European Journal of Pharmaceutics and Biopharmaceutics 70 (2008) 828–838

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Quality by design – Spray drying of insulin intended for inhalation

Morten Jonas Maltesen [a,b,*], Simon Bjerregaard [b], Lars Hovgaard [b], Svend Havelund [b], Marco van de Weert [a]

[a] Department of Pharmaceutics and Analytical Chemistry, University of Copenhagen, Copenhagen, Denmark
[b] Department of Preformulation and Delivery, Novo Nordisk A/S, Måløv, Denmark

ARTICLE INFO

Article history:
Received 4 June 2008
Accepted in revised form 27 July 2008
Available online 8 August 2008

Keywords:
Spray drying
Insulin
Inhalation
Design of experiments
Multivariate data analysis
Quality by design
Particle characteristics

ABSTRACT

Quality by design (QBD) refers to a holistic approach towards drug development. Important parts of QBD include definition of final product performance and understanding of formulation and process parameters. Inhalation of proteins for systemic distribution requires specific product characteristics and a manufacturing process which produces the desired product. The objective of this study was to understand the spray drying process of insulin intended for pulmonary administration. In particular, the effects of process and formulation parameters on particle characteristics and insulin integrity were investigated. Design of experiments (DOE) and multivariate data analysis were used to identify important process parameters and correlations between particle characteristics. The independent parameters included the process parameters nozzle, feed, and drying air flow rate and drying air temperature along with the insulin concentration as a formulation parameter. The dependent variables included droplet size, geometric particle size, aerodynamic particle size, yield, density, tap density, moisture content, outlet temperature, morphology, and physical and chemical integrity. Principal component analysis was performed to find correlations between dependent and independent variables. Prediction equations were obtained for all dependent variables including both interaction and quadratic terms. Overall, the insulin concentration was found to be the most important parameter, followed by inlet drying air temperature and the nozzle gas flow rate. The insulin concentration mainly affected the particle size, yield and tap density, while the inlet drying air temperature mainly affected the moisture content. No change was observed in physical and chemical integrity of the insulin molecule.

1. Introduction

Quality by design (QBD) encompasses designing and developing formulations and manufacturing processes which ensure predefined product specifications. An important part of QBD is to understand how process and formulation parameters affect product characteristics, and subsequent optimisation of these parameters with respect to the final specifications [1]. Therefore, critical parameters should be identified in order to monitor these parameters online in the production process. Thus, QBD is a holistic concept where final product specifications, manufacturing process and critical parameters are included in order to ease the final approval and the ongoing quality control of a new drug [2].

Pulmonary delivery has been stated as an attractive alternative to the subcutaneous and intravenous administration routes for proteins, which are presently the most frequent used administration routes for proteins. The large surface area and a thin lung epithelium in the deep lungs make the absorption of proteins easier compared with other noninvasive routes [3]. The deposition of particles in the deep lungs is controlled by a number of parameters including geometry of the airways, breathing behaviour of the individual and clearance mechanisms of the lungs. As a result, the inhaled particles must be designed to reach the deep lungs by overcoming the different natural defence mechanisms. The most important parameter for the deposition of particles in the deep lungs is the aerodynamic particle size, which should be in the range of 1–4 μm for optimal delivery [3]. Particles with the optimal aerodynamic particle size can be manufactured in several different ways, including milling and spray drying [4]. Spray drying has attracted great attention in the recent years and currently the only approved systemic protein drug administered through the lungs is manufactured by spray drying [5,6]. However, this product was recently removed from the market due to disappointing sales.

The advantages of spray drying are many and include the possibility to control particle size and particle size distribution, as well as other particle characteristics. In addition, the heat stress to which the proteins are exposed during the drying process is often negligible due to the short residence time in the drying chamber [4,7]. Furthermore, spray drying is a one step continuous drying process, which utilises less energy than freeze drying and thus makes it an attractive manufacturing process in the industry [8].

* Corresponding author. Department of Pharmaceutics and Analytical Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark. Tel.: +45 35 33 61 40; fax: +45 35 33 60 30.
E-mail address: mjm@farma.ku.dk (M.J. Maltesen).

0939-6411/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2008.07.015

Spray drying of insulin is popular in the industry, but little has been published regarding process knowledge and design. Exubera is produced by spray drying an insulin solution combining several excipients [6], while Stahl et al. examined the effect of different process parameters when spray drying an insulin solution without excipients [9]. However, the insulin concentration has so far not been incorporated in the experimental design, and the effect of insulin concentration has therefore not been addressed.

Design of experiments (DOE) is a well-established method for identifying important parameters in a process and optimising the parameters with respect to certain specifications [10]. Several studies have utilised DOE on the spray drying process [9,11,12], where the effect of process parameters on various particle characteristics have been studied. However, these studies have all focused on single prediction equations obtained from the statistical analysis and have not utilised multivariate data analysis.

The present study focuses on a deeper understanding of the spray drying process of insulin. The effect of the formulation parameter insulin concentration is investigated, in addition to the process parameters nozzle, feed and drying air flow rate and drying air temperature. The dependent variables include well-described variables such as geometric particle size, density, morphology, outlet temperature, moisture content, and physical and chemical degradation. However, less well-described variables such as aerodynamic particle size and droplet size are addressed as well. DOE and multivariate data analysis are utilised to generate maximum information and lead to a better understanding of the spray drying process of insulin.

2. Materials and methods

2.1. Materials

2Zn human insulin was kindly supplied by Novo Nordisk A/S. Deionized water was filtered using a Millipore system (Millipore, Billerica, MA, USA). Insulin solutions were prepared by lowering the pH to 2.5 with 0.2 M icecold HCl, which is below the isoelectric point of the insulin monomer (5.3) and hexamer (6.4) [13]. After dissolution of the insulin, the pH was adjusted to 8.0 with 0.2 M icecold NaOH and the concentration was adjusted to 60 mg/mL. For 30 and 5 mg/mL the stock solution was diluted with water.

2.2. Spray drying

Spray drying was performed with a Büchi B-290 spray dryer (Büchi Labortechnik AG, Postfach, Switzerland). The spray drying process was performed according to standard procedures and the design was similar to the process illustrated by Mosen et al. [14]. The humidity of the inlet drying air was controlled and kept below 20%. The nozzle, a two fluid design with nitrogen as atomising gas used in a co-current mode, was standard equipment provided with the Büchi B-290 spray dryer. The orifice diameter was 0.7 mm. Spray dried particles were separated from the drying air by a high-performance cyclone provided by Büchi. The process parameters investigated were nozzle gas flow rate (7.3–17.5 L/min), feed flow rate (1.8–5.25 mL/min), drying air temperature (75–220 °C) and aspirator capacity (80–100%) (see Table 1 for details). The temperature of the outlet drying gas (T_{out}) was measured between the drying chamber and the cyclone. The spray dried powders were stored in vials at 5 °C and at a relative humidity of 20%.

Table 1
Process and formulation parameters included in the CCF design

Parameter		Low level	Centre level	High level
Nozzle gas flow rate (L/min)	N	7.3	11.1	17.5
Feed flow rate (mL/min)	F	1.8	3.6	5.25
Inlet air temperature (°C)	T_{in}	75	150	220
Aspirator capacity (%)	A	80	90	100
Insulin concentration (mg/mL)	I	5	30	60

2.3. Droplet size

The droplet size and droplet size distribution were analysed by laser diffraction using a Malvern Spraytec (Malvern Instruments Ltd., Malvern, UK). The nozzle was placed in horizontal position and the laser was focused in the centre of the spray. Gravitational settling of the droplets was neglected due to the high velocity of the spray compared to the short distance between nozzle tip and laser beam. The distance between nozzle and Fourier lens was 50 mm and the distance between nozzle tip and the laser beam was 30 mm. The setup was calibrated with water before each experiment. The running time for each experiment was 30–60 s with a sampling frequency of 2 Hz. The mass median diameter is used as the droplet diameter (D).

2.4. Aerodynamic particle size

The aerodynamic particle size was analysed by a time of flight principle with an Aerodynamic Particle Sizer 3321 (TSI Incorporated, Shoreview, MN, USA). The aerodynamic particle size is given as mass median aerodynamic diameter (MMAD).

2.5. Geometric particle size

The median particle size was analysed by laser diffraction with a Helos system (Sympatec GmbH, Clausthal-Zellerfeld, Germany). The powder was dispersed in isopropanol with Tween 20 and the optical density of the dispersion was adjusted to approx-

Table 2
CCF experimental design

Run No.	Parameters				
	N (L/min)	F (mL/min)	T_{in} (°C)	A (%)	I (mg/mL)
1	11.1	5.25	150	90	30
2	17.5	5.25	75	80	5
3	17.5	5.25	75	100	60
4	7.3	5.25	75	100	5
5	11.1	3.6	150	90	30
6	17.5	3.6	150	90	30
7	11.1	3.6	75	90	30
8	11.1	3.6	150	90	30
9	17.5	5.25	220	80	60
10	7.3	3.6	150	90	30
11	11.1	1.8	150	90	30
12	7.3	5.25	220	80	5
13	11.1	3.6	220	90	30
14	17.5	1.8	220	100	60
15	7.3	1.8	220	100	5
16	17.5	1.8	75	80	60
17	7.3	5.25	75	80	60
18	11.1	3.6	150	90	30
19	11.1	3.6	150	90	30
20	7.3	1.8	220	80	60
21	17.5	5.25	220	100	5
22	11.1	3.6	150	90	5
23	11.1	5.25	150	90	30
24	17.5	1.8	75	100	5
25	11.1	3.6	150	90	60
26	11.1	3.6	150	100	30
27	7.3	1.8	75	100	60
28	11.1	3.6	150	80	30
29	7.3	1.8	75	80	5
30	17.5	1.8	220	80	5
31	7.3	5.25	220	100	60

imately 10% prior to measuring. The used wavelength was 632.8 nm. The particle size was given as the mass median diameter (MMD).

2.6. Density

The poured (d_p) and tapped densities (d_t) of the powder were measured with a tapped density tester (Antech Solutions Ltd., Waterford, Ireland). The analysis was performed according to the test for apparent volume in the European Pharmacopeia [15], except a 10 mL glass cylinder was used due to lack of sufficient protein powder. The powder was gently poured through a funnel into the glass cylinder to a volume of approximately 5 mL. The volumes were read before and after 1250 taps. The density before tapping was designated poured density and the density after tapping was designated tapped density.

2.7. Particle morphology

The particle shape and surface morphology were investigated by scanning electron microscopy (SEM) using a Quanta 200 (FEI Company, Hillsboro, OR, USA). The acceleration voltage was 10 kV and magnifications from 1000× to 20,000× were used for all samples. The powder was sprinkled on a SEM stub, covered with adhesive carbon tape and sputter coated with gold prior to scanning. The signal is composed of secondary electrons.

The interior of the spray dried particles was analysed by focused-ion-beam (FIB) milling using a Zeiss CrossBeam 1540 FIB/SEM dual beam system (Carl Zeiss NTS GmbH, Oberkochen, Germany). Milling of the spray dried particles was achieved using gallium ions accelerated at 30 kV and a current of 100 pA. The ion beam was mounted at an angle of 54° to the electron beam. The setup allowed imaging with secondary electrons from the sample. To minimize electrical charging (samples were not coated) electrons were accelerated at a low voltage of 3 kV.

2.8. Moisture content

The moisture content in the powder samples was analysed by thermogravimetric analysis with a TGA 7 (Perkin-Elmer Inc., Waltham, MA, USA) with nitrogen purging. The powder sample weighing approximately 2 mg was heated from 20 to 250 °C at a rate of 10 °C/min. The weight loss observed between 20 and 160 °C was assigned to water evaporation from the powder and the weight change in per cent was defined as the moisture content of the powder. Weight losses occurring after 160 °C were probably due to thermal decomposition of insulin observed by browning and subsequent blackening of the powder.

2.9. Process yield

The yield (Y) was defined as the ratio between the amount of powder obtained corrected for moisture content and the amount of protein introduced in the liquid feed. The unit was per cent by weight (%).

2.10. Degradation products

The content of soluble irreversible aggregates, i.e., high molecular weight proteins (HMWP), was analysed by size exclusion chro-

Table 3
Overview of dependent variables measured for the 31 experiments

ID Exp. No.	Responses											
	T_{out} (°C)	D (μm)	MMD (μm)	MMAD (μm)	Y (%)	d_p (g/cm^3)	d_t (g/cm^3)	Moisture (%)	SEM type	HMWP (%)	Desamido (%)	OIRC (%)
1	63	16.40	4.86	3.56	0.71	0.12	0.23	5.17	2	0.20	1.10	0.09
2	32	9.81	3.84	1.54	0.45	0.12	0.16	7.45	3	0.30	0.89	0.14
3	44	14.75	6.64	3.62	0.80	0.24	0.26	8.46	1	0.24	0.85	0.10
4	37	24.25	4.94	2.83	0.43	0.13	0.16	7.91	3	0.28	0.87	0.09
5	54	15.99	4.15	3.08	0.82	0.12	0.19	5.69	3	0.21	0.92	0.12
6	57	11.65	3.73	2.57	0.75	0.11	0.15	5.14	2	0.22	0.88	0.11
7	43	15.99	4.52	3.30	0.87	0.16	0.21	7.75	2	0.24	0.85	0.09
8	52	15.99	4.42	2.80	0.75	0.11	0.17	5.69	2	0.21	0.86	0.09
9	63	14.75	5.99	3.13	0.75	0.11	0.16	4.81	1	0.20	0.86	0.09
10	58	26.08	6.30	3.77	0.81	0.13	0.24	5.62	2	0.21	0.86	0.11
11	59	15.55	3.92	2.72	0.79	0.11	0.17	4.82	2	0.21	0.86	0.11
12	65	24.25	5.08	2.44	0.78	0.07	0.11	6.54	3	0.23	0.92	0.14
13	67	15.99	4.51	2.89	0.75	0.10	0.17	5.00	2	0.21	0.86	0.11
14	69	13.90	5.75	2.42	0.68	0.09	0.13	4.90	1	0.22	0.88	0.10
15	81	23.40	3.83	2.01	0.67	0.08	0.11	4.29	3	0.29	0.89	0.10
16	44	13.90	6.68	3.02	0.73	0.17	0.21	7.22	1	0.22	0.90	0.13
17	38	29.18	21.35	5.81	0.50	0.15	0.19	9.23	1	0.22	0.89	0.10
18	49	15.99	4.20	3.09	0.75	0.12	0.18	6.88	2	0.21	0.90	0.13
19	53	15.99	4.36	3.15	0.78	0.13	0.21	6.22	2	0.21	0.89	0.09
20	77	28.33	9.42	4.12	0.78	0.11	0.17	5.32	2	0.32	0.88	0.10
21	67	9.81	2.78	1.74	0.55	0.10	0.13	5.68	3	0.25	0.88	0.09
22	57	13.75	3.15	1.78	0.58	0.10	0.14	8.73	3	0.27	0.89	0.12
23	51	16.40	4.23	3.01	0.83	0.13	0.19	6.75	2	0.25	0.92	0.10
24	39	8.97	2.53	1.66	0.49	0.09	0.11	8.32	3	0.30	0.96	0.11
25	42	18.69	7.85	3.57	0.81	0.14	0.19	8.05	1	0.22	0.90	0.13
26	51	15.99	4.63	3.15	0.81	0.11	0.19	7.01	2	0.22	0.90	0.09
27	44	28.33	13.65	5.19	0.81	0.17	0.26	7.14	1	0.22	0.90	0.13
28	42	15.99	4.79	3.49	0.83	0.16	0.20	9.00	2	0.26	0.92	0.11
29	39	23.40	4.96	3.35	0.82	0.12	0.17	7.20	3	0.29	0.90	0.10
30	64	8.97	2.23	1.38	0.31	0.08	0.10	4.78	3	0.25	0.91	0.12
31	67	29.18	11.37	3.93	0.80	0.09	0.14	5.06	1	0.22	0.94	0.11
Min	32	8.97	2.23	1.38	0.31	0.07	0.10	4.29	1	0.20	0.85	0.09
Max	81	29.18	21.35	5.81	0.87	0.24	0.26	9.23	3	0.32	1.10	0.14

The abbreviations are explained in Section 2.

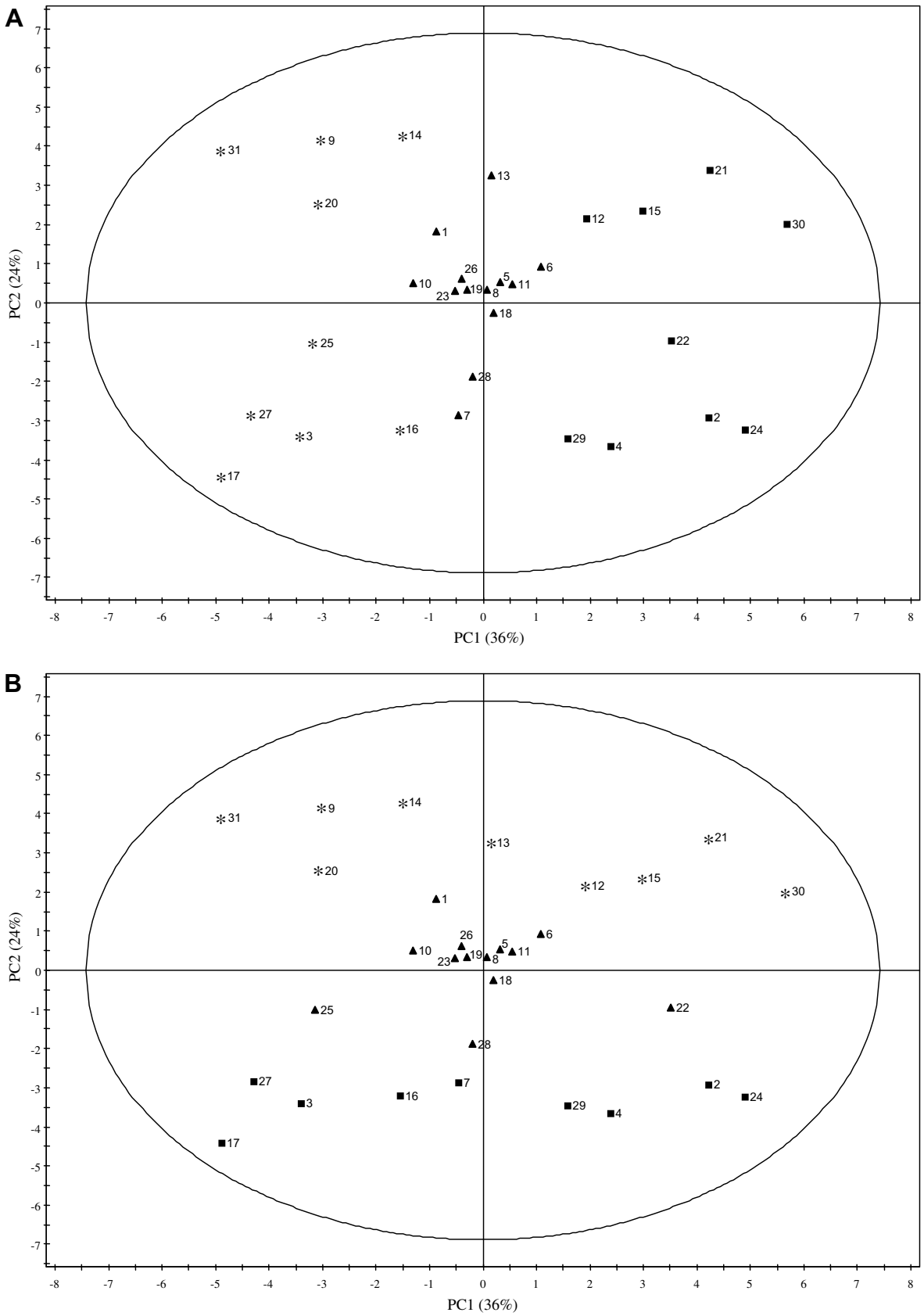

Fig. 1. Score plot from the principal component analysis with PC1 on the horizontal and PC2 on the vertical axis. Each experiment is placed according to the influence of PC1 and PC2. The ellipse indicates a 95% confidence interval. (A) Score plot labelled according to the insulin concentration: 5 mg/mL (■), 30 mg/mL (▲), and 60 mg/mL (∗). (B) Score plot labelled according to inlet drying air temperature 75 °C (■), 150 °C (▲), and 220 °C (∗).

matography (SEC-HPLC) on a Waters Alliance system (Waters Corporation, Milford, MA, USA). The samples were reconstituted to a concentration of 4 mg/mL, and 50 μL was injected on the column (Insulin HMWP column, 7.8 × 300 mm, Waters Corporation, Milford, MA, USA) at a flow rate of 0.5 mL/min. The eluent was isocratic and consisted of 15% (v/v) glacial acetic acid and 20% (v/v) acetonitrile. The sample temperature was kept at 7 °C while the column was equilibrated to room temperature. Detection was performed at 276 nm.

The content of deamidation products (desamido) and other insulin related compounds (OIRC) was analysed by reversed phase high-performance liquid chromatography (RP-HPLC) on a Waters Alliance system (Waters Corporation, Milford, MA, USA). The samples were reconstituted to a concentration of 4 mg/mL, and 10 μL was injected on the column (Sunfire C18, 3.5 μm, 150 mm × 4.6 mm, Waters Corporation, Milford, MA, USA) at a flow rate of 1.0 mL/min. The method is based on an isocratic elution with 0.8% (v/v) sodium sulphate, 0.1% (v/v) phosphoric acid and 22.7% acetonitrile. The sample temperature was kept at 7 °C while the column was equilibrated to 35 °C. Detection was performed at 214 nm. The desamido content includes Asp^{B3}, $isoAsp^{B3}$, and Asp^{A21}.

2.11. Experimental design

A central composite face centred design (CCF) was created in SAS-JMP (SAS Institute Inc., Cary, USA) to investigate the parameters nozzle gas flow rate (N), feed flow rate (F), inlet drying air temperature (T_{in}), inlet drying air flow rate (A) and insulin concentration (I). Due to factor constraints some factor levels were modified. The different levels are given in Table 1. The CCF was chosen since it is possible to obtain a full quadratic prediction model from the results. The design is described in details by Myers and Montgomery [16]. The CCF used consisted of 31 total experiments. Sixteen experiments represented a two-level fractional factorial design (2^{5-1} design). Ten experiments were star points with a high and low level for each parameter, and five experiments were centrepoints. The levels of the different process parameters were chosen to yield the largest possible design space. The ranges of nozzle gas flow rates and inlet drying air temperatures were chosen as broad as possible and feed flow rate and aspirator rate were chosen so the drying process was feasible. The experimental design and conditions are given in Table 2 including process parameters and insulin concentration.

Prediction models were estimated using the least squares method and are given according to the following equation

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii}(X_i - \mu_i)^2 + \sum \beta_{ij}(X_i - \mu_i)(X_j - \mu_j) + \varepsilon \quad (1)$$

where Y is the predicted response, β is the parameter estimate, μ is the mean of the parameter, X is the independent parameter and ε is the residual error. Second-order parameter estimates were centred to obtain independent main effects. Parameters which were found significant at a 95% confidence level were included in the final prediction models. Main effects which were present in the significant second-order effects were included, even if they were not significant to themself. In order to evaluate the importance of each effect, t-ratios were used and the effect was tested. Models were accepted when there was no lack of fit, no correlation in the residual plots and the residuals were normally distributed.

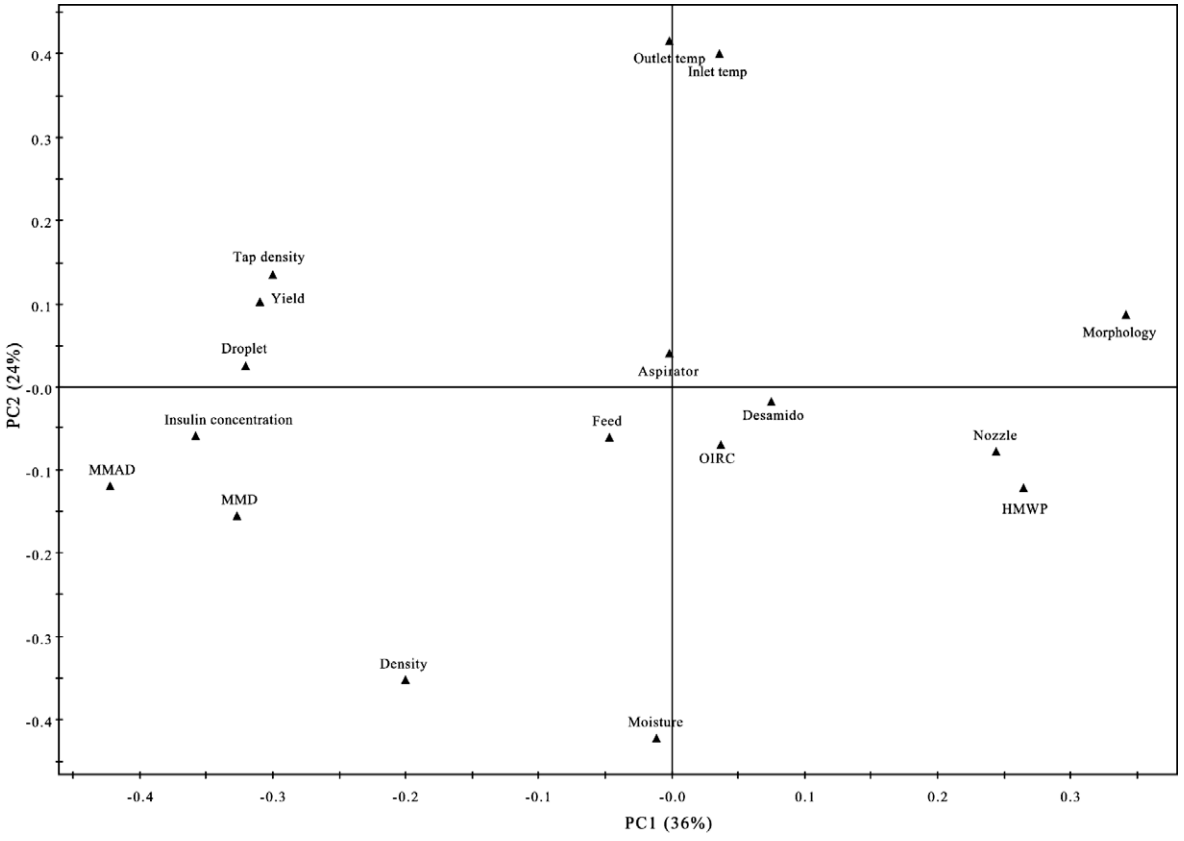

Fig. 2. Loading plot of PC1 and PC2. A loading plot describes the relationship between observations (experiments) and variables. High score on the horizontal plane indicates a high influence on PC1, whereas a high score on the vertical plane indicates a high influence on PC2. Variables located close to each other on PC1 or PC2 are positively correlated and variables located opposite each other on PC1 or PC2 are negatively correlated.

Table 4
Importance of process and formulation parameters, interaction terms and quadratic terms on the responses

Parameter	T_{out}	D	MMD	MMAD	Y	d_p	d_t	Moisture	SEM	HMWP	Desamido	OIRC
N		**−17.27**	−8.92	−14.55	−4.55	*0.75*	*−1.44*	*1.40*		*−0.90*		
F	*−1.73*[a]	2.22	*2.59*	3.35	*−1.62*	*1.76*	*0.07*	2.09		*−1.68*		
T_{in}	**12.85**[b]		−3.68	−8.46	*1.41*	**−9.36**	−6.29	**−9.66**		*−1.36*		
A	*1.73*		*−1.74*	−2.50	*0.18*	*0.28*		*−1.36*		*−0.57*		
I		6.27	**11.34**	**21.64**	**8.27**	6.42	**7.38**	−1.12	**−15.88**	**−4.80**		
$N \times N$		6.66	5.79	2.57				−3.88				
$N \times F$					4.81	3.11	3.23					
$N \times T_{in}$			2.45	4.76	−3.37							
$N \times A$				3.00	2.42			3.11				
$N \times I$			−4.81	−3.54	5.05	2.20				−2.25		
$F \times F$	2.71[c]							−3.88				
$F \times T_{in}$					6.01							
$F \times A$			−2.71			2.92						
$F \times I$				2.15			3.18			−2.31		
$T_{in} \times T_{in}$												
$T_{in} \times A$												
$T_{in} \times I$			−2.85	−3.14		−3.97	−2.38			2.56		
$A \times A$	−2.19			2.37				3.28				
$A \times I$			2.10		2.91							
$I \times I$				−4.46	−7.98		−4.79	4.34		4.48		
R^2	0.88	0.97	0.94	0.98	0.94	0.89	0.85	0.89	0.90	0.74	0.24	0.23

The t-ratio for each term is stated. The abbreviations are explained in Section 2.
[a] Parameter t-ratios in italic are not significant but are present in higher order factors and are therefore included in the model.
[b] Parameter t-ratios in bold indicate that the parameter is the most significant factor for the measured variable.
[c] Underscored t-ratios indicate that the parameter is the second most significant factor for the measured variable.

2.12. Principal component analysis

Principal component analysis (PCA) computed with Simca-P v. 11.5 (Umetrics AB, Umeaa, Sweden) was used to visualize differences and correlations in the spray dried samples. The variables were scaled to unit variance and centred prior to the PCA. PCA is a projection method used to reduce complexity and to visualize patterns in complex data sets. The data are arranged in N rows (observations) and K columns (variables) and the number of dimensions in the full data set is K. A vector is introduced in the K dimensional space which spans the largest variation. This vector is denoted the first principal component (PC). Once the first PC has been identified (PC1), the second vector orthogonal to the first PC and accounting for the second largest variation is estimated (PC2). The algorithm continues until no additional variation is explained by adding additional PCs. A new coordinate system, where the principal components form the axes, is introduced and the original data set (X) can be described according to the following equation

$$X = TP^{T} + E \quad (2)$$

where P is the principal components, T is the coordinates of each observation projected onto the principal components and E is the residual error. A score plot is based on the coordinates for each observation (T), while a loading plot is based on the direction of the principal components (P). An introduction to PCA is given by Wold et al. [17].

3. Results and discussion

Results of the measured particle characteristics and HPLC analyses of the spray dried insulin are given in Table 3. All 31 spray drying experiments produced white noncohesive powders. There was a visible but varying amount of powder deposited on the walls in the drying chamber and the cyclone in all 31 experiments, but the specific amount was not addressed further. The insulin flow rate varied between 9 and 315 mg/min depending on the insulin concentration and feed flow rate. As a consequence the time for the spray drying experiments varied with a factor 35 between the shortest and the longest run. This difference is important when designing the final manufacturing process.

3.1. Principal component analysis

In order to obtain a visual overview of interactions between process parameters and variables, principal component analysis was performed. The main objective of PCA is to reduce complexity of the data set by introducing principal components. In the score plot of PC1 and PC2 there is no evident clustering of the experiments, which is in accordance with the nature of DOE, since the design space is constructed to be as large as possible. When the experiments are labelled according to inlet temperature or insulin concentration a clear trend is observed. The largest separation is observed along PC1, which coincides with the differences in insulin concentration (Fig. 1A). Experiments with a low insulin concentration are grouped to the right and experiments with a high insulin concentration are grouped to the left. The experiments are grouped according to the inlet drying air temperature along PC2, where experiments with high inlet drying air temperature are located at the top, and experiments with a low drying air temperature are located at the bottom (Fig. 1B). The two first principal components explain 36% and 24%, respectively, of the total variation in the data set. The third principal component explains 14% of the total variation, which primarily is due to the variation in nozzle gas flow rate and droplet size (not shown). Thus, the first three principal components describe 74% of the total variation in the data set, which originates from insulin concentration (PC1), inlet drying air temperature (PC2) and nozzle gas flow rate (PC3).

To further visualise the correlation between process parameters and measured variables a two dimensional loading plot can be obtained for PC1 and PC2 (Fig. 2). A loading plot describes the most important variables for each sample. Thus, the importance of the different variables is described as the projection of the variable in the loading plot on the horizontal axis (PC1) and the vertical axis (PC2). Parameters and variables located close to each other or at opposite sides are correlated. In contrast, parameters or variables located perpendicular to each other are not correlated. Insulin con-

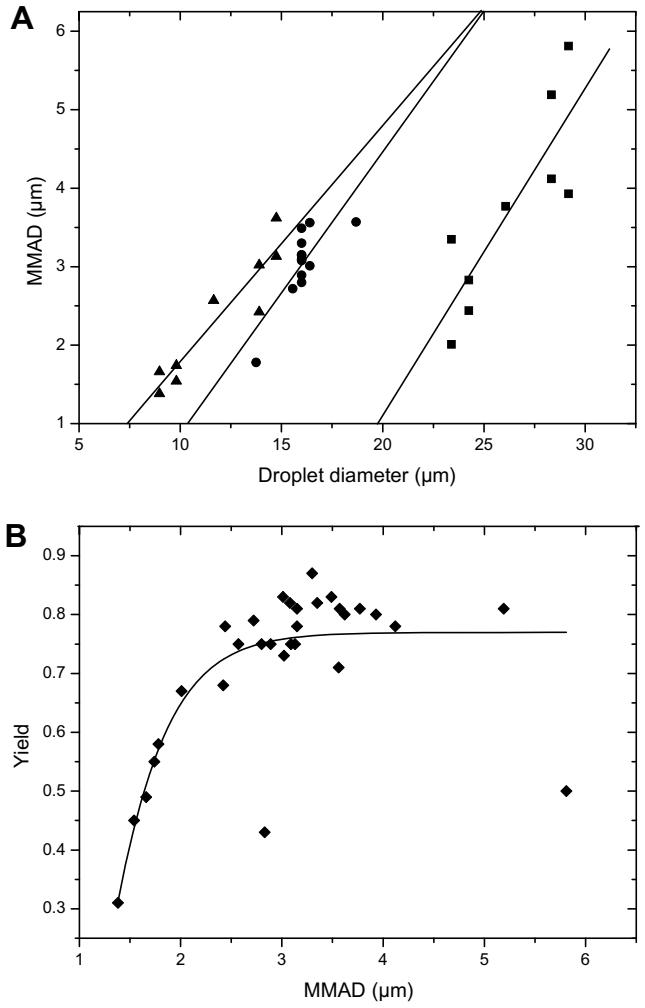

Fig. 3. (A) Correlation between droplet diameter and aerodynamic particle size. The experiments are labelled according to the nozzle gas flow rate: 7.3 L/min (■), 11.1 L/min (●), and 17.5 L/min (▲). (B) Correlation between yield and aerodynamic particle size of spray dried powders.

centration is the process parameter with the highest numerical score on PC1, which is in agreement with the score plot, where the experiments are grouped horizontally according to the insulin concentration. Variables which are strongly dependent on the insulin concentration are also placed with a high numerical score on the PC1 axis, whereas variables less dependent on the insulin concentration are placed with a low numerical score. Variables mainly influenced by the insulin concentration include MMAD, MMD, yield, tap density, droplet size, HMWP, and morphology (SEM) (Fig. 2). MMAD, MMD, yield, tap density and droplet size all increase with increasing insulin concentration, whereas HMWP decreases with increasing insulin concentration. The process parameter with the highest numerical score on PC2 is the inlet drying air temperature, which again is in good agreement with the score plot where the experiments are grouped vertically according to the inlet drying air temperature on the PC2 axis. The inlet drying air temperature mainly affects the outlet air temperature and the moisture content of the spray dried powders (Fig. 2). The variables tap density, yield and MMD are mainly dependent on the insulin concentration and to the same extent as indicated by similar scores on PC1. However, the variables are separated according to PC2 as a result of the different effects of the drying air temperature on the three variables (Fig. 2). Powder density is influenced equally by insulin concentration and inlet drying air temperature. The process parameters aspirator rate and feed flow rate are located near the origin together with the variables OIRC and desamido content (Fig. 2). The location near zero indicates that neither PC1 nor PC2 describes the variation in these parameters and variables. Therefore, the variation is not related to other parameters or variables located in other parts of the coordinate system. Thus, aspirator rate and feed flow rate are the least important parameters for the spray drying process of insulin under the given conditions. It should be mentioned that it is not possible to identify quadratic terms in PCA.

PCA indicates that, in relation to QBD, the insulin concentration and inlet drying air temperature must be designated critical parameters for the spray drying process of insulin intended for inhalation. These two parameters are the most important factors for the final particle characteristics and should thus be optimised. Furthermore, these two parameters should be monitored closely during the manufacturing process, since variations will likely result in significant variations in the final batch quality.

3.2. Particle characteristics

To further evaluate the effect of the process and formulation parameters, a statistical prediction model for each dependent variable was obtained including interaction and quadratic terms. Interaction terms indicate that the effect on a variable produced by changing one parameter level depends on the level of the other parameter in the interaction term. Quadratic terms indicate a minimum or approach to a minimum if the term is positive or a maximum or approach to maximum if the term is negative. An overview of the prediction models is given in Table 4. The variation explained by the prediction models ranged from 0.85 to 0.98 for the particle characteristics.

3.2.1. Insulin concentration

The PCA analysis indicated that the variables MMD, MMAD, yield, tap density, HMWP content, and morphology were mainly affected by the insulin concentration. These findings are in good agreement with the obtained prediction equations where the insulin concentration is the most significant parameter for the variables MMD, MMAD, yield, tap density, HMWP content, and morphology. The significant effect of the insulin concentration can in most cases be explained by the critical concentration of the solid during the spray drying process. The critical concentration is considered to be the solubility of the spray dried solid at the wet bulb temperature [18,19] and is mainly dependent on formulation parameters which influence the solubility [20]. Thus, in addition to the insulin concentration, parameters affecting the solubility of insulin, such as the concentration of NaCl and ethanol, should be included when a new spray drying process is designed.

First it should be noted that there is a correlation between droplet size and MMAD (Fig. 3A). This finding supports the assumption that one droplet dries to one particle [14]. Thus, the nozzle gas flow rate is an important process parameter, since it has the most significant effect on the droplet size due to an increase in kinetic energy available for the atomisation process. A negative correlation was observed between nozzle gas flow rate and droplet size which levelled off at high nozzle gas flow rates. The reason for the nonlinear dependency is probably that a lower limit for the droplet size is reached at the investigated energy levels, and thus, any further increase in nozzle gas flow rate does not decrease the droplet size. Furthermore, the droplet size is dependent on the insulin concentration due to an increase in viscosity at higher insulin concentrations.

The particle sizes MMAD and MMD are some of the most complex particle characteristics to predict, and are affected by different

parameters. Even with the energy input from the nozzle gas flow rate the particle size is mainly affected by the insulin concentration in the feed solution. The effect levels off at high insulin concentrations for the MMAD, whereas a linear relationship was observed between the insulin concentration and the MMD. The reason for the significant effect of the insulin concentration is due to an increased solid content in the droplets. A correlation between solid content and particle size is expected under the assumption that one droplet dries to one particle. The linear correlation between MMD and insulin concentration can be explained by a shell or crust formation when a specific concentration is reached, due to the effect on the solvent evaporation [8,21]. In contrast, the aerodynamic particle size also depends on the drying conditions after the critical concentration is reached.

The insulin concentration had the most significant effect on the yield. The yield and the insulin concentration were positively correlated with the effect levelling off at high insulin concentrations. The effect of the insulin concentration on the yield can be explained by the limitations of the cyclone used in the spray drying experiments. The efficiency of the cyclone has previously been reported to drop dramatically when spray drying particles in the size range for inhalation [14]. In this study the yield was highly dependent upon the aerodynamic particle size below 2.5 μm (Fig. 3B). To increase the yield of the spray drying process a different type of cyclone should be used to ensure a better separation for particles in the desired size range. Another possibility is to use a filter instead of a cyclone [18,19].

Three distinct morphologies were observed for the spray dried powders including spherical shaped particles (I), wrinkled particles (II), and highly folded particles (III). The morphology was designated based on SEM pictures without knowledge of the experimental conditions (Fig. 4A–C). The morphology of the particles was solely dependent on the insulin concentration. High insulin concentrations resulted in particles with type I morphology, medium insulin concentration resulted in particles with type II morphology and low insulin concentrations resulted in particles with type III morphology. These findings are in agreement with other studies, where high solid concentration resulted in smooth particles and low solid concentration resulted in wrinkled particles [11]. For the highly folded particles it was difficult to differentiate distinct particles and it visually looked as if smaller particles had merged into greater particles. The aerodynamic particle sizer is able to deagglomerate most dry particles, and since the larger size of the merged particles was not observed during the MMAD analysis, the merging was probably a phenomenon related to the SEM analysis procedure. In the literature the morphology of spray dried proteins has primarily been stated as highly folded particles corresponding to type III

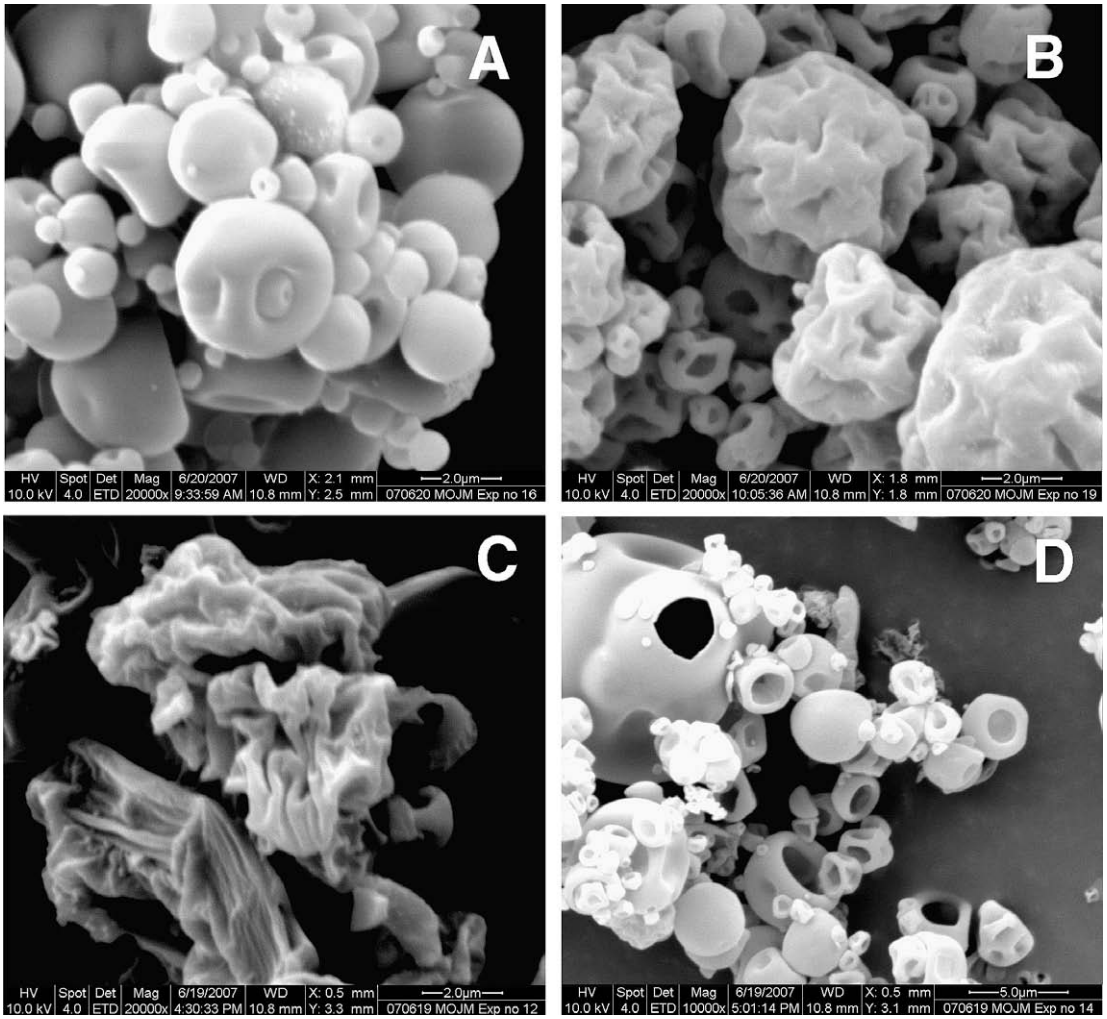

Fig. 4. Scanning electron microscopy pictures of spray dried insulin resulting in different morphologies. (A) SEM picture of spray dried insulin obtained from a 60 mg/mL feed solution (designated type 1 morphology). (B) SEM picture of spray dried insulin obtained from a 30 mg/mL feed solution (designated type 2 morphology). (C) SEM picture of spray dried insulin obtained from a 5 mg/mL feed solution (designated type 3 morphology). (D) SEM picture of spray dried insulin where the shell is blown.

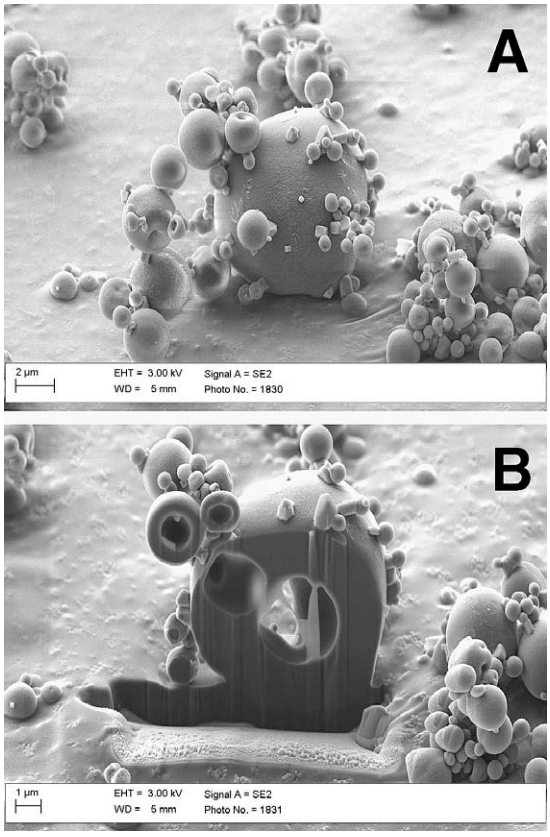

Fig. 5. Scanning electron microscopy pictures of spray dried insulin obtained from a 60 mg/mL feed solution. (A) SEM picture before FIB milling. (B) SEM picture after FIB milling. The more crystalline entities are sodium chloride crystals.

[6,11,14,21–23] but spherical shaped particles (I) are not uncommon [11,21,22,24]. In order to evaluate the morphology further, the interior of the particles was analysed with FIB milling [25,26]. After milling off the surface, distinct holes appeared in the interior of the particles. The holes were randomly distributed with the majority located in the middle of the particles (Fig. 5). Furthermore, the formed shell seemed to be solid without pores (Fig. 5). In this study, the morphology was solely dependent on the insulin concentration and not on the rate of evaporation, which is dependent on the temperature, solvent and humidity as reported by Ameri and Maa [21]. It is reasonable that the liquid feed composition, and not just the concentration, has a pronounced effect on the morphology of the spray dried particles, which can be explained by a film formation at the surface of the droplet [21]. Thus, an increase in feed concentration results in a concentrated film at the surface and therefore smoother and spherical shaped particles. At lower concentration the film is more diluted and is not able to maintain the spherical form. The film properties are primarily dependent on the protein and surfactant used in the feed solution and will reflect interactions at the solvent-air interface [20,22].

3.2.2. Inlet temperature

The outlet air temperature is mainly influenced by the inlet air temperature as seen in both PCA and the prediction model for the outlet air temperature. In addition to the inlet air temperature, feed flow rate and aspirator rate have a minor, but significant, effect according to the prediction model. Feed flow rate had a negative effect on the outlet air temperature levelling off at high feed flow rates. Aspirator rate had a positive effect on the outlet air temperature levelling off at high aspirator rates. The findings are reasonable, since both high inlet air temperature and aspirator rates increase the energy available for the drying process and thus are expected to increase the outlet air temperature [8]. An increase

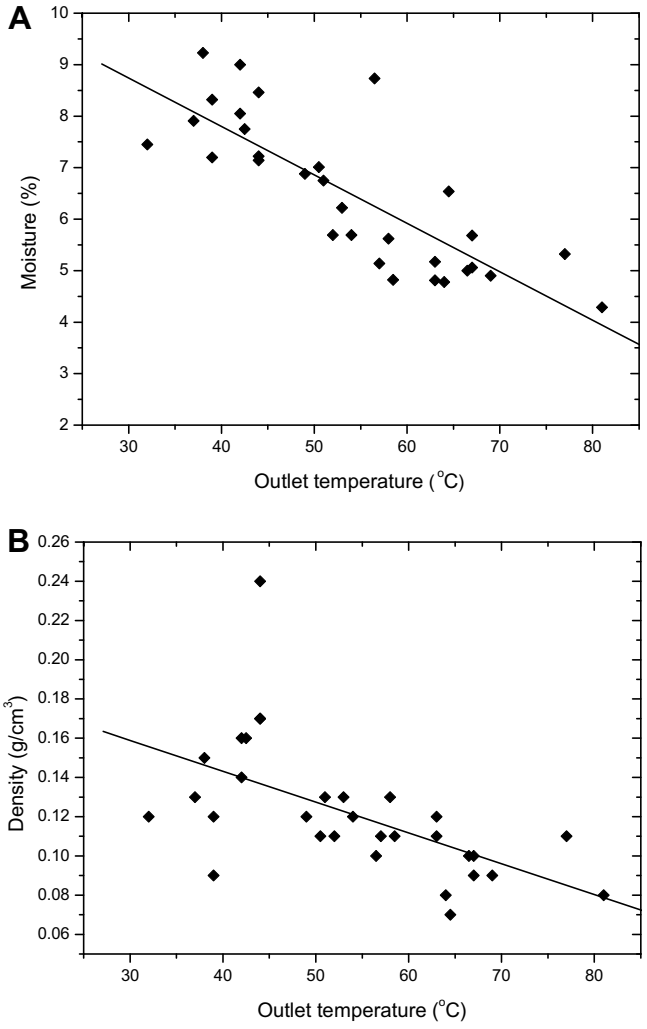

Fig. 6. Effect of outlet air temperature. (A) Correlation between outlet temperature and moisture content in spray dried powders. (B) Correlation between density and outlet air temperature.

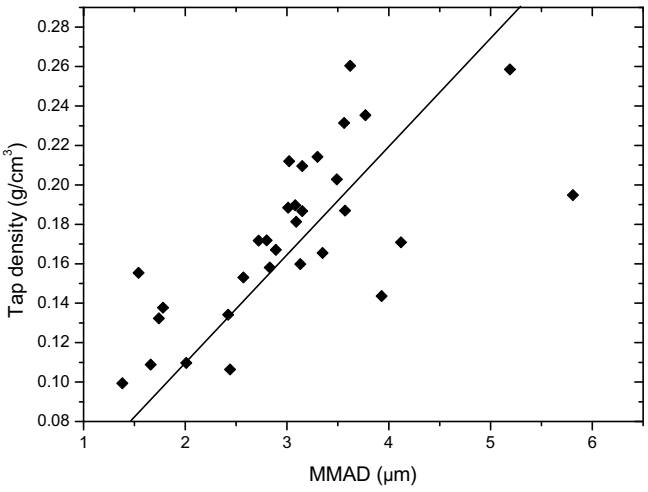

Fig. 7. Tapped density as a function of aerodynamic particle size.

in feed flow rate increases the amount of solvent available for evaporation and thus decreases the outlet air temperature. In general, the mass transfer of water vapour from the droplet and the heat transfer from the drying air to the droplet are similar during the initial phase of the drying period. This drying period is termed the constant drying rate phase and is characterised by a constant droplet temperature and steady state appearance. At the critical concentration a shell or crust is formed at the surface and the mass transfer becomes limited [19,21]. The consequence is a higher heat transfer compared to mass transfer, which results in a higher particle temperature while the drying rate decreases (falling drying rate phase) [8]. An increase in feed rate and solvent available for evaporation prolongs the constant drying rate phase. This is due to a longer time period before the critical concentration is reached, and decreases the falling drying rate phase, thus decreases the outlet temperature. In contrast, an increase in inlet air temperature shortens the period before the critical concentration is reached and thus increases the outlet air temperature. In addition, the outlet air temperature is dependent on the total residence time of each particle, which must be taken into account when scaling up the process.

The moisture content in the spray dried powders varied between 4.29% and 9.23%. As may be expected, the inlet air temperature was the most important parameter and was negatively linearly correlated with the moisture content. This was found with both PCA and statistical analysis. The maximal temperature of drying air in contact with the particles during the drying process is that of the outlet air temperature [8]. Thus, the final drying of the particle is forced by the temperature difference between the temperature when the critical concentration in the droplet is reached and the outlet drying air temperature, making the outlet drying air temperature an important parameter for drying capacity and moisture content (Fig. 6A) [9]. Most of the solvent in the droplet is evaporated during the constant drying rate phase, before the critical concentration is reached and the solvent mobility is restricted. This moisture removal in the constant drying phase is mainly dependent on temperature, humidity and solvent [8,21]. The final moisture content of spray dried particles is mainly affected during the falling drying rate phase after the critical concentration has been reached and the particle has been formed. Thus, the moisture content is dependent on the nature of the solid as well as the temperature, humidity, and solvent. Furthermore, the particle residence time and the time point of particle formation are important parameters for moisture content and especially when scaling up the spray drying process [8]. It has been shown previously that moisture content of the powder, in addition to the ability to take up water, is important for the long term stability of proteins [6,27].

3.2.3. Insulin concentration and inlet temperature

Both the insulin concentration and the inlet temperature have a significant influence on the poured and tapped density as seen from PCA and statistical analysis. The poured densities ranged from 0.07 to 0.24 g/cm^3 and the tapped densities ranged from 0.10 to 0.26 g/cm^3, which is in agreement with the literature [23]. A high inlet temperature results in a shell formation at the surface of the droplet before all solvent evaporates [20]. This is a consequence of the nonsteady state phase, where the solvent is not transferred fast enough to the surface of the droplet. The shell formation explains the hollow interior of the spray dried particles (Fig. 5). The trapped solvent inside the shell still needs to evaporate and if the shell is impenetrable and flexible the volume of the particles increases [8]. The increase is driven by the outlet air temperature (Fig. 6B) and the result is enlarged inflated particles with a lower density. In extreme cases the outer shell can burst and the particle becomes damaged. For these burst particles it is observed that the inflated particles are hollow and not solid (Fig. 4D). In addition, an increased outlet temperature decreases the moisture content in the dried particles, which tends to decrease the density [8]. The insulin concentration is linearly correlated with the particle density, as an increase of solid content in the droplet increases the density of spray dried particle. The tap density is linearly correlated with the aerodynamic particle size (Fig. 7). This finding is in agreement with the definition of aerodynamic particle size and is supported in the literature [23,28].

3.3. Protein integrity

The protein integrity is essential for the use of proteins as drugs and must be addressed in new formulations. The content of HMWP ranged from 0.20% to 0.32% in spray dried powders. The HMWP content was mainly correlated with the insulin concentration, and levelled off at higher insulin concentrations. The explained variation in the HMWP content by the prediction model was low (R^2 = 0.74). The contents of desamido insulin and OIRC were 0.85–1.10% and 0.09–0.14%, respectively. There was no significant correlation between the process parameters and the desamido insulin and the OIRC contents in neither PCA nor statistical analysis. These results indicate that there is no significant increase in covalent aggregates or chemical degradation for the present data set. The variations reported were small and insignificant compared with overall variation in the data set and the control (HMWP 0.70%, desamido 1.47%, and OIRC 0.10%). Thus, neither the nozzle gas flow rate nor the inlet temperature caused any degradation, which has been reported earlier [9,24,29,30]. This is surprising, since the maximal inlet drying air temperature used in our experiments was 220 °C, which is high for spray drying of proteins. On the other hand, the process parameters resulted in an outlet drying air temperature of no higher than 81 °C, while others have shown that insulin is stable during spray drying up to an outlet temperature of 120 °C [9].

4. Conclusion

Characteristics of spray dried insulin particles were described by design of experiments and multivariate data analysis. The investigated process parameters were nozzle gas flow rate, feed flow rate, drying air flow rate, inlet drying air temperature, and insulin concentration. Insulin concentration was found to be the most important parameter for the powder characteristics, followed by the inlet drying air temperature and the nozzle gas flow rate. Feed flow rate and aspirator rate were not found significant. Insulin integrity was analysed by RP-HPLC and SEC-HPLC, but no relationship was found between process parameters and degradation products. The results indicate that formulation parameters are at least as important as process parameters when spray drying proteins. In particular, parameters affecting the critical concentration are important when designing a proper process for spray drying proteins.

Design of experiments and multivariate data analysis proved to be useful tools for QBD and were able to identify important parameters and variable correlations.

Acknowledgement

We thank Pia Wahlberg for assistance with focused-ion-beam measurements.

References

[1] L.X. Yu, Pharmaceutical quality by design: product and process development, understanding and control, Pharm. Res. 25 (2008) 781–791.

[2] P. McKenzie, S. Kiang, J. Tom, A.E. Rubin, M. Futran, Can pharmaceutical process development become high tech, AIChE J. 52 (2006) 3990–3994.
[3] J.S. Patton, P.R. Byron, Inhaling medicines: delivering drugs to the body through the lungs, Nat. Rev. Drug Discov. 6 (2007) 67–74.
[4] S.A. Shoyele, S. Cawthome, Particle engineering techniques for inhaled biopharmaceuticals, Adv. Drug Deliv. Rev. 58 (2006) 1009–1029.
[5] S. Bellary, A.H. Barnett, Inhaled insulin (Exubera): combining efficacy and convenience, Diab. Vasc. Dis. Res. 3 (2006) 179–185.
[6] S. White, D.B. Bennett, S. Cheu, P.W. Conley, D.B. Guzek, S. Gray, J. Howard, R. Malcolmson, J.M. Parker, P. Roberts, N. Sadrzadeh, J.D. Schumacher, S. Seshadri, G.W. Sluggett, C.L. Stevenson, N.J. Harper, EXUBERA: pharmaceutical development of a novel product for pulmonary delivery of insulin, Diab. Technol. Ther. 7 (2005) 896–906.
[7] A.H. Chow, H.H. Tong, P. Chattopadhyay, B.Y. Shekunov, Particle engineering for pulmonary drug delivery, Pharm. Res. 24 (2007) 411–437.
[8] K. Masters, Spray Drying in Practice, SprayDryConsult International ApS, Copenhagen, Denmark, 2002.
[9] K. Stahl, M. Claesson, P. Lilliehorn, H. Linden, K. Backstrom, The effect of process variables on the degradation and physical properties of spray dried insulin intended for inhalation, Int. J. Pharm. 233 (2002) 227–237.
[10] L. Xie, H. Wu, M. Shen, L.L. Augsburger, R.C. Lyon, M.A. Khan, A.S. Hussain, S.W. Hoag, Quality-by-design (QbD): effects of testing parameters and formulation variables on the segregation tendency of pharmaceutical powder measured by the ASTM D 6940-04 segregation tester, J. Pharm. Sci. (2008), doi:10.1002/jps.21320 (Epub ahead of print).
[11] K.B. Prinn, H.R. Costantino, M. Tracy, Statistical modeling of protein spray drying at the lab scale, AAPS PharmSciTech 3 (2002) E4.
[12] P. Tewa-Tagne, G. Degobert, S. Briancon, C. Bordes, J.Y. Gauvrit, P. Lanteri, H. Fessi, Spray-drying nanocapsules in presence of colloidal silica as drying auxiliary agent: formulation and process variables optimization using experimental designs, Pharm. Res. 24 (2007) 650–661.
[13] N.C. Kaarsholm, S. Havelund, P. Hougaard, Ionization behavior of native and mutant insulins: pK perturbation of B13-Glu in aggregated species, Arch. Biochem. Biophys. 283 (1990) 496–502.
[14] K. Mosen, K. Backstrom, K. Thalberg, T. Schaefer, H.G. Kristensen, A. Axelsson, Particle formation and capture during spray drying of inhalable particles, Pharm. Dev. Technol. 9 (2004) 409–417.
[15] European Directorate for the Quality of Medicines and Healthcare, Pharmaceutical Technical Procedures 2.9.15 Apparent Volume, The European Pharmacopoeia, vol. 6, 2007.
[16] R.H. Myers, D.C. Montgomery, Response Surface Methodology: Process and Product Optimization using Designed Experiments, Wiley, New York, 2002.
[17] S. Wold, K. Esbensen, P. Geladi, Principal component analysis, Chemometr. Intell. Lab. Syst. 2 (1987) 37–52.
[18] J. Elversson, A. Millqvist-Fureby, G. Alderborn, U. Elofsson, Droplet and particle size relationship and shell thickness of inhalable lactose particles during spray drying, J. Pharm. Sci. 92 (2003) 900–910.
[19] J. Elversson, A. Millqvist-Fureby, Particle size and density in spray drying-effects of carbohydrate properties, J. Pharm. Sci. 94 (2005) 2049–2060.
[20] R. Vehring, W.R. Foss, D. Lechuga-Ballesteros, Particle formation in spray drying, J. Aerosol Sci. 38 (2007) 728–746.
[21] M. Ameri, Y.F. Maa, Spray drying of biopharmaceuticals: stability and process considerations, Dry. Technol. 24 (2006) 763–768.
[22] Y.F. Maa, S.J. Prestrelski, Biopharmaceutical powders: particle formation and formulation considerations, Curr. Pharm. Biotechnol. 1 (2000) 283–302.
[23] Y. You, M. Zhao, G.L. Liu, X. Tang, Physical characteristics and aerosolization performance of insulin dry powders for inhalation prepared by a spray drying method, J. Pharm. Pharmacol. 59 (2007) 927–934.
[24] C. Anandharamakrishnan, C.D. Rielly, A.G.F. Stapley, Effects of process variables on the denaturation of whey proteins during spray drying, Dry. Technol. 25 (2007) 799–807.
[25] D. Heng, P. Tang, J.M. Cairney, H.K. Chan, D.J. Cutler, R. Salama, J. Yun, Focused-ion-beam milling: a novel approach to probing the interior of particles used for inhalation aerosols, Pharm. Res. 24 (2007) 1608–1617.
[26] S.H. Moghadam, R. Dinarvand, L.H. Cartilier, The focused ion beam technique: a useful tool for pharmaceutical characterization, Int. J. Pharm. 321 (2006) 50–55.
[27] A.A. Elkordy, R.T. Forbes, B.W. Barry, Stability of crystallised and spray-dried lysozyme, Int. J. Pharm. 278 (2004) 209–219.
[28] C. Bosquillon, P.G. Rouxhet, F. Ahimou, D. Simon, C. Culot, V. Preat, R. Vanbever, Aerosolization properties, surface composition and physical state of spray-dried protein powders, J. Control. Release 99 (2004) 357–367.
[29] M. Mumenthaler, C.C. Hsu, R. Pearlman, Feasibility study on spray-drying protein pharmaceuticals: recombinant human growth hormone and tissue-type plasminogen activator, Pharm. Res. 11 (1994) 12–20.
[30] M. Yang, S. Velaga, H. Yamamoto, H. Takeuchi, Y. Kawashima, L. Hovgaard, M. van de Weert, S. Frokjaer, Characterisation of salmon calcitonin in spray-dried powder for inhalation. Effect of chitosan, Int. J. Pharm. 331 (2007) 176–181.