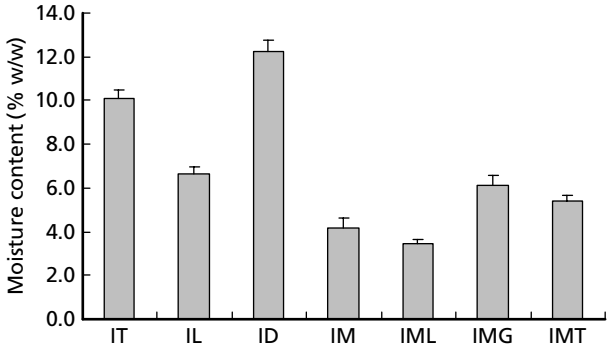

Figure 3 Moisture content of powders prepared by spray drying. IT, insulin/trehalose; IL, insulin/lactose; ID, insulin/dextran; IM, insulin/mannitol; IML, insulin/mannitol/L-leucine; IMG, insulin/mannitol/glycine; IMT, insulin/mannitol/threonine.

The corresponding moisture absorption profiles for the dry powders at 45, 55, 65, 75 and 85% RH are presented in Figure 4. The lactose sample was found to adsorb a significant amount of water at RH levels of 45, 55 and 65% (weight change about 9.5, 11.5 and 14.2%, respectively). While the RH increased to about 75%, the trehalose formulation showed the highest water affinity (21.8%). In contrast, the lowest water binding capacity of the seven samples was obtained for insulin spray dried with mannitol, the weight change of which

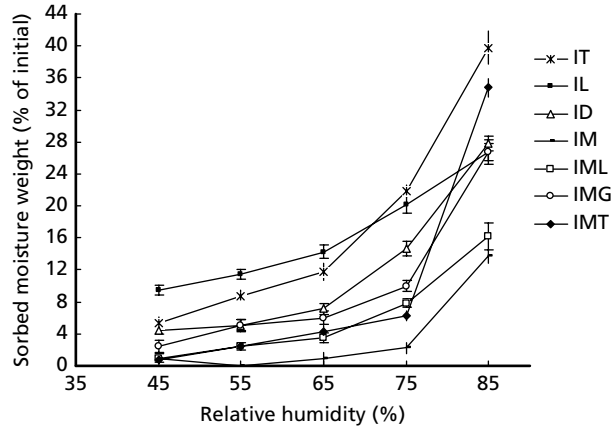

Figure 4 Moisture adsorption profile of insulin dry powders at 45–85% relative humidity at 25°C. IT, insulin/trehalose; IL, insulin/lactose; ID, insulin/dextran; IM, insulin/mannitol; IML, insulin/mannitol/L-leucine; IMG, insulin/mannitol/glycine; IMT, insulin/mannitol/threonine.

was negligible as the RH increased to 75%. It is clear that, as the RH increases to 85%, there is a marked increase in the weight change of all formulations. The presence of amino acids increases the water absorption properties of the powders compared with the formulation with mannitol alone. In these formulations, spray drying insulin with L-leucine produced a powder with a substantially lower moisture absorption uptake compared with those formulated with glycine and threonine. When the RH increased from 75% to 85%, the powders with threonine exhibited the greatest weight increase, from 6.3% to 27.8%, compared with other formulations ($P < 0.05$), except the powders containing trehalose.

Moisture will increase powder agglomerates via interparticulate capillary forces, which will reduce the dispersibility of the dry powder. That is why the characteristics of the excipients should be considered in the design of formulations. It can be concluded that mannitol has good anti-hygroscopic properties for insulin dry powders for inhalation, because powders with mannitol are less sensitive to moisture at high RH. It is also suggested that good packaging is necessary to protect insulin dry powders from moisture absorption.

In-vitro aerosol deposition

TSI has been widely used for evaluating the lung deposition of inhalation dry powders in-vitro (Braun et al 1996; Rabbani & Seville 2005; Young et al 2005). Particles with an aerodynamic diameter lower than the cut-off diameter of 6.4 μm are likely to reach the lower stage (Hallworth & Westmoreland 1987). The particles that can reach stage 2 are expected to be deposited in the central regions of the lung after inhalation. The respirable fraction was selected to evaluate the aerosolization performance of the powders. In Figure 5 the deposition values of different insulin powders in the TSI are shown, expressed as a percentage of the total dose loaded into the capsules. The amount of insulin deposited on two stages of the TSI varied for different powders.

Figure 5 shows that almost all samples had a high delivery efficiency of insulin, with a respirable fraction in excess of 50% from the TSI, with the exception of the powders generated from insulin/mannitol/glycine and insulin/mannitol/threonine. The respirable fraction was further reduced from 56.2% (insulin/mannitol formulation) to 11.2% and 23.5%, respectively, for these two formulations. Some large agglomerates of insulin/mannitol/glycine (58.8%) and insulin/mannitol/threonine (45.5%) were found remaining in capsules following aerosolization. This agglomeration was probably attributed to their sensitivity to moisture and the electrostatic attraction between particles, which resulted in poor dispersibility to entrain into the air stream in the TSI. The effect on aerosolization, however, was found to be strongly dependent on the type of amino acids used. It is interesting to note that the presence of L-leucine enhances the aerosolization performance of the powders, giving a respirable fraction as high as 67.3% of the total dose in the TSI. The respirable fraction was significantly ($P < 0.05$) increased by the addition of L-leucine compared with that produced by mannitol alone, demonstrating an enhanced dispersibility and dose emission of the insulin dry powders. The reason why L-leucine can effectively increase the aerosolization performance of the spray dried powders may be attributed to its surfactant-like properties (Gliniski et al 2000), which allow L-leucine to change the surface tension of the solvent systems of insulin and form particles with a small size and low density, thus affecting the aerosolization properties of the powders. Compared with the powders of formulation insulin/lactose, insulin/dextran and insulin/mannitol, the insulin/trehalose powder exhibited a higher respirable fraction (63.0%). No significant difference in respirable fraction was observed among insulin/lactose, insulin/dextran and insulin/mannitol powders. These results indicate that L-leucine can improve the aerosolization properties of the insulin dry powders, and suggest that these powders would produce enhanced deposition in the lower regions of the lung. It should be mentioned that the insulin/lactose powders possessed the highest fraction in stage 1 (30.6%), most likely due to the large median aerodynamic diameter.

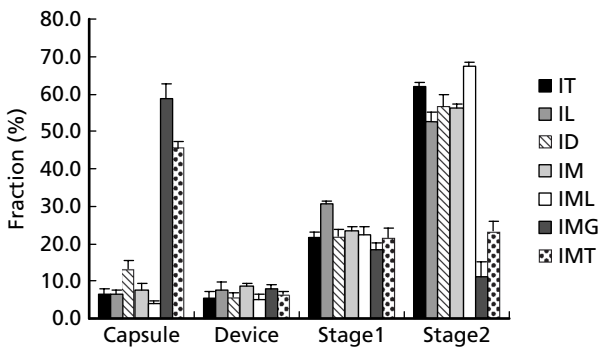

Figure 5 Comparison of the in-vitro deposition properties among different insulin inhalation dry powders from a twin-stage impinger. IT, insulin/trehalose; IL, insulin/lactose; ID, insulin/dextran; IM, insulin/mannitol; IML, insulin/mannitol/L-leucine; IMG, insulin/mannitol/glycine; IMT, insulin/mannitol/threonine.

Conclusion

The present study has shown that in all formulations insulin was hardly degraded during spray drying, and the composition of the particles significantly influences the physical characteristics and aerosolization performance of insulin dry powders for inhalation. The presence of L-leucine can reduce the particle size and aerodynamic diameter of the insulin powders, and dramatically improve the deposition profiles of insulin. Although glycine is a good low density additive, it exhibits poor aerosolization properties with a tendency to agglomerate. It has been shown that L-leucine can be used to enhance the aerosolization behaviour of insulin dry powders for inhalation. Compared with lactose, mannitol and dextran, trehalose appears to be an excipient for insulin dry powders for inhalation owing to its fine physical characteristics and good performance in the TSI.

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