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Research paper

Pulmonary delivery of therapeutic peptides via dry powder inhalation: effects of micronisation and manufacturing

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Abstract

Pulmonary drug delivery is increasingly appreciated as a route of administration for systemically acting proteins and peptides. A respirable particle size of the drug is a key requirement, but the fragile nature of many proteins may be a limitation for the application of conventional production processes. The aim of this study was to examine the effect of different micronisation processes on the degradation and aerodynamic properties of the GnRH-antagonist cetrorelix in order to enable its application by a dry powder inhaler (Novolizer®). A modified pearl mill was used for milling in fluid propellant. Furthermore, a spray drying procedure was established using a novel process of atomisation and drying. Adhesive mixtures of lactose and 5–20% of micronised cetrorelix-acetate were prepared. Analysis by laser light scattering, HPLC, Karl Fischer, cascade impactor and scanning electron microscopy were performed to characterise the manufactured powders. Both micronisation procedures succeeded in producing small range particle size distributions, suitable for deep lung deposition (D50 = 1.6 μm for milling and 3.3 μm for spray drying). The pearl milled cetrorelix showed promising results when delivered by the Novolizer®: a reproducible and highly efficient dispersion of the drug was achieved (around 60% of aerosolised drug <5 μm). The spray dried drug was not suitable when processed as adhesive mixture.

Keywords: Peptides; Micronisation; Dry powder inhaler; Cetrorelix; Spray drying; Pearl mill; Pulmonary

1. Introduction

In the search for needle-free delivery, the administration of systemic acting drugs by inhalation is a promising alternative that has generated increasing interest over the past decade [1–3]. The thin alveolar epithelium of the lung can be effectively targeted by delivering the drug as an aerosol, with a mass median aerodynamic diameter less than 5 μm [4,5]. Many proteins and peptides with a molecular weight below 30 kDa can rapidly pass the alveolar membrane to enter the bloodstream, resulting in a high drug absorption that is achieved without the need for enhancers which are necessary for many other non-invasive routes [6].

Preparing dry powder formulations for inhalation is an attractive and appreciated proposition because many solubility and stability issues can be avoided [7,8]. Furthermore, a dry powder aerosol offers the capacity to provide a wide range of single doses per inhalation. Further advantages are the low susceptibility to microbial growth and the suitability for both water soluble and insoluble drugs [9]. However, the small particle size necessary to achieve effective lung deposition causes problems in processing (poor flowability) and redispersion (strong agglomeration and adhesion) [10,11].

Protein therapeutics require an accurate dose consistency, physico-chemical stability as well as a constant and efficient deposition in the respiratory tract [12]. Moreover, some may require the administration of relatively high doses. The production of a high-efficiency inhalation device therefore is a major issue. All passive dry powder inhalers rely on the inspiratory flow from the patient's inhalation.

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Hence, respirable fractions of the drug may be dependent on different air flow rates through the device. Since potent macromolecules are often very expensive, the development of highly efficient de-agglomeration systems within the inhaler in combination with specially designed formulations for low energy dispersion is important [13].

The majority of drugs in powders for inhalation is prepared by jet milling which can also be used for the preparation of fine particles of protein and peptide molecules [7,14,15]. Spray drying has been recognised as a successful process to generate protein-containing powders in a single step from solutions [16]. Within the spray drying process, an aerosol is usually generated by pneumatic nozzles. There are some distinct disadvantages associated with these nozzle systems, such as a control over the mean droplet size, a broad droplet distribution, and the risk of clogging in the case of suspensions. Ultrasonic nozzles are able to generate droplets with a more uniform size which could lead to a relatively homogeneous size distribution of the produced powders [17]. Using such powders may result in a more accurate delivery to the airways [18]. Supercritical fluid technology [19] and spray freeze drying [20] are also promising techniques for producing small particles of peptides and proteins. A method of liquid phase milling in fluid propellant for the preparation of peptide suspensions for pressurised metered dose inhalers has been recommended by Adjei [21]. Lizio [22] improved this technique using a modified and abrasive resistant pearl mill. Fine protein particles with a volumetric mean diameter of 3.1 μm could be produced without the generation of degradation products or contaminants deriving from the milling process. The system also allowed a direct manufacturing of fine drug suspensions for systemic aerosol delivery by pressurised metered dose inhalers [23].

The aim of the present study was to enable the pulmonary administration of a systemically acting drug using a multidose dry powder inhaler (Novolizer®). Micronisation by liquid phase milling in a pearl mill was performed as described for pressurised metered dose inhaler application in a previous study [24]. Furthermore, a unique spray drying technique was established using a novel process of atomisation and drying. Two different manufacturing techniques have been used to produce homogeneous and easy dispersible powder blends. As a model drug for this study, the decapeptide cetrorelix (GnRH-antagonist) was chosen which is already present on the market (Cetrotide® 0.25/3.0 mg subcutaneous) for the controlled ovarian superstimulation in assisted reproductive technique [25]. This choice was made for the following reasons. After subcutaneous injection, the absolute bioavailability of cetrorelix is approximately 85% [26]. Its pulmonary absorption and a clear pharmacological response after intratracheal instillation to rats have been investigated by Lizio [24]: In animal experiments an absolute bioavailability up to 75% could be achieved without the use of any absorption enhancers. On the basis of these results, it was estimated that a single dose of an inhalable product should generate a respirable dose (particles <5 μm) of at least 0.3 mg.

The in situ metered Novolizer® was used as lung delivery system for all performed studies. This dry powder inhaler is available on the European market and up to now approved for pulmonary drug administration of locally acting drugs [27]. The inhaler embodies a patented air classifier concept which uses the inspiration flow with a high efficiency and therefore enables an effective aerolisation of even highly cohesive materials [11,13]. Additionally, the device's low to medium flow resistance makes inhaling easy. Thus, even asthmatic children generate a peak inspiratory flow through the Novolizer® which exceeds 76 l/min [28].

2. Materials and methods

2.1. Materials

Cetrorelix-acetate was synthesised for preclinical pharmacokinetic studies in the laboratories of Degussa AG (Hanau, Germany). Crystalline alpha lactose monohydrate (CapsuLac® 60) was fabricated by Meggle GmbH (Wasserburg, Germany). The pearl mill micronisation was performed in Solkane® 227 (Heptafluorpropane, HFA 227, Solvay Fluor und Derivate GmbH, Hannover, Germany). Anhydrous ethanol and ethanol 96% were applied during pearl mill micronisation and supplied by Merck Eurolab GmbH (Darmstadt, Germany). Acetonitrile and trifluoracetic acid were used for HPLC analyses and supplied by Merck Eurolab GmbH.

2.2. HPLC analysis of cetrorelix-acetate

All quantitative analyses of cetrorelix-acetate were performed by reversed phase HPLC provided with a Hewlett Packard 1100 evaluation unit (Agilent Technologies Inc., Avondale, USA). The separation column was purchased from Macherey-Nagel GmbH (Düren, Germany). Details are summarised in Table 1. Also, potential degradation products resulting from the micronisation processes have been considered by HPLC.

2.3. Determination of water content

The water content of three selected formulations was analysed according to the Karl Fischer moisture method of the European Pharmacopoeia (method A) [29]. The measurements were performed with a Titrino® 701 KF (Metrohm Ltd, Herisau, Switzerland).

2.4. Scanning electron microscopy

Scanning electron microscopy (SEM) was performed by Infracor GmbH (Marl, Germany). The surface morphology

Table 1
Parameters of HPLC analysis

Separation column		Instrumental parameter	
Column material	Nucleosil C18 120-3	Injection volume (μl)	15
Particle size (μm)	3	Flow rate (ml/min)	1.0
Length of column (mm)	125	Oven temperature (°C)	45
Diameter of column (mm)	4.0	Detection wavelength (nm)	226
		Attenuation (mAU)	1000
Mobile phase	Demineralised water (ml)	Acetonitrile (ml)	Trifluoracetic acid (ml)
Eluent A: (58%)	970	30	1
Eluent B: (42%)	300	700	1

of the manufactured drug-carrier-mixtures was observed with a Philips XL30 ESEM (Philips Electron Optics, Eindhoven, The Netherlands) at a voltage of 10 kV for the pearl milled and 5 kV for the spray dried batches. Small amounts of powder samples were mounted on sample stubs and coated under vacuum with gold/palladium in a Balzers SCD004 sputter coater unit (BAL-TEC GmbH, Witten, Germany).

2.5. Particle size distribution

The particle size distribution of the micronised cetrorelix batches was determined by laser light scattering. Using a wet dispersion method the analyses were performed on a Master Sizer X (Malvern Instruments GmbH, Herrenberg, Germany), equipped with a 45 mm focus lens which covers a particle size range of 0.1–80 μm. Small amounts of powder were suspended in a mixture of cyclohexane and Span® 85. In order to deaggregate particles, the samples were treated for a short time in an ultrasonic bath before measurement. Each sample was assayed in triplicate. The standard algorithm of Malvern software based on the Mie theory was used for calculating the volumetric particle size distribution. The key parameters D10, D50 (median diameter) and D90 represent the volume diameters at the 10th, 50th and 90th percentiles of this distribution.

2.6. Micronisation by spray drying

The spray drying of cetrorelix-acetate was targeted at producing a narrow particle size range around 3.0 μm. A pilot scale spray dryer was used, constructed and operated by Degussa AG (Hanau, Germany). The spray generator at the top of the spray tower was composed of an atomiser nozzle and the required control logic. In order to control the process by optical criterion, the atomisation process was watched by a CCD-camera. The air passage was optimally adapted by a novel technology aimed at the production of monosized droplets caused by Rayleigh jet breakup conditions (filed for patent). Proteinaceous solutions of 0.016% (w/w) of cetrorelix-acetate were prepared in acetic acid (94% w/w) and continuously fed to the spray dryer at a solution flow rate of 1.35–1.55 kg/h and an aspiration of 30–33 Nl/h. During spray drying an inlet temperature of 155–165 °C and a product temperature of 49–57 °C were kept.

2.7. Micronisation by milling

A pearl mill (Dispermat® SL-C 12, VMA-Getzmann GmbH, Reichshof, Germany) was specially modified for the present study: Its milling chamber was provided with iridium-stabilised ZrO_2 as abrasion-resistant material. This was also used for coating the rotating pearls (diameter of 0.6 mm). Further on, special linking tubes for in-process testing and sample collection were positioned on the reflux tube. A cryostat (N8-KT90W, Gebrüder HAAKE GmbH, Karlsruhe, Germany) for temperatures down to −70 °C was coupled with the pearl mill and ethanol 96% was used as coolant. At the beginning of the micronisation process, cetrorelix-acetate was suspended within fluid propellant (Solkane® 227) using the share forces of an UltraTurrax® at 8000 rpm (IKA Werke, Janke & Kunkel GmbH, Staufen, Germany). The resulting suspension was filled into the modified Dispermat®. The milling process was performed according to the operative parameters described elsewhere [24]. The suspension of milled cetrorelix-acetate in Solkane® 227 was finally filled into a flask and rotated at room temperature until the fluid propellant was completely evaporated.

2.8. Preparation of adhesive mixtures for inhalation experiments

A free flowing and coarse lactose (CapsuLac® 60) was added as a carrier over which the drug particles could be distributed. The carrier and also the preparation process of the drug mixture were chosen to achieve a homogeneous distribution of the active ingredient within the powder blend.

The bulk homogeneity of each mixture was tested by taking 10 random samples (mass corresponding to the device metered mass). The drug content was determined by HPLC as described above. Afterwards, the blends were filled into the powder cartridges of the dry powder inhaler described below.

Table 2
Overview of the prepared adhesive mixtures with CapsuLac® 60 as carrier and different drug loads of pearl milled and spray dried cetrorelix-acetate, respectively

	Batch number	Preparation method	Load of cetrorelix-acetate (% w/w)	Calculated load of cetrorelix (% w/w)
Pearl mill micronisation + dry mixing	PM-20	A	20	18.4
	PM-15		15	13.8
	PM-10		10	9.2
	PM-5		5	4.6
Spray dry micronisation + dry mixing	SD-20	A	20	17.0
	SD-15		15	12.8
	SD-10		10	8.5
	SD-5		5	4.3
Pearl mill micronisation + wet mixing	PMS-10	B	11.5	10.6

Further on, sufficient flow properties for an accurate dose metering were targeted, as well as an effective de-agglomeration of the powder mixture into a mono-particulate aerosol. Table 2 gives an overview of the investigated batches which were manufactured by two different preparation methods.

2.8.1. Preparation method A (dry mixing procedure)

Micronised cetrorelix-acetate was added to the lactose-carrier and initially blended with a metallic spoon to break-up large agglomerates. After passing through a 250 μm stainless steel sieve, the mixture was blended for 60 min at 40–50 rpm using a free fall mixer. Powder blends of four different mass fractions of cetrorelix-acetate (5–20%) were prepared in batches of 20 g with pearl milled drug (batches PM-20, PM-15, PM-10, PM-5) as well as spray dried drug (batches SD-20, SD-15, SD-10, SD-5).

2.8.2. Method B (wet mixing procedure)

A suspension of cetrorelix-acetate in fluid propellant was prepared using the share forces of an UltraTurrax® for 2 min at 8000 rpm (IKA Werke, Janke and Kunkel GmbH, Staufen, Germany). The mixture was afterwards filled into a flask which contained lactose also suspended in fluid propellant. The lactose-cetrorelix suspension was rotated at room temperature (70–100 rpm) until the fluid propellant was completely evaporated. The resulting dry powder contained 11.5% cetrorelix-acetate (batch PMS-10).

2.9. Dry powder inhaler

All studies on inhalation performance were carried out with the Novolizer® (Sofotec GmbH, Frankfurt, Germany). Fig. 1 presents the main components of the device. The inhaler's cartridges were filled with the powder blends of lactose and micronised cetrorelix and placed in the device. During each actuation, the metering cavity of a cartridge was filled with formulation from the powder reservoir. A reproducible dosing was achieved through an optimal interaction between the device metering system and the powder formulation's rheological properties. In our study the metering performance of each formulation was investigated by weighing 20 doses metered by actuation. The drug dose fell into the powder channel when released from the metering cavity. During inhalation the cyclone-based circulation chamber caused a de-agglomeration of drug and carrier. Ideally an aerosol cloud of non-agglomerated single drug particles left the inhaler.

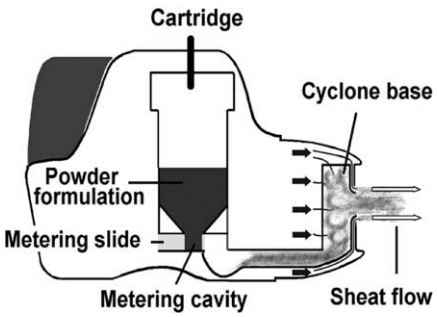

Fig. 1. Schematic design of the dry powder inhaler (Novolizer®) used within the study. The metering cavity of the cartridge is filled with formulation from the powder reservoir. A cyclone-based circulation chamber causes the de-agglomeration of drug and carrier.

2.10. Measurement of aerodynamic particle size distribution

A cascade impactor (Multistage Liquid Impinger, Copley MSLI, Copley Scientific, Nottingham, UK) was used for the in vitro measurements of fine drug particles discharged as aerosol cloud from the Novolizer®. For each analysis, two doses per batch were released from the Novolizer® and drawn through the MSLI at a flow rate corresponding to a pressure drop of 4 kPa over the inhaler (approx. 70 l/min). The fractions of cetrorelix deposited on the multistage liquid impinger's throat, stages 1–4 and filter, were collected in 30% (w/w) acetic acid and assayed by HPLC. The total dose of particles with aerodynamic diameters smaller than 5 μm was calculated and considered as the fine particle dose (μg) or fine particle fraction (% of total drug dose). Each batch was analysed in triplicate according to standard European Pharmacopoeia conditions [30].

To investigate the influence of the inhalation flow on the powder dispersion and the resulting fine particle fraction, the aerodynamic particle size analysis was determined under different airflow rates. By adapting the sampling time it was assured that a constant total volume of 4 l was drawn through the multistage liquid impinger during each measurement. The study was performed at a constant pressure drop of 4 kPa and airflow rates of 50, 70, 90 and 100 l/min were selected.

3. Results and discussion

3.1. Physical characteristics of pearl milled and spray dried cetrorelix-acetate

The resulting volumetric particle size distributions of pure cetrorelix-acetate after micronisation by pearl milling as well as spray drying are presented in Fig. 2 and Table 3. The pearl mill produced particles with a volume median diameter (D50) of 1.6 μm and 89% of all particles were smaller than 5 μm. The spray drying procedure led to a median diameter of 3.5 μm with 83% of the particles smaller than 5 μm.

All micronised batches of cetrorelix-acetate were analysed for degradation products resulting from the micronisation process. HPLC analysis showed that after micronisation by pearl milling no degradation products could be detected. It was observed that during spray drying the amount of an ornithin-derivative of cetrorelix increased with increasing temperature at the product filter. A sample exposed to nearly 60 °C at the product filter contained 1.3% of the ornithin-derivative whereas in a sample exposed to not more than 50 °C the amount of derivative was determined to be 0.6%. The batch of spray dried drug used for the further inhalation studies contained 0.7% ornithin-derivative. From the above results, it can be seen that particularly the pearl milling procedure was an effective but mild micronisation technique for cetrorelix.

The morphology and surface structure of the lactose-cetrorelix mixtures were analysed by SEM. Two batches have been selected for presentation in Fig. 3, each with a drug fraction of 20% cetrorelix-acetate. The pure milled cetrorelix-acetate consists of agglomerates of coarse and fine particles with high surface rugosity. After spray drying, the active ingredient had the typical appearance of spherical and smooth spray dried particles. A comparison of SEM pictures of the cetrorelix-lactose mixtures illustrates a completely different drug-to-carrier behaviour: Fig. 3a and b of a 20% formulation with pearl milled cetrorelix-acetate (PM-20)

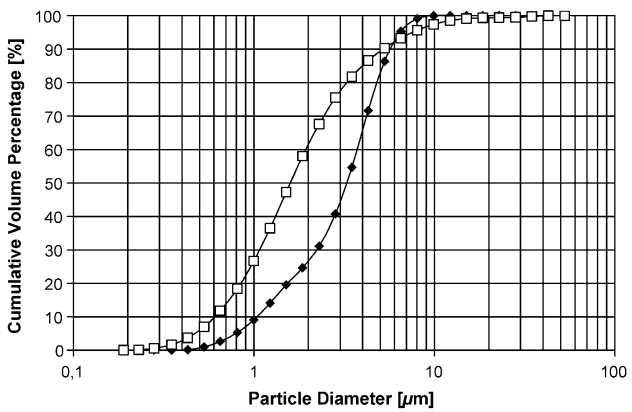

Fig. 2. Volumetric particle size distribution of pure cetrorelix-acetate after micronisation by ◆ spray drying and □ pearl milling procedure.

Table 3
Key data of the volumetric particle size distribution of pure pearl milled and spray dried cetrorelix-acetate: particle size which contains 10 (D10) and 90% (D90) of the total volume of material, median diameter (D50) and % of total volume material smaller than 5 μm

Micronised cetrorelix-acetate	D10 (μm)	D50 (μm)	D90 (μm)	<5 μm (%)
Pearl milled	0.60	1.58	5.15	89
Spray dried	1.03	3.25	5.65	83

shows that the lactose surface is almost completely covered with cetrorelix particles. Depending on the loading rate, the carrier was more or less completely surrounded and smoothed by the fines of the drug. Regarding the formulations with spray dried material (Fig. 3c and d), the carrier surface is sparsely covered with particles of the drug, as shown for the 20% formulation (SD-20). Obviously the selected lactose carrier and the mixing procedure were insufficient to distribute the spray dried particles over the carrier's surface and to break up the drug's strong agglomerates.

3.2. Performance of the formulations

Reproducible metering of an exact dose from the cartridge's powder bulk was determined for each formulation. The results are presented in Fig. 4. All inhalation powders containing pearl milled or spray dried cetrorelix-acetate, can be handled from low up to high percentage formulations with acceptable influence of the drug load on the metered masses: None of the determinations was outside the specified range of 75–125% of the mean value. However, most formulations with spray dried cetrorelix (SD-20, SD-15, SD-10) showed high relative standard deviations (>5%). This difference between spray dried and pearl milled batches may be explained by the scanning electron micrographs (Fig. 3c and d): a significant fraction of the spray dried cetrorelix remained agglomerated and was not bound to the carrier surface at all. This may cause a disturbance of the formulation's free flowing behaviour and a risk of segregation. In contrast to this, a drug load of 5–20% pearl milled cetrorelix could be homogeneously distributed over the carrier's surface. Neither the formulation's flow behaviour nor its resulting dose uniformity (relative standard deviation of around 2.5%) was significantly affected by loading with the pearl milled cetrorelix.

The fine particle fractions that were generated from the different formulations by the Novolizer® are given in Fig. 5. The correspondent fine particles doses are presented in Fig. 6. Obviously, the dispersion characteristic is strongly dependent on the previous micronisation and mixing procedure. The formulations with pearl milled cetrorelix prepared according to method A (PM-5/10/15/20) released at least 28.5% of the loaded drug dose as fine drug particles (Fig. 5). The 20% blend of milled drug (PM-20) delivered

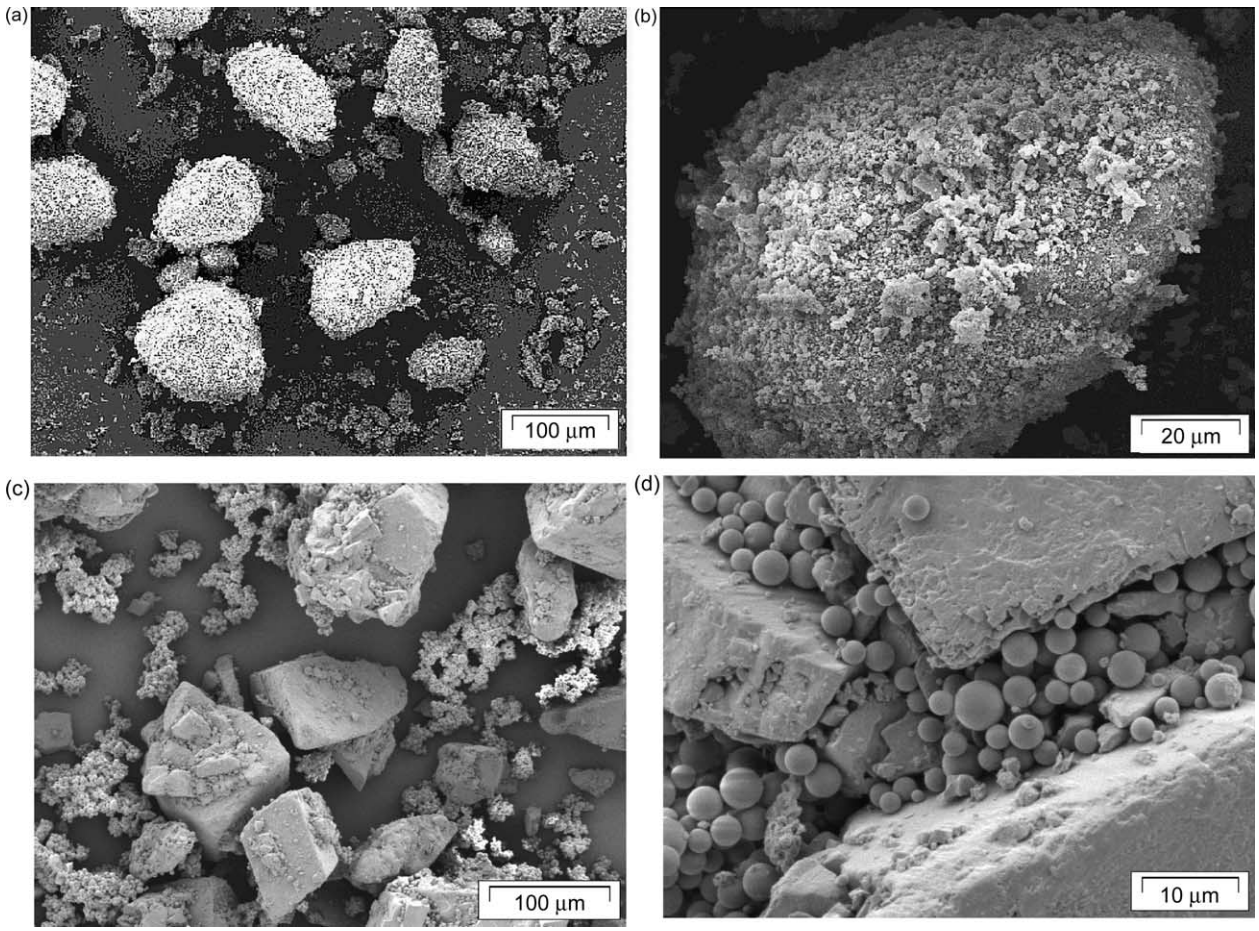

Fig. 3. Scanning electron micrographs of adhesive mixtures (preparation method A) containing CapsuLac® 60 as lactose carrier. (a, b) Mixture with 20% of pearl milled cetrorelix-acetate. (c, d) Mixture with 20% of spray dried cetrorelix-acetate.

45.8% of the dose of particles <5 μm in size. As can be seen from Fig. 6 only the drug load of 5% milled cetrorelix-acetate (PM-5) did not reach the target dose of at least 0.3 mg per single dose application.

In contrast to these very promising data the dispersion characteristics of batches with spray dried cetrorelix showed a poor generation of fine drug particles: a maximum of 16% fine particle fraction was found (Fig. 5). None of the formulations came up to the target of 0.3 mg/dose (Fig. 6). Even for the 20% drug load (SD-20) less than 0.1 mg/dose was within the desired particle size range. Approximately 40% of the spray dried drug remained in the throat of

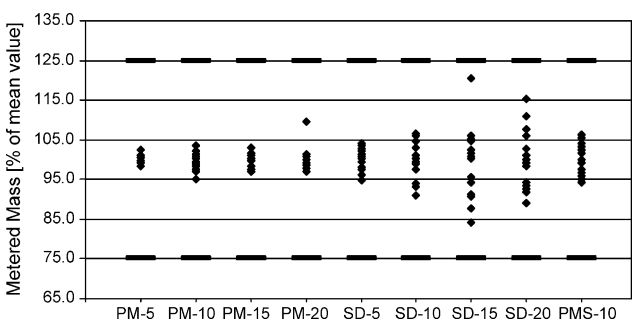

Fig. 4. Metering performance of adhesive mixtures of CapsuLac® 60 with different mass fractions of cetrorelix-acetate: (a) pearl milled 5% (PM-5), 10% (PM-10), 15% (PM-15), 20% (PM-20); (b) spray dried: 5% (SD-5), 10% (SD-10), 15% (SD-15), 20% (SD-20); (c) pearl milled and suspended: 10% (PMS-10). Metered masses in percent of the mean value of the respective batch ($n = 10$). Dashed lines: specified upper and lower limits of the metered mass (75–125% of mean value).

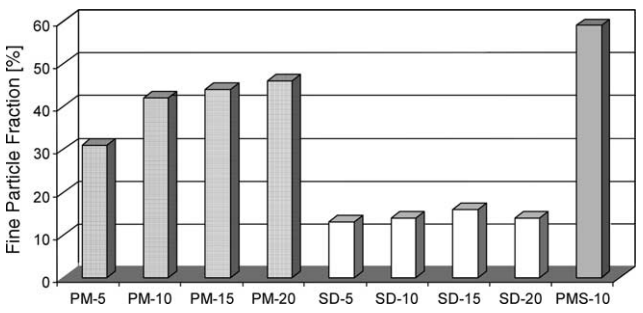

Fig. 5. Fine particle fraction (FPF) of the cetrorelix inhalation powders released from the Novolizer® and collected by multistage liquid impinger. FPF values (mass fraction of total drug dose) include particles with an aerodynamic diameter <5 μm. Formulations contained a mass fraction of cetrorelix-acetate: (a) pearl milled 5% (PM-5), 10% (PM-10), 15% (PM-15), 20% (PM-20); (b) spray dried: 5% (SD-5), 10% (SD-10), 15% (SD-15), 20% (SD-20); (c) pearl milled and suspended cetrorelix-acetate: 10% (PMS-10).

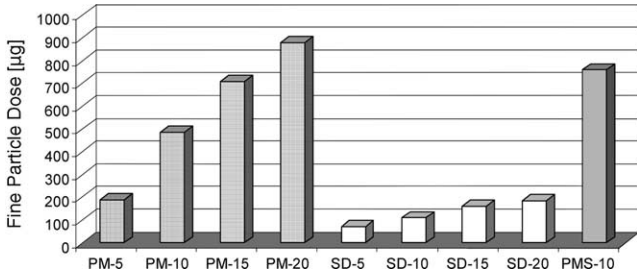

Fig. 6. Fine particle dose (FPD) of the cetrorelix inhalation powders released from the Novolizer® and collected by multistage liquid impinger. FPD values (μg) include particles with an aerodynamic diameter <5 μm. Formulations contained a mass fraction of cetrorelix-acetate: (a) pearl milled 5% (PM-5), 10% (PM-10), 15% (PM-15), 20% (PM-20); (b) spray dried: 5% (SD-5), 10% (SD-10), 15% (SD-15), 20% (SD-20); (c) pearl milled and suspended cetrorelix-acetate: 10% (PMS-10).

the multistage liquid impinger. Again, the observed results could be attributed to agglomerates of the spray dried drug. Thus, it appears that the de-agglomeration of powders containing pearl milled drug is quite more effectively performed than de-agglomeration of the spray dried drug.

The preparation step of resuspending the pearl milled cetrorelix in fluid propellant and afterwards mixing it with lactose (method B) turned out to be a significant improvement of preparation method A. As can be seen from Fig. 5, highest fine particle fractions of about 59% were obtained for the formulation PMS-10. Compared to formulation PM-20 (20% drug load via method A), almost the same fine particle drug dose of around 800 μg was achieved for the PMS-10 with a drug load of 10% only (Fig. 6). This is an important factor especially for expensive drugs.

The strong enhancement in fine particle dose of drug prepared via the suspension of drug and carrier (method B) may be explained by electrostatic phenomena. Probably, the wet mixing procedure reduced the electrostatic charge of the pearl milled cetrorelix and improved its de-agglomeration tendency. Therefore, the in vitro deposition profile of the formulation PMS-10 showed a better de-agglomeration and dispersion of cetrorelix when compared to the correspondent dry mixed formulations. Another explanation for the improvement found might be a difference in the interaction between drug and carrier, caused by the different mixing process. It could be possible that the wet mixing process results in less press-on forces compared to the dry mixing process. As a result the mixture produced by wet mixing shows a higher de-agglomeration [11]. Moisture content of the mixtures (see Table 4) may also adversely affect the fine

Table 4
Water content analysed by Karl Fischer moisture method

	PM-10 (%)	SD-10 (%)	PMS-10 (%)
Water content	5.07	5.76	5.27

Formulations contained a mass fraction of 10% cetrorelix-acetate from pearl milling (PM-10), spray drying (SD-10) or pearl milling and suspension (PMS-10).

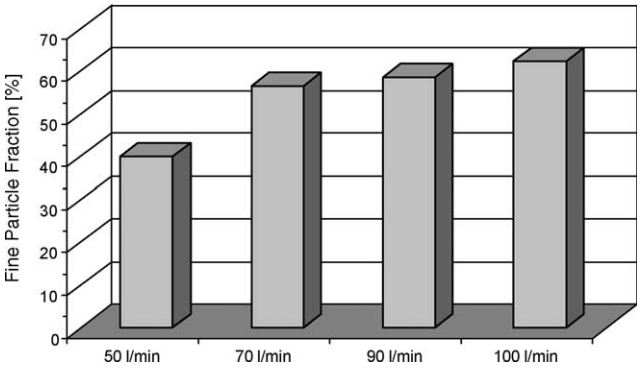

Fig. 7. Influence of different airflow rates on the aerodynamic particle size distribution. Fine particle fraction (FPF) of the formulation PMS-10 (pearl milled cetrorelix via wet suspension) released from the Novolizer® and collected by multistage liquid impinger. FPF values (mass fraction of total drug dose) include particles with an aerodynamic diameter <5 μm.

particle dose. In the present study this can be one reason for the low de-agglomeration of the formulation SD-10 with spray dried material (FPF 14%, water content 5.76%). However, moisture does not explain the strong difference in fine particle results of formulation PM-10 (FPF 42%, water content 5.07%) and formulation PMS-10 (FPF 59%, water content 5.27%). Exploring the influence of amorphicity in this connection will be part of a further study.

The formulation PMS-10 was taken for evaluating the influence of the inhalation flow rate on the powder formulations' de-agglomeration. This formulation with 10% pearl milled and subsequently suspended cetrorelix was selected because of its promising fine particle results and the profitable ratio of expected bioavailable to non-available drug amount. Only a flow rate of 50 l/min resulted in a slightly reduced fine particle fraction of about 40% (Fig. 7). A very low recovery of drug substance (emitted dose below 80% of the nominal dose) due to an insufficient delivery and material adhesion within dosage passage may be the main reason. At normal operating flow rates of 70–100 l/min a constant fine particle fraction of 57–62% of the drug was found. These results seem to indicate that within the range of airflow rates typically generated by a patient through the Novolizer®, the inhalation manoeuvre of a patient may not have a significant impact on the respirable drug amount.

4. Conclusions

Pearl milling was an effective but mild micronisation technique for the decapeptide cetrorelix. Formulated as highly loaded adhesive mixture, high fine particle fractions could be generated with the de-agglomeration system of a multidose dry powder inhaler (Novolizer®). The best performance was shown by a formulation manufactured via wet mixing of a drug-carrier-suspension (formulation PMS-10). Fine particle fractions of about 60% were

obtained. Within the range of airflow rates typically generated in vivo through the Novolizer®, no significant effect on the dispersion and the resulting amount of respirable drug was determined.

Cetrorelix micronised by spray drying was not suitable when subsequently processed as adhesive mixture. For those mixtures a rather low generation of respirable drug was determined (16% fine particle fraction at maximum). This could be explained by the distinct formation of agglomerates visible on scanning electron micrographs. These drug agglomerates could not be de-aggregated during mixing with a carrier or its subsequent inhalation.

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