

Precipitation of insulin microparticles for pulmonary delivery

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Summary

For systemic delivery of proteins the inhalation of drugs was found to be an appropriate alternative to injections. Especially in the therapy of diabetes an inhalable powder formulation of insulin could facilitate the application. The aim of this study was to produce an optimised insulin powder formulation suitable for pulmonary delivery and to compare it to the marketed, but recently withdrawn, product Exubera®. The focus was laid on particles smaller than 2 µm due to the fact that those are able to reach the alveoli and therefore the bloodstream. Insulin powders were produced by spray drying aqueous solutions or suspensions of precipitated drug, tested in combination with the Aerolizer® device concerning particle size and aerodynamic behaviour and compared to Exubera®. It was found that the particle fraction smaller 2 µm could be considerably increased. For the Exubera® device a very low ex-device fraction was found, due to the fact that about 35% of the drug adhered to the device and the blister package. In summary, precipitated insulin particles combined with the delivery from a standard capsule-based inhaler were at least as effective in vitro as the marketed Exubera® product.

Introduction

To avoid the often unpleasant but necessary injection of insulin in diabetes therapy, it has been searched for alternative, non-invasive ways of application for decades. The lung with its large surface area, good vascularization and low proteolytic activity is predestined for the systemic delivery of peptides and proteins.¹ The absorption process of proteins through the lung epithelium is not yet completely understood, though transcytosis and specific protein transporters seem to be responsible for insulin absorption across the alveolar capillary and epithelial cells.^{2,3} As a result of the increased understanding of the absorption process and pulmonary delivery, the first insulin product for inhalation was launched in May 2006 (Exubera®, Pfizer Inc., USA).

Requirements for inhalable powders are first of all a suitable particle size and good dispersibility through the inhalation manoeuvre of a patient. Particles above 5 µm are known to be deposited in the upper airways and not to reach the deeper regions of the lung. Systemically effective drugs which have to attain the alveoli, should even have a mass median aerodynamic diameter of less than 2 µm.⁵ To achieve such a particle size several methods of powder production as for example spray drying are well established. Micronization via milling presents the standard method but is less suited for protein powders due to the high energy input and often leads to bad flow properties and a broad particle size distribution.⁶ Narrower particle size distributions combined with a smaller median diameter can be achieved if particles are precipitated via solvent change.⁷ Using such an approach, the drug is not exposed to mechanical stress and typically results in a powder with good aerosolization properties and dispersibility.

The aim of this study was to produce an optimised insulin powder suitable for pulmonary delivery and to compare it to the marketed product. Due to the fact that a systemic drug delivery is required, the mean particle size should be smaller than 2 µm. Insulin particles were prepared in two different ways: spray dried out of solution and spray dried out of suspension. To form a suspension the drug was dissolved in double distilled water and precipitated with a water-miscible non-solvent. In combination with the Aerolizer® device, a standard capsule-based inhaler, the powders were compared to Exubera® with respect to geometric particle size and aerodynamic behaviour. A significant difference in particle properties among the preparation methods and among the devices was expected.

Materials and Methods

Human Insulin

The API in this study was recombinant human insulin (Wanbang Biochemical Pharmaceutical Company, Xuzhou, China) with 28.4 insulin human units in each mg and less than 0.4% zinc.

Exubera®

Exubera® is a spray dried powder for inhalation in blister packages containing human insulin, mannitol, glycine, sodium citrate and sodium hydroxide. It is combined with a special inhalation device where the blister packages with dosages of 1 mg or 3 mg are emptied via air pressure. The powder is dispersed into a spacer that holds a mouthpiece for inhalation. The 1 mg dose strength was taken from the market (batch no. 50025B). The device was used as described in the patient information leaflet.

Micronization technique

Screening tests revealed that methanol is an optimal non-solvent system for the precipitation process (data not shown). Insulin was dissolved in double distilled water at room temperature to a concentration of 1% (w/w). This solution was adjusted to pH 6.75. The respective amount of methanol was added to the insulin solution into a beaker while stirring with a magnetic stirrer (500 rpm) at a ratio of 1+1, resulting in an insulin concentration of 0.5% (w/w). Storage experiments revealed that particle growth of about 15nm/day took place after one day of storage. Accordingly, the spray drying of the precipitated insulin suspension was conducted 2-3 hours after

precipitation. To have a direct comparison to the Exubera[®] product, two batches were spray dried out of an aqueous solution in concentrations of 1% and 0.5%.

For spray drying, a Mini-Büchi B-290 (Büchi, Switzerland) with a small cyclone was used to enable collection of particles in the low micron range. A two fluid nozzle with an inner diameter of 0.7 mm was used for spraying and gas flow was in the same direction as product flow. Spray drying conditions were 100°C inlet temperature, 50°C outlet temperature and 100% aspirator power (atomising gas flow of 40 m³/h).

Particle size

Particle size of the spray dried powders was measured by laser diffraction (HELOS, Sympatec, Germany). The sample was suspended in middle-chain triglycerides (MCT), sonicated for 30 minutes in an appropriate dilution (optical concentration 10–30%) and transferred to a 6 mL cuvette for measurement. In the data shown, x50 indicates the volume median diameter, x90 is the 90% quantile of the respective particle size distribution.

Aerodynamic particle size

Every produced batch was tested with the Aerolizer[®] device, the Exubera[®] insulin powder was tested with the commercial device. The aerodynamic particle size was determined using a Next Generation Pharmaceutical Impactor (NGI, MSP Corp., MN, USA) at a flow rate of 100 L/min for the Aerolizer[®] device and 56 L/min for Exubera[®], respectively, corresponding to a pressure drop of 4 kPa over the device as demanded in the European Pharmacopeia. The powders were directly weighed into capsules (hydroxypropylmethylcellulose capsules size 3, Qualicaps, Alcobendas, Spain). The number of capsules used per run in the NGI was adapted to the sensitivity of the HPLC method used and corresponded to a total drug mass in the NGI of about 3 mg of insulin (three capsules at 1 mg). All NGI stages were coated with a coating fluid (Brij 35 in a mixture of ethanol 96% and glycerol) in order to minimize particle bouncing.

Capsules were placed in the Aerolizer[®] device and pierced as directed in the user instructions. The NGI was operated once and afterwards it was visually controlled whether the capsule was emptied. If this was not the case, a second actuation was conducted. The Exubera[®] device was prepared and actuated as described in the patient information leaflet.

All capsules and blister packs were collected after emptying and washed in 0.001 M HCl to determine the residual drug amount. All NGI stages as well as the throat were washed with 0.001 M HCl to dissolve the drug on the stages for HPLC analysis. The complete Aerolizer[®] device and the deagglomeration unit together with the spacer of the Exubera[®] device were washed with 0.001 M HCl to determine the drug amount remaining in the inhalers. All measurements were performed in triplicate. The calculations for the particle fractions smaller than 5 µm and 2 µm, respectively, were carried out with the Copley CITDAS software (Copley Scientific, Nottingham, UK).

High performance liquid chromatography (HPLC)

Determination of residual drug amount and analysis of NGI measurements was carried out using HPLC (Agilent Technologies, Santa Clara, USA). A RP-18 column (LiChroChart 125-4, LiChroSpher 100, 5 µm, Merck, Germany) with pre column (LiChroChart 4-4, LiChroSpher 100, 5 µm, Merck, Germany) was used as stationary phase which was conditioned to 25°C in a column oven. As mobile phase a mixture of acetonitrile and buffer (0.01 M KH₂PO₄; 0.1 M Na₂SO₄) 27:73 adjusted to pH 3.0 was used. Flow rate was 1 mL/min; detection wavelength was 215 nm. Quantification was carried out by an external standard method. Linearity was checked between 10 and 500 µg/mL.

Results and discussion

Particle size

The powders analysed in this study were produced using two different production approaches: in case of Exubera[®] and the batches spray dried out of solution the particle formation took place during the spray drying process. In case of the batch spray dried out of suspension the particle development is completely different due to the fact that the primary particles are already formed during the precipitation. Significant differences in many attributes (morphology, flowability, aerodynamic behaviour) between the powders were observed. Also, differences between the powders spray dried out of solution were detected. Laser diffraction analyses showed an average particle size of 3.34 µm for the 1% (w/w) solution and 2.13 µm for the 0.5% (w/w) solution (Figure 1). The common understanding of the spray drying process is that single droplets containing a dissolved substance dry within some milliseconds to form a single solid particle. The resulting particle size of the dried particles is mainly influenced by the drug concentration within the droplet whereas size and morphology largely depend on the drying conditions and the properties of the drug. Due to the fast drying process within the spray-dryer hollow spheres are formed leading to a very low bulk density of the powder. A decreasing amount of drug in the solution and, accordingly, in each single droplet during the drying process resulted in smaller particle sizes (Figure 1).

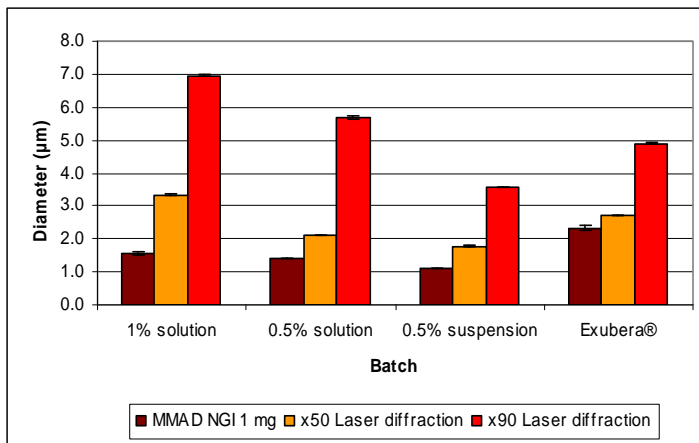


Figure 1: Particle size determined via laser diffraction and calculated using the NGI deposition data, n=3

the fact that not the whole particle fraction is considered for the calculation but only the amount of drug that could be recovered in the impactor. Larger particles could have remained in the capsule or the device. Owing to their low density the hollow spheres of the first two batches can follow the airstream into the impactor for a long distance and, therefore, have a smaller aerodynamic diameter. The small MMAD of the precipitated particles can not be explained with a low density. In this case a better deagglomeration of the primary particles during the inhalation process is presumable.

The Exubera® powder shows a moderate average particle size of about 2.72 µm but by far the highest MMAD. Due to the fact that the production process of the commercial insulin powder is not disclosed it can be hardly compared directly to the other powders. However, the found high MMAD is noteworthy.

Recovery

To gain further insight into the particle behaviour during the inhalation process the amount of drug remaining in the device and the capsule/blister was determined. When delivered with the Aerolizer® device, the powders do not differ significantly in their adhesion behaviour. The amount of drug remaining in the inhaler was in all cases in the range of 8-9% (Figure 2). The found marginal differences are linked to the construction of the inhaler. The Aerolizer® device is characterized by a short mouth piece and a simple deagglomeration chamber. The Exubera® device in contrast contains a complex unit for the piercing of the blister and the aerosolization of the powder via compressed air. After deagglomeration the powder reaches the integrated spacer having a large inner surface where it can be adhered to. This leads to an overall device retention of about 15%.

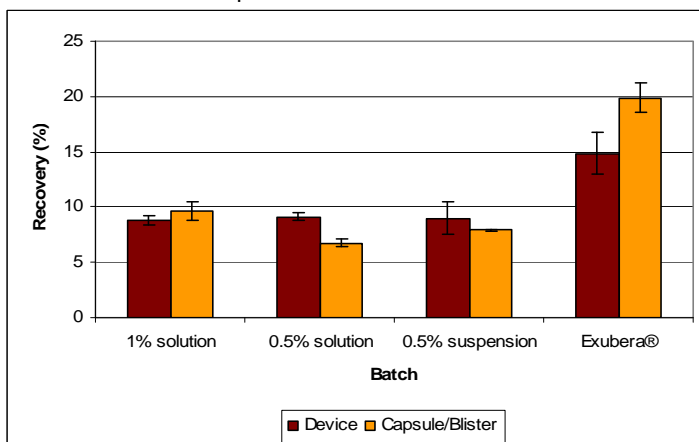


Figure 2: Amount of drug remaining in the device and capsule or blister after actuation of a 1 mg dosage, n=3

different way of emptying. The capsule is pierced at both sides and empties during rotation in the air stream. The aluminium blister is pierced at the upper side and emptied using an air blast. Powder deposition is enhanced by irregular cuts into the aluminium blister resulting in a blister retention of 20% (Figure 2).

In summary, overall insulin recovery was excellent for the impactor testing when device and capsule/blister retention are taken into account

Fine particle fraction

Usually the drug amount smaller than 5 µm is called fine particle fraction (FPF). Since the systemic effect of the drug is important, the particle fraction smaller than 2 µm is considered in this study and from now on called systemic FPF and was calculated in two different ways. The so-called relative systemic FPF is the particle fraction smaller than 2 µm related to the ex-device fraction, the absolute systemic FPF is the amount of particles smaller than 2 µm in relation to the nominal drug dose.

Figure 3 shows an increasing relative systemic FPF for the batches produced from the 1% solution, the 0.5% solution and the 0.5% suspension, respectively. This finding correlates well with the geometric particle size as

Particles spray-dried out of suspension were found to be smaller than the other batches though the absolute insulin concentration is the same as in the 0.5% solution. In this case, one sprayed droplet contains several solid insulin particles and some dissolved drug (the yield of precipitated insulin is about 64%). The diluted drug dries onto the primary particles (average size: about 1.5 µm) or glues several primary particles together. As a result, no hollow spheres but small agglomerates are formed. This presumably leads to smaller particles with a higher bulk density.

The mass median aerodynamic diameter (MMAD) calculated using the NGI deposition data is in every case smaller than the particle size determined via laser diffraction. This could be expected due to

the fact that not the whole particle fraction is considered for the calculation but only the amount of drug that could be recovered in the impactor. Larger particles could have remained in the capsule or the device. Owing to their low density the hollow spheres of the first two batches can follow the airstream into the impactor for a long distance and, therefore, have a smaller aerodynamic diameter. The small MMAD of the precipitated particles can not be explained with a low density. In this case a better deagglomeration of the primary particles during the inhalation process is presumable.

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To gain further insight into the particle behaviour during the inhalation process the amount of drug remaining in the device and the capsule/blister was determined. When delivered with the Aerolizer® device, the powders do not differ significantly in their adhesion behaviour. The amount of drug remaining in the inhaler was in all cases in the range of 8-9% (Figure 2). The found marginal differences are linked to the construction of the inhaler. The Aerolizer® device is characterized by a short mouth piece and a simple deagglomeration chamber. The Exubera® device in contrast contains a complex unit for the piercing of the blister and the aerosolization of the powder via compressed air. After deagglomeration the powder reaches the integrated spacer having a large inner surface where it can be adhered to. This leads to an overall device retention of about 15%.

The difference between the amount of drug remaining in the capsule (Aerolizer®) and the blister (Exubera®) is also highly significant and can be explained by the

different way of emptying. The capsule is pierced at both sides and empties during rotation in the air stream. The aluminium blister is pierced at the upper side and emptied using an air blast. Powder deposition is enhanced by irregular cuts into the aluminium blister resulting in a blister retention of 20% (Figure 2).

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Figure 3 shows an increasing relative systemic FPF for the batches produced from the 1% solution, the 0.5% solution and the 0.5% suspension, respectively. This finding correlates well with the geometric particle size as

determined by laser diffraction (Figure 1). In spite of a moderate particle size the Exubera[®] device generates by far the lowest relative systemic FPF. This is mainly attributed to the fact that up to 28% of the drug are deposited

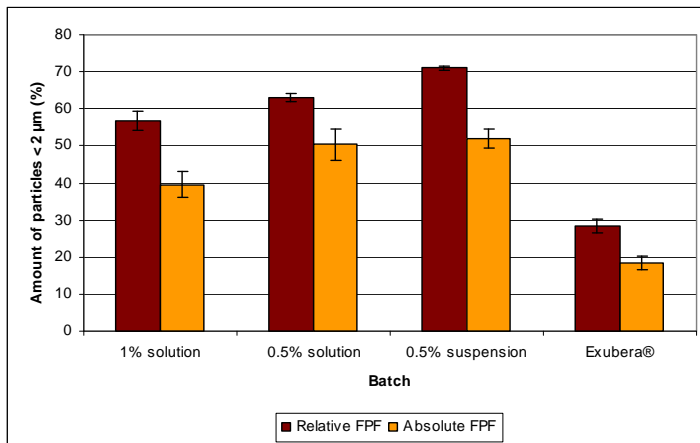


Figure 3: Particle fraction < 2 μm related to the ex-device fraction (relative FPF) and related to the amount of applied drug (absolute FPF), n=3

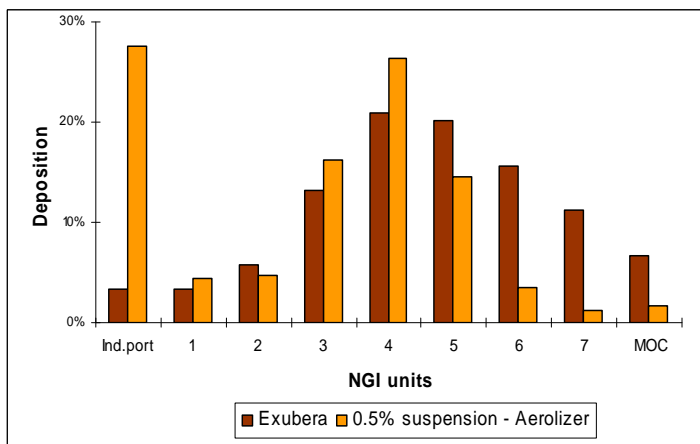


Figure 4: NGI deposition of insulin, Exubera[®] and precipitated drug in combination with the Aerolizer[®] device

in the induction port (Figure 4) which again leads to the conclusion that the device is not able to accomplish an adequate particle deagglomeration. On the other hand, a high amount of the precipitated batch follows the air stream to the lower stages of the NGI resulting in a relative systemic FPF of about 70% caused by the none-cohesive, small primary particles with good deagglomeration properties, even when delivered in a simple device as the Aerolizer[®].

As a matter of fact the absolute systemic FPF is the parameter which is of most practical relevance, since it can be taken as an indirect indicator of the insulin amount reaching the alveoli. Due to the fact that the ex-device fraction using the Exubera[®] device hardly reaches 65%, the found 28% relative systemic FPF result in only 18% absolute systemic FPF. For the batches produced from the 0.5% solution and the 0.5% suspension the absolute systemic FPFs adjust to about 50% as a consequence to the smaller ex-device fraction of the precipitated particles. Still the absolute systemic FPF is more than twice as high for the self produced batches as for Exubera[®].

Conclusion

There are different ways to produce insulin powders with a particle size in the respirable range. Spray drying leads to products with good aerosolization properties and is a simple and well-understood technique. The particle size after spray drying depends partly on the concentration of the drug if sprayed from a solution. A lower drug concentration induces a decrease in particle size. This process can be optimised by precipitating the drug before spray drying. This leads to

small particles with a good dispersibility and, therefore, a high fine particle fraction. It could be shown that a small inhaler with a simple but effective deagglomeration unit positively influences the ex-device fraction.

In summary, precipitated insulin particles combined with the delivery from a standard capsule-based inhaler were at least as effective in vitro as the marketed Exubera[®] product. With an optimised powder having an increased particle fraction smaller than 2 μm more insulin may reach the deeper lung. Therefore, a lower dose may be used for an effective diabetic therapy.

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