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Insulin-micro- and nanoparticles for pulmonary delivery

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ABSTRACT

The pulmonary application of insulin via oral inhalation turned out to be a promising option due to the large surface area and good vascularisation the lung is offering for the systemic delivery of peptides and proteins. To have a systemic effect, inhaled particles need to attain the alveoli and should therefore have a mass median diameter of less than 2 μm. To achieve such a particle size for dry powders spray drying of drug solutions is a common method. In this study, a nano-precipitation of the drug prior to spray drying was carried out using the solvent change method. The produced powders were compared to powder produced out of a solution and to the marketed product Exubera®. The Aerolizer® device was used representing a simple capsule-based dry powder inhaler.

It could be shown that the insulin yield of the precipitation process highly depends on the used pH-value and the amount of non-solvent. Also the particle size after spray drying decreases with increasing amount of non-solvent. Aerodynamic assessment of insulin powders showed that the precipitated insulin particles behave superior to powders spray dried from solution with respect to particles smaller than 2 μm. The deposition pattern of the originator powder delivered with the Exubera® device showed significantly lower fine particle fractions and higher residues in comparison to the Aerolizer® device.

In summary, precipitated insulin particles combined with the delivery from a standard capsule-based inhaler were found to be 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 could be used for an effective diabetic therapy.

1. Introduction

In spite of the many improvements in diabetes therapy, the use of injections and the associated fear of the needle may cause a certain reluctance to the parenteral application. As a consequence, some type 2 diabetes patients delay the start of the insulin therapy (Scholze, 2005). Besides a poor patient compliance, further disadvantages of the insulin injections are fluctuating absorption and time of action caused by change of the injection-area (Parhofer, 2004), the necessity of cooling the insulin solutions/suspensions, the intricate handling of the pens and the social stigma that occurs with syringe-use (Simon and Kissel, 2001). Therefore, researches have been looking for decades for alternative, non-invasive ways of insulin application.

For centuries nicotine and anaesthetics have been good examples for the systemic use of drugs administered via the lungs (Gonda, 2000). Also more than 80 years ago an effect of inhaled insulin on the blood sugar has been discovered (Gänsslen, 1925). The large surface area, good vascularisation, immense capacity for solute exchange and ultra-thinness of the alveolar epithelium are unique features of the lung that can facilitate systemic delivery via pulmonary administration of peptides and proteins (Agu and Ugwoke, 2001). Another important advantage in comparison to oral application is a lower proteolytic activity in the lung and, therefore, less degradation of the protein in the application area (Gonda, 2007). Currently, the absorption process of proteins in the lung is not completely understood. Transcytosis seems to be the primary mechanism of insulin absorption across the alveolar capillary and epithelial cells (Klonoff, 1999). Other researchers report about specific protein transporters which could facilitate the absorption process (von Wichert and von Wichert, 2006). The relative bioavailability of aerosolized insulin compared to subcutaneous injected insulin is highly depending on the delivery device and ranges from 10% to 20% (Laube et al., 1998). The onset of action of inhaled insulin is significantly faster as compared to a subcutaneous injection (Heinemann et al., 1997).

In general, a dry powder inhaler (DPI) is superior for protein preparations as compared to an aqueous product. The usage of a DPI provides better user-friendliness due to a significant longer storage stability (Chew and Chan, 2002) and the fact that a storage and distribution in a cool-chain are not necessary. After years of development Pfizer launched the first insulin for inhalation (Exubera®) in May 2006. It is a powder containing fast acting human insulin in combination with a novel inhalation device, which disperses the

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powder from a blister into an implemented spacer. Exubera® is licensed for the treatment of type 1 and type 2 diabetes. In comparison to a subcutaneous injection a ten times higher dose is necessary to achieve a similar depression of blood sugar (Parhofer, 2004). This fact and the complex inhalation device increase the therapy costs three to five times in comparison to the standard therapy. In addition to hypoglycaemia, side effects can be cough or, in rare cases, dyspnoe (Pfizer, 2006).

Studies have shown that inhaled insulin is effective, well tolerated and provides glycaemic control comparable to a conventional subcutaneous regimen (Hollander and Blonde, 2004; DeFronzo and Bergenstal, 2005). Also, no unexpected safety concerns have been reported in the first studies (Barnett, 2004). Still long term studies have to show if there is a significant risk to come down with lung cancer by the use of inhaled insulin as the FDA warned in April 2008 for patients with smoking background. However, in case of Exubera® the expected increase in patient compliance appeared to fail. The inhalation of insulin did not appeal to the diabetes patients and Exubera® was not accepted by both, physicians and patients. Two years after launch, the product has been withdrawn from the market due to commercial failure. The bad acceptance can partially be ascribed to the complex and eye-catching device. Also unreadiness of the attending doctors to deviate from the standard therapy and a reluctance of the patients to use the complex device may have supported the failure. An enhancement concerning size and handling of the inhaler is considered the right approach for an optimisation of patient compliance. The capsule-based Aerolizer® which has been used in this study combines both characteristics. It is handy, inconspicuous and easy to use.

Inhalable powders have to fulfil certain requirements; the increase of the respirable fraction with a decreasing average particle diameter is a fundamental matter of fact (Hickey et al., 1996). The general assumption is that particles smaller than 5 μm are not deposited in the upper airways but are able to penetrate into the lung (Gonda, 2003). Systemically effective drugs which have to attain the alveoli, should even have a mass median aerodynamic diameter of less than 2 μm (Lucas et al., 1999). Because of the high agglomeration tendency caused by the comparatively huge surface area of very small particles, the necessity of good flow properties and dispersibility by airflow turned out to be a considerable technological challenge. The common way of producing small particles is milling with a high energy input. The mechanical strain during micronization may be high enough to increase the free enthalpy of the particles resulting in structural defects and instability of the proteins. Calorimetric measurements have shown that this part can be up to 10% of the used mechanical energy (Redeker, 2003). Micronization via milling leads to triboelectrification of the powders, a broad particle size distribution, an increase of the non-polar surface area and, hence, to poor flow properties (Feeley et al., 1998). The high energy input can cause disruptions in the crystal lattice and physical or chemical instability (Rasenack et al., 2004) which has to be considered especially in the case of sensitive peptides or proteins like insulin. For the production of inhalable protein/peptide particles spray drying is predominantly used to provide greater control over physical characteristics of the powder (Edwards, 2002). But there are also other promising methods suitable for the production of inhalable protein powders. For example spray freeze drying (van Drooge et al., 2005) to avoid heat induced degradation of very sensitive drugs or micronization using carbon dioxide assisted nebilisation (Sievers et al., 2003).

In this study, precipitation via a solvent change method was chosen to produce particles in the low micrometer range. These particles receive no mechanical stress and, therefore, show good flow properties and dispersibility. In comparison to milling – e.g. by using a jet mill – narrower particle size distributions combined with a smaller median diameter can be achieved (Rasenack et al., 2003). The advantage of these particles is the nanoparticular structure which is completely different to the morphology of typical spray dried particles and leads to better aerodynamic behaviour. The active ingredient has to be solved in an appropriate solvent and precipitated by a fast addition of a miscible non-solvent. This leads at first to a suspension that can be spray dried to a flowable powder. The suspensions and powders have been analyzed concerning mass median diameter and mass median aerodynamic diameter via photon correlation spectroscopy (PCS), laser diffraction (LD) and Next Generation Pharmaceutical Impactor (NGI) and were compared to Exubera®. The yield of precipitated insulin has been quantified via HPLC.

2. Materials and methods

2.1. Materials

2.1.1. Human insulin

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

2.1.2. 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 device was used as described in the patient information leaflet.

2.2. Micronization technique

Screening tests revealed that methanol is the optimal non-solvent system for the precipitation process as applied for this study (data not shown). Insulin was dissolved in double distilled water at room temperature to a concentration of 1% (m/m). This solution was adjusted to pH 6.75. The respective amount of methanol was added entirely to the insulin solution in ratios of 1 + 4 to 4 + 4 (m + m) (resulting in methanol contents of 20–50% m/m). Higher methanol concentrations caused an immediate and intense particle agglomeration and were therefore not considered further in this study. The resulting suspensions were stirred for another 5 min and then analyzed in terms of particle size distribution and yield. Storage experiments revealed that particle growth of about 15 nm/day took place after one day of storage (Klingler and Müller, 2007). Accordingly, spray drying of the precipitated insulin suspensions was conducted within 2–3 h after precipitation. To have a direct comparison to the Exubera® product, one batch (batch no. 600) was spray dried without precipitation.

For spray drying, a Mini-Büchi B-290 (Büchi, Switzerland) with a high-performance 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 40 m^3/h). These conditions were found to cause low degradation (0.36%) and moderate residual moisture content (5.1%) if used for aqueous insulin solutions (Stahl et al., 2002). Due to the lower boiling point of methanol in comparison to water the residual moisture content in this case was expected to be even lower. The product flow varied from 3.3 g/min to 7.8 g/min (Table 1), which was necessary to adjust the outlet temperature. In other reports, the product flow was found to have no influence on the particle size (Elversson et al., 2003). According to the HPLC-data no

Table 1
Batch production parameters.

Batch	Insulin solution 1% (g)	Methanol (g)	Product flow (g/min)	Yield of spray dried product (%)
600	50.0	–	3.3	69.4
601	40.0	10.0	6.25	54.5
602	40.0	20.0	6.0	50.5
603	40.0	30.0	7.8	44.25
604	40.0	40.0	7.3	50.0

degradation of the protein could be observed during the process (data not shown).

2.3. Characterisation

2.3.1. Particle size in suspension

The particle size distribution of precipitated insulin particles in methanol/water mixtures was analyzed via dynamic light scattering also known as photon correlation spectroscopy (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK). Five minutes after precipitation a sample of about 1.5 mL was transferred to a polystyrene cuvette and measured after 2 min equilibration time at a temperature of 25 °C.

2.3.2. Particle size after spray drying

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 min in an appropriate dilution (optical concentration 10–30%) and transferred to a 6 mL cuvette for measurement. In the data shown, x_{50} indicates the volume median diameter while x_{90} and x_{99} are the percent quantiles of the respective particle size distribution.

2.3.3. Scanning electron microscopy (SEM)

SEM pictures were taken using a Philips XL 20 (Philips B.V., Eindhoven, The Netherlands) scanning electron microscope. Samples were fixed on an aluminium stub with conductive double-sided adhesive tape (Leit-Tabs, Plano, Wetzlar, Germany) and coated with gold in an argon atmosphere (50 Pa) at 50 mA for 50 s (Sputter Coater, Bal-Tec, Liechtenstein).

2.3.4. Aerodynamic particle size

For estimation of the aerodynamic behaviour of the insulin particles, the powder had to be dispersed into an air stream. The Aerolizer® device was used for this formulation because different dosages can be applied very easily and effectively. Every produced batch was tested with this 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, which leads to a run time of 2.4 s and 4.3 s, respectively, for an inspiration volume of 4 L. This flow rate was chosen as it refers to a pressure drop of 4 kPa over the device as demanded in the European Pharmacopoeia. 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 or one capsule at 3 mg). All NGI stages were coated with a coating fluid in order to minimize particle rebounce.

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, resulting in 10 samples per run [capsule, throat, stages 1–7, multiorifice collector (MOC)]. 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).

2.3.5. Drug quantification

2.3.5.1. Yield. To determine the yield of precipitated drug, 1.0 mL of the suspension was centrifuged (Heraeus Sepatech, Osterode, Germany) for 10 min at 17,000 rpm resulting in a force of 25,000 × *g*. The supernatant was separated from the residue and drug quantification was performed by HPLC as described below.

2.3.5.2. Saturation solubility. For the determination of the saturation solubility at different pH-values a saturated insulin solution (with clearly visible residue of unsolved drug) in double distilled water adjusted to pH 3.0 with diluted hydrochloric acid was used. This solution was adjusted with diluted sodium hydroxide solution in steps of 0.5 to pH 7.0. It was ensured that a residue of unsolved insulin remained in the solution at all time. Samples of about 1 mL were taken at every step after 5 min pH equilibration and treated like the yield samples. The samples for determination of the saturation solubility in methanol were taken after 30 min ultrasonication of a methanolic insulin suspension and also handled as described above.

2.3.5.3. High-performance liquid chromatography (HPLC). Determination of drug content 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 KH_2PO_4; 0.1 M Na_2SO_4) 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 μg/mL and 500 μg/mL.

2.4. Statistical analysis

All experiments were performed in triplicate unless indicated otherwise. The statistical analysis was performed using a two-side Student's *t*-test to calculate the probability of error *p*. Differences between the data are indicated according to the following legend:

0	No significant difference ($p \geq 0.05$)
*	Significant difference ($0.05 > p \geq 0.01$)
**	Significant difference ($0.01 > p \geq 0.0001$)
***	Significant difference ($p < 0.0001$)

To preserve the lucidity, not every calculatable probability of error is graphically presented.

3. Results and discussion

3.1. Characterisation of precipitated insulin in suspension

As every protein, insulin contains several functional groups differently charged and with different acid or basic behaviours depending on the pH-value. At a specific pH the net charge of the

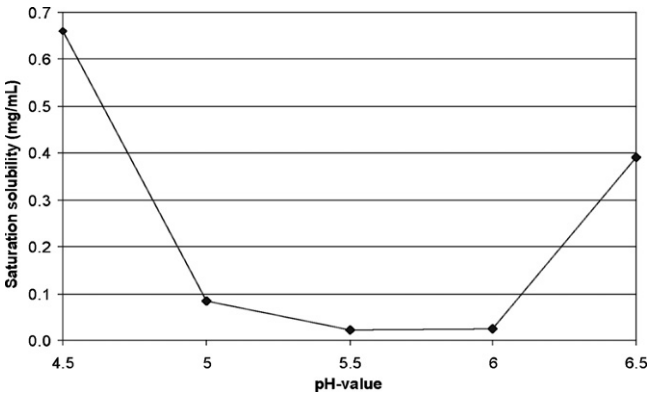

Fig. 1. Saturation solubility of insulin at different pH-values, $n = 1$.

amino acids is balanced leading to a formal uncharged molecule (isoelectric point, IEP). Due to this, the molecule becomes more non-polar and the solubility in a polar solvent like water decreases (Fig. 1). In the case of insulin the IEP is between pH 5.5 and 6.0. Precipitation via pH-shift is possible, but the non-polar molecules show a strong agglomeration tendency which leads to big flaky particles. These are not suitable for a pulmonary application (experimental data not shown) and, hence, this approach was not followed further.

The precipitation via solvent change took place at a pH of 6.75 where insulin has a saturation solubility of about 10 mg/mL. This pH was necessary to combine a good stability of the drug in solution and an appropriate drug concentration to force a sufficient supersaturation during the precipitation. At higher pH-values the good solubility of insulin would cause an uneconomical high drug loss. In the organic non-solvent methanol, insulin was found to have a solubility of 0.13 mg/mL. It was expected that rising methanol contents cause a continuous increase in the yield of precipitated drug due to the poor solvent power of methanol. Surprisingly, a non-continuous rise of the yield was observed when the methanol–water ratio was increased. Determination of the pH in the precipitation medium revealed that methanol causes a shift towards higher pH-values if mixed with buffered aqueous solutions (Fig. 2, left, tested with phosphate buffer pH 5.5). This leads to a better solubility of insulin in the aqueous part of the suspension and results in a stagnation of the yield increase and to an optimum of about 65% precipitated drug.

The particle size of the precipitated insulin particles was found to be highly dependent on the non-solvent-to-solvent ratio. Precipitation carried out with an excess of water (ratio methanol:water from 1 + 4 to 3 + 4; see Fig. 2, right) led to insulin nanoparticles not exceeding an average particle size of 200 nm. At a methanol/water ratio of 4 + 4 a sudden and intense increase of particle size can be detected (Fig. 2, right). At this concentration, methanol is able to weaken or destroy the hydrate layer of the nanoparticles. This effect has already been described for acetone (Bergeron et al., 2003). The loss of hydrate cover causes an increase of non-polar surface and leads to agglomeration of the particles.

3.2. Characterisation of the spray-dried products

3.2.1. Scanning electron microscopy

If insulin is spray dried out of solution, resulting powders have a characteristic morphology. The drying starts at the outside of the atomised droplets. Solvent evaporates and the concentration of dissolved substance increases until the solubility limit is reached. A solid shell forms on which further dried substance can attach (Charlesworth and Marshall, 1960). Losing water, the insulin particles become visco-elastic and collapse to an erythrocyte-like shape (Fig. 3, left), leading to a powder with acceptable flow properties and a low bulk density (0.114 g/cm^3). The Exubera® powder also possesses these typical structure elements leading to the assumption that it is as well spray dried out of a solution. Batch 604 (precipitated, 4 + 4) differs significantly from batch 600. Some rudimental hollow spheres can be observed, but the majority of the particle collective has a different shape. In this case the particle development is completely different due to the fact that the primary particles are already formed during the precipitation. One sprayed droplet contains several solid insulin particles and some dissolved drugs. The diluted drug dries onto the primary particles or glues several primary particles together. As a result, no collapsed spheres but small agglomerates are formed which consist of insulin nanoparticles. Accordingly, particles have a higher bulk density (0.163 g/cm^3).

3.2.2. Particle size

Fig. 4 shows a decreasing average particle size of the spray-dried particles with increasing methanol amount. The more methanol is used for precipitation, the lower is the absolute insulin concentration in the resulting suspension. Assuming that in the spray drying process one droplet dries to one particle, a lower drug amount per droplet would induce smaller particles (Elversson et al., 2003). This theory applies for solutions as well as for suspensions.

Batches 603 and 604 do not differ as significantly in their average particle size as the other batches, though the particles in suspension

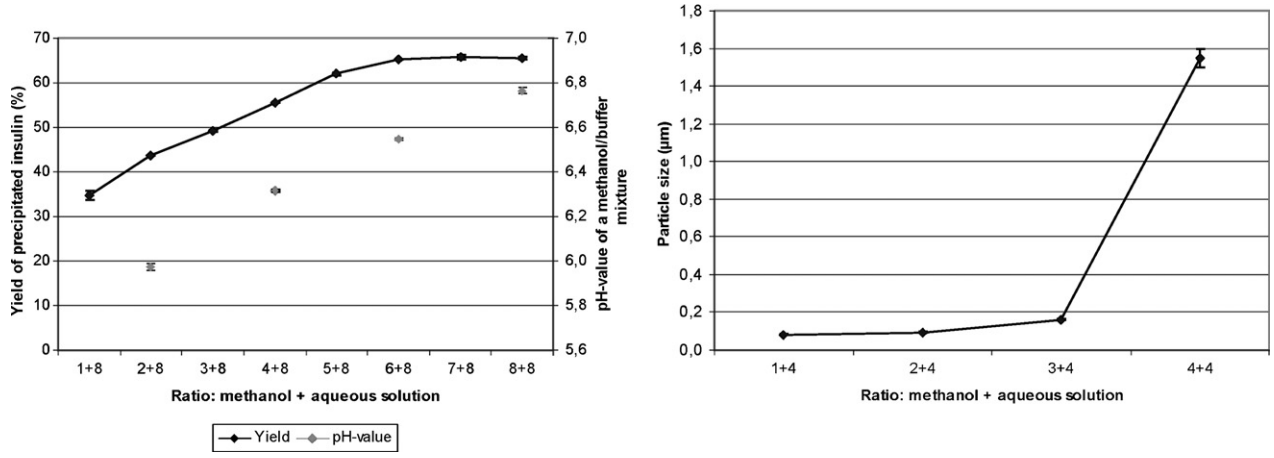

Fig. 2. Precipitation of insulin – yield and pH-value (left) – particle size in suspension (right); $n = 3$, error bars indicating standard deviation.

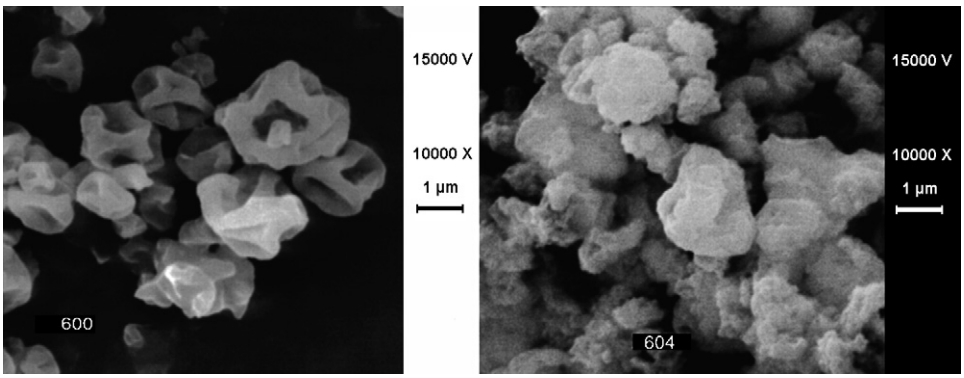

Fig. 3. SEM pictures of spray dried batches 600 and 604.

vastly varied. Batch 603 contained primary particles in nanosize which were agglomerated during spray drying, in case of batch 604 the agglomerates were already formed during precipitation. The similar average sizes of particles in suspension (1.55 μm) and after spray drying (1.78 μm) for batch 604 indicate, that there is only one agglomerate per droplet. Otherwise the resulting particles would be at least twice as big. The stagnating mean particle size could be caused by the physical conditions of the spray dryer. Though a high-performance cyclone was used, particles in the low micron range cannot be separated completely, resulting in nearly similar particle size distributions for batches 603 and 604.

The mean particle size of the Exubera® powder is with 2.72 μm located between batches 600 and 601 and therefore fulfils the size conditions for an inhalable powder. As the spray drying parameters for the commercial insulin powder are not known, a direct comparison to the other powders is not possible.

3.3. Aerodynamic behaviour

To determine the amount of drug reaching the lung, the site of insulin deposition after actuation of the device has to be investigated. A significant reduction of the ex-device fraction is caused by drug remaining in the capsule and in the blister, respectively. The residual amount of insulin was in the cases of batches 600–604 in the range of 80–120 μg (Fig. 5, left). Due to the constant inner surface of the capsules and an assumed complete coverage of this surface with insulin, the different dosages do not cause significantly different residual drug amounts. The cohesiveness of the batches 600–604 also does not differ enough to show an effect.

The difference between the amount of drug remaining in the capsule (Aerolizer®) and the blister (Exubera®) is in the most cases significant and can be explained by the different way of emptying. The capsule is pierced at both sides and empties during rotation into the air stream whereas the aluminium blister of the Exubera® product 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 250 μg (dosage 1 mg) and 425 μg (dosage 3 mg).

The ex-device fraction was found to be around 70% for all tested formulations which is considered non-optimum (Fig. 5, right). From batches 600 to 604 a trend to lower ex-device fractions can be observed. Apart from statically charged powder sticking to the inside of the impactor some of the powder got lost leaving the NGI towards the vacuum pump. The latter leads to the assumption, that during the inhalation process the agglomerates are disrupted again into the primary particles. The low ex-device fraction of Exubera® can be explained by the high blister retention.

The relative fine particle fraction (FPF) was calculated as percentage of the ex-device fraction smaller than a defined particle size. In spite of different mean particle sizes, the relative FPFs <5 μm of batches 601–604 do not differ significantly (Fig. 6, left). Though there is a small increase in FPF to >90% if the drug is precipitated,

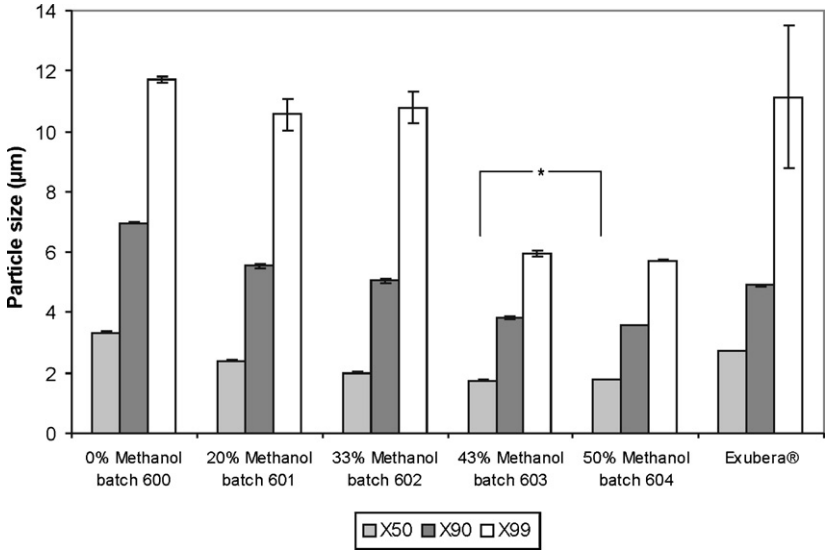

Fig. 4. Particle size of the dry powders determined via laser diffraction; x_{50}, x_{90} and x_{99} describing the percent quantiles of the respective particle size distribution; $n = 3$, error bars indicating standard deviation; highly significant x_{50} differences ($p < 0.0001$) between batches 600, 601, 602, Exubera® and 603/604 not shown.

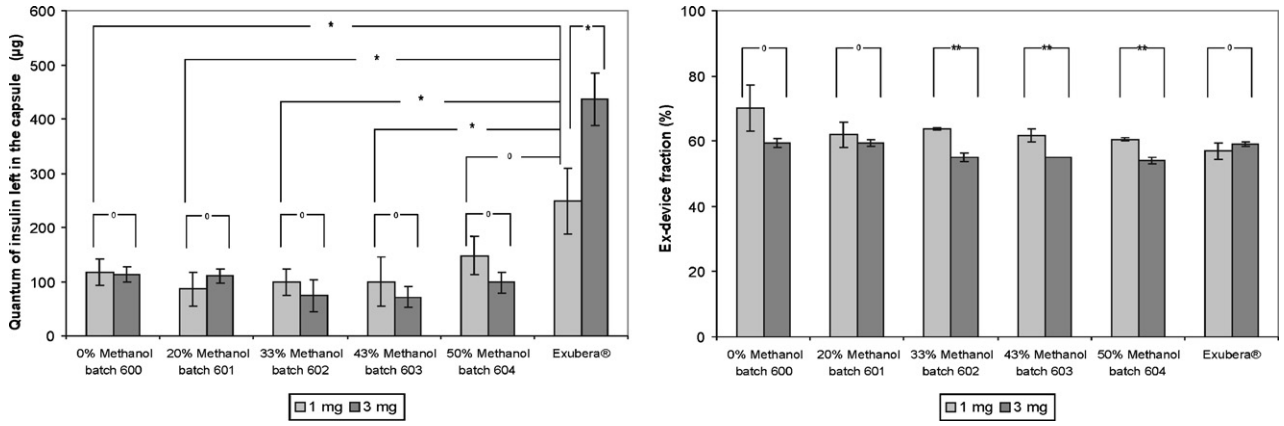

Fig. 5. Total amount of drug remaining in the capsule/blister after actuation (left) and ex-device fraction (right); $n = 3$, error bars indicating standard deviation.

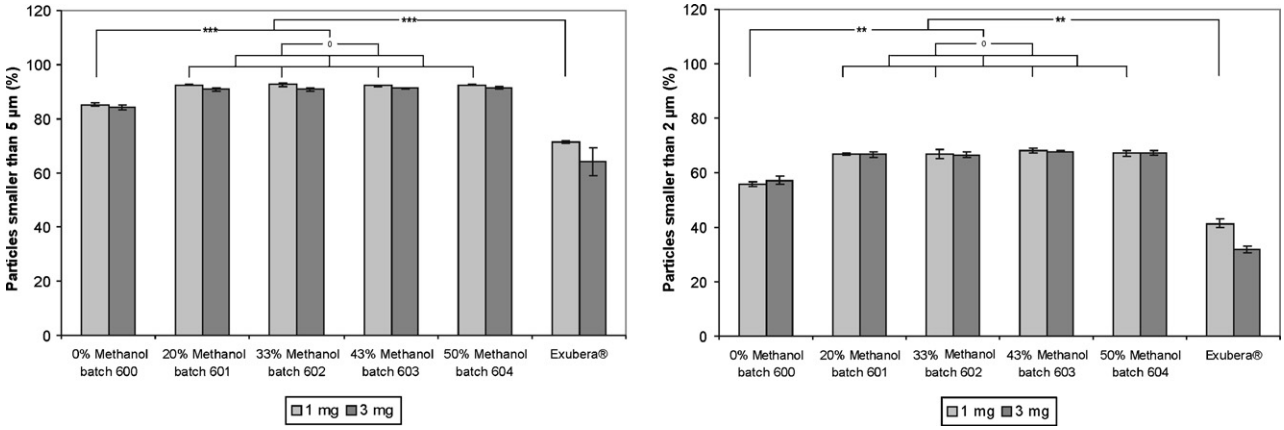

Fig. 6. Relative fine particle fractions smaller than 5 µm (left) and smaller 2 µm (right); $n = 3$, error bars indicating standard deviation.

there seems to be only little room for further improvement. In these ranges a further downsizing of the particles has little effect on the respirable fraction. In fact, the deagglomeration behaviour of the powder and the deagglomeration ability of the inhaler are of particular importance. The difference between batch 600 and the batches 601–604 is highly significant ($p < 0.0001$). This leads to the assumption that the precipitation step can affect the properties of the resulting particles and enhance the aerosolization behaviour. In spite of a comparable particle size the fine particle fraction of the Exubera® powder is up to 20% lower than the powders tested with the Aerolizer® device.

The amount of particles reaching the alveoli is of special interest in this study. As base for a calculation the cut-off point for these particles is set at 2 µm and is defined as systemic FPF. Even with respect to this systemic FPF, the batches 601–604 do not differ significantly and are located around 67% for both strengths (Fig. 6, right). As expected, the relative systemic FPF of batch 600 is about 10% lower due to the larger particles. With relative systemic FPFs of 42% for the 1 mg dosage and 32% for the 3 mg dosage Exubera® has by far the smallest amount of particles < 2 µm leading to the assumption of a poor deagglomeration in this device. It was also found during aerodynamic analysis that about 25% of the insulin remains in the throat of the NGI, whereas a comparable powder (batch 600) used with the Aerolizer® device has only a throat retention of about 6.5% (Fig. 7). This cannot be an effect of particle size, due to the fact that the mean particle size of batch 600 is larger than the Exubera® powder, but has to be related to an inappropriate deagglomeration and/or a higher cohesiveness of the powder.

It was also observed for Exubera® powders that the 3 mg strength showed a lower FPF for both chosen cut-off points which was not the case for the powders delivered with the Aerolizer® device (Fig. 6). An explanation may be given by examination of the blister emptying process: for both strengths the same amount of air is used to blow the powder out of the pierced blister resulting in a higher aerosol density for the 3 mg strength and, therefore, a worse deagglomeration.

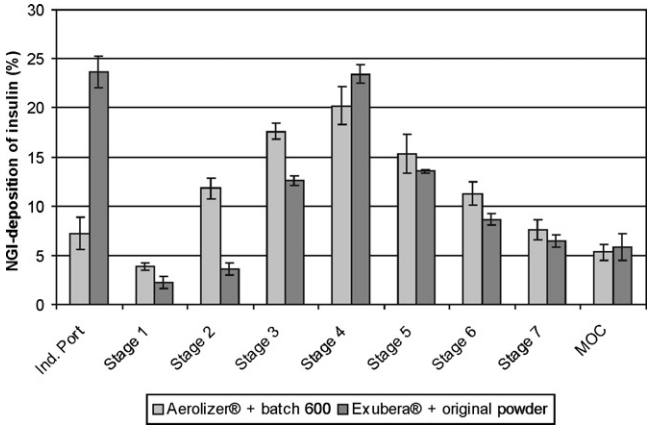

Fig. 7. NGI deposition of Exubera® with original powder compared to the Aerolizer® device with spray dried insulin, dosage 1 mg; $n = 3$, error bars indicating standard deviation.

4. Conclusions

Insulin particles were successfully precipitated into the nanometer range. For the precipitation using the solvent change method the pH-value is of particular importance. It can be assumed that other proteins in comparable size can be precipitated in the same way if the isoelectric point is considered for the precipitation process. The amount of non-solvent also influences primary particle size and behaviour, a mixture of solvent to non-solvent 1 + 1 causes particle agglomeration in suspension.

The concentration of drug in suspension and solution has a significant influence on the resulting particle size after spray drying. The less substance a sprayed droplet contains the smaller the dried particles.

No major differences between the powders spray dried out of water/methanol mixtures containing precipitated insulin were found as to the aerodynamic behaviour. Using the Aerolizer® device and HPMC capsules there is no significant difference in the residual amount of insulin in the emptied capsules, the relative FPF <5 μm and the relative systemic FPF.

The Exubera® device in combination with its original powder has by far the worst aerodynamic performance and the highest blister residue. Assuming that particles should be smaller than 2 μm to end up in the alveoli and including the ex-device fraction of about 58% to the relative systemic FPF of 41%, it can be calculated that about 23.8% of the insulin in the Exubera® 1 mg blister reach these deep regions in the lungs. Referring to data published by Pfizer about 25% of the administered drug reaches the alveoli (Pfizer Pharma, 2006) which is comparable to the results of this study.

By optimisation of the powder formulation and the device the amount of drug reaching the alveoli and, therefore, having a systemic effect can be increased. According to that the dosage could be decreased to gain the same effect, lowering the therapy costs significantly.

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