International Journal of Pharmaceutics 408 (2011) 248–256

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical Nanotechnology

New insights into respirable protein powder preparation using a nano spray dryer

K. Bürki[a], I. Jeon[a], C. Arpagaus[b], G. Betz[a,*]

[a] Industrial Pharmacy Laboratory, Department of Pharmaceutical Sciences, University of Basel, Mülhauserstrasse 51, 4056 Basel, Switzerland
[b] BÜCHI Labortechnik AG, Meierseggstrasse 40, 9230 Flawil, Switzerland

ARTICLE INFO

Article history:
Received 2 September 2010
Received in revised form 4 February 2011
Accepted 8 February 2011
Available online 16 February 2011

Keywords:
Spray drying
Nano spray dryer
Inhalation
β-Galactosidase
Trehalose

ABSTRACT

In this study the Nano Spray Dryer B-90 (BÜCHI Labortechnik AG, Flawil, Switzerland) was evaluated with regard to the drying of proteins and the preparation of respirable powders in the size range of 1–5 μm. β-Galactosidase was chosen as a model protein and trehalose was added as a stabilizer. The influence of inlet temperature, hole size of the spray cap membrane and ethanol concentration in the spray solution was studied using a 3^3 full factorial design. The investigated responses were enzyme activity, particle size, span, yield and shelf life. Furthermore, the particle morphology was examined.

The inlet temperature as well as the interaction of inlet temperature and spray cap size significantly influenced the enzyme activity. Full activity was retained with the optimized process. The particle size was affected by the hole size of the spray cap membrane and the ethanol content. The smallest cap led to a monodisperse particle size distribution and the greatest yield of particles of respirable size. Higher product recovery was achieved with lower inlet temperatures, higher ethanol contents and smaller cap sizes. Particle morphology differed depending on the cap size. The protein exhibited higher storage stability when spray dried without ethanol and when a larger spray cap size was used.

1. Introduction

Peptides and proteins are of increasing importance in today's drug therapy. Due to the recent and fast developments in biotechnology they have become available in large quantities and the sales volume of biopharmaceuticals has increased constantly over the last decades.

Currently, almost all commercially available protein formulations are designed for injection-based application (Sollohub and Cal, 2010). This invasive administration is a major inconvenience for patients and limits the product's potential on the market. The development of non-invasive applications is, therefore, desirable. Many routes of administration have been investigated and pulmonary delivery has shown to be a promising one. It is non-invasive and painless, which leads to a better patient compliance. The alveolar region of the lung offers a huge surface area (approximately 100 m^2) for systemic absorption of the drug. It has only a thin epithelial barrier and is free of mucus and cilia. Furthermore, pulmonary delivery allows avoiding the first-pass metabolism (DeFelippis et al., 2007). Different therapeutic proteins and peptides have been reported to show substantial systemic absorption in animals when they are administered to the lung (Agu et al., 2001). However, for an efficient deposition of the dry powder in the alveolar region, a particle size of 1–5 μm is required (Gonda, 2004).

A major challenge during processing and storage of proteins is their high sensitivity to physicochemical stress. Common methods to increase the stability of proteins are drying and the use of stabilizing excipients (Maltesen and van de Weert, 2008). So far, freeze drying has been the standard process to dry labile biopharmaceuticals (Chan and Chew, 2007; Sollohub and Cal, 2010). Contrary to freeze drying, spray drying is a fast and economic single step drying method which can be designed as a continuous process (Masters, 1972a). Despite the high temperatures of the drying gas, spray drying is well suited for heat-sensitive materials. The droplet temperature remains relatively low, due to the cooling effect of the evaporating solvent (Masters, 1972b). Another advantage of spray drying is that it is possible to control particle size and morphology by varying process parameters and formulation (Masters, 1972a). This is of great importance for the preparation of powders for inhalation. Therefore, spray drying can be considered as an interesting alternative to freeze drying for the preparation of respirable protein powder.

Previous studies of various research groups showed that the extent of protein degradation during spray drying is strongly dependant on the process conditions and the formulation. Namely, inlet temperature, spray rate and the addition of excipients are of

* Corresponding author. Tel.: +41 61 381 0720; fax: +41 61 381 04 30.
E-mail addresses: Imjak.Jeon@unibas.ch (I. Jeon), arpagaus.c@buchi.com (C. Arpagaus), Gabriele.Betz@unibas.ch (G. Betz).

0378-5173/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2011.02.012

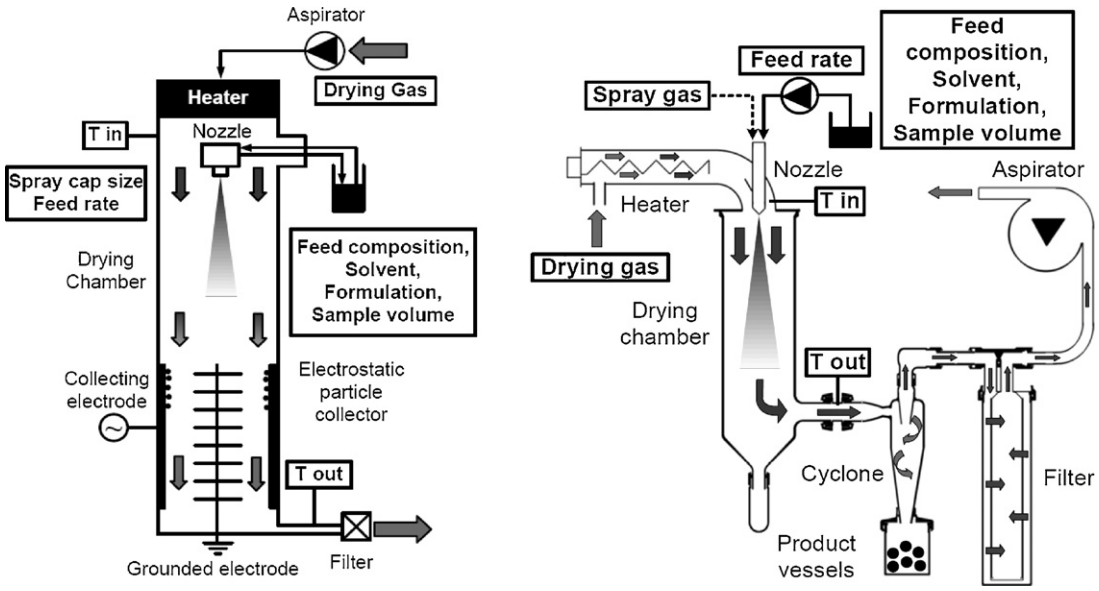

Fig. 1. Nano Spray Dryer B-90 and Mini Spray Dryer B-290 from BÜCHI Labortechnik AG.

major importance (Branchu et al., 1999; Broadhead et al., 1994; Maury et al., 2005a). Process parameters and formulation also have an impact on the particle morphology and the aerodynamic properties of the powder (Cabral-Marques and Almeida, 2009; Chan et al., 1997; Jalalipour et al., 2008; Maa et al., 2007; Schüle et al., 2008).

Product recovery is another parameter which has to be kept in mind in optimization. Low yields are still a drawback in the development of spray dried pharmaceuticals because active ingredients are expensive and only available in small amounts during the early stages of development (Broadhead et al., 1994). The recovery for small particles – as needed for inhalation – is especially low because they are not efficiently separated in cyclone collectors due to their low mass (Prinn et al., 2002).

To overcome this technical challenge, BÜCHI Labortechnik AG has recently developed the Nano Spray Dryer B-90 (Fig. 1). It is designed to generate very fine droplets resulting in particle sizes between 300 nm and 5 μm. By means of a piezoelectric driven vibrating membrane in the spray head, millions of precisely sized tiny droplets are generated every second. The dried particles are separated by the use of an electrostatic particle collector with high particle recovery rates even for nanoparticles of milligram sample amounts. The Nano Spray Dryer B-90 can be used to evaluate spray drying during the early stages of product development for a variety of applications including spray drying of solutions, nanoemulsions, nanosuspensions as well as structural transformations or micro- and nanoencapsulations. The Nano Spray Dryer B-90 has been assessed in previous studies for the preparation of submicron particles of polymeric wall materials, the encapsulation of nano-emulsions (Li et al., 2010) as well as the drying of pharmaceutical excipients and model drugs (Schmid et al., 2010). Recently, Nano Spray Dryer B-90 has been applied for producing protein nanoparticles (Lee et al., 2011).

The aim of this study was to evaluate the Nano Spray Dryer B-90 with regard to the drying of proteins, β-galactosidase, together with trehalose as a stabilizer. According to literature, trehalose is described to prevent protein degradation by glassy immobilization and/or water replacement (h-bonds) as possible mechanisms (Crowe et al., 1996; Anhorn et al., 2008; Maltesen et al., 2008; Maury et al., 2005a,b; Schüle et al., 2008).

It was the objective to optimize process settings and formulation to obtain particles of respirable size (1–5 μm) with full enzyme activity at maximised yield. For this purpose a 3^3 full factorial design was applied to study the effects and interactions of inlet temperature, ethanol content in the spray solution and spray cap type on the above mentioned product characteristics. In addition, the influence of process parameters and formulation on the particle morphology was examined and the shelf life of the samples was compared after accelerated storage test.

Table 1
Levels of process parameters.

Parameters	Levels		
	Low (−)	Intermediate (0)	High (+)
T [°C]	80	100	120
E [%][a]	0	10	20
C [μm]	4.0	5.5	7.0

T: inlet temperature; *E*: ethanol content; *C*: hole size of the spray cap membrane.
[a] Percentage (v/v) of the solvent.

2. Materials and methods

2.1. Materials

β-Galactosidase from *Aspergillus oryzae* (activity: 9.9 U/mg) and D-(+)-trehalose dihydrate from *Saccharomyces cerevisiae* were purchased from Sigma–Aldrich (Buchs, Switzerland). O-Nitrophenyl β-D-galactopyranoside, sodium carbonate anhydrous and ethanol were purchased from Fluka Chemie (Buchs, Switzerland). Disodium hydrogen phosphate dihydrate was supplied from Merck (Darmstadt, Germany), citric acid mohohydrate from Hänseler (Herisau, Switzerland) and acetonitrile from Biosolve B.V (Valkenswaard, The Netherlands).

2.2. Methods

2.2.1. Design of experiments

A 3^3 full factorial design was employed to investigate the effects of the inlet temperature of the drying air, the ethanol content of the spray solution and the hole size of the spray cap membrane. The three levels of these parameters are presented in Table 1. The high and low levels of the inlet air temperature were set to 120 °C and 80 °C, respectively, which are the highest operable temperatures of the Nano Spray Dryer B-90 and the lowest temperature allowing a

reasonable process when spray drying a water-based feed. For the ethanol content, the levels were defined as 0%, 10% and 20% (v/v) of the solvent amount. 20% is still in the safe range below explosion limit. The hole size of the spray cap membrane used were 4 μm, 5.5 μm and 7 μm which are the three available sizes for the Nano Spray Dryer B-90. A total number of 27 experiments were carried out. The examined response variables were residual enzyme activity, median particle size, span, yield and activity loss during storage. The objective was to produce a powder with full enzyme activity at maximised yield. Particle size was desired to be in the respirable range (1–5 μm). Therefore, the target range for the median particle size was defined as 2–4 μm. The statistical analysis of the data and the optimization of the parameters were conducted using the software STAVEX® (AICOS AG, Switzerland).

2.2.2. Spray drying

2.2.2.1. Spray drying with the Nano Spray Dryer B-90. The runs of the experimental design were spray dried using a Nano Spray Dryer B-90 (Fig. 1) (BÜCHI Labortechnik AG, Flawil, Switzerland) with the long version of the drying chamber. This spray dryer has a vibrating membrane in the spray cap to atomize the feed and the particles are collected by an electrostatic particle collector. The controllable parameters are the inlet temperature, the hole size of the vibrating membrane in the spray cap, the flow rate of the drying gas as well as the relative spray rate. Inlet temperature and spray cap size were varied according to the experimental design (see Table 1). Compressed air was used as the drying gas and the flow rate was set to about 100–110 L/min. The relative spray rate was set to 100% for most of the runs but had to be reduced for certain experiments, in order to obtain a reasonably dry product in the collector. To prevent heating of the liquid sample in the course of pump circulation, the sample vessel was kept in an ice bath.

Additional experiments were carried out to measure the residual enzyme activity directly after atomization. For this purpose, the spray solution was collected in a vial after passing through the spray cap membrane. The drying air was not heated for these experiments.

2.2.2.2. Spray drying with the Mini Spray Dryer B-290. Two additional experiments were performed using a Mini Spray Dryer B-290 (Fig. 1) (BÜCHI Labortechnik AG, Flawil, Switzerland). This spray dryer has a 0.7 mm two fluid nozzle for atomization of the feed solution and a cyclone is used to collect the particles. The controllable parameters are the inlet temperature, the solution feed rate, the flow rate of the atomizing gas and the flow rate of the drying gas. For both experiments, the feed rate was 6 g/min, the flow rate of the atomizing air was 667 L/h and the flow rate of the drying air was 35 m^3/h. The inlet temperature was set to 140 °C and 160 °C, respectively.

2.2.3. Preparation of the liquid feed

For the experiments with the Nano Spray Dryer B-90, 166.7 mg of β-galactosidase and 368.4 mg of D-(+)-trehalose dihydrate were dissolved in the solvent, resulting in a total weight of 10 g. This led to a protein to trehalose ratio of 1:2 (w/w) and a solid content of the feed of 5%. The solvent used was distilled water or a mixture of distilled water and ethanol, according to the experimental design (see Table 1).

For the two experiments with the Mini Spray Dryer B-290, the solvent was distilled water. The amounts of feed solution, which were sprayed, were 27 g and 33 g, respectively.

2.2.4. Activity assay

An adapted version of the activity assay from Kuny and Leuenberger (2003) was used to determine the residual enzyme activity. The spray dried powder was dissolved in phosphate-citrate buffer (pH 4.5). 90 μL of this solution were added to 1590 μL of a substrate solution (6.6 mM O-nitrophenyl β-D-galactopyranoside in phosphate-citrate buffer) and incubated for 10 min at 30 °C. After 10 min, 420 μL of a Na_2CO_3-solution (1 M) were added to stop the reaction. The incubation was conducted in duplicate. The absorbance of the product O-nitrophenol (ONP) was measured spectrophotometrically at 420 nm (DU 530 UV/VIS Spectrophotometer, Beckmann, Krefeld, Germany).

Two calibration curves were established with untreated, purchased protein. The first one correlates the absorption with the relative ONP concentration. The second curve shows the relationship between the relative ONP concentration and the enzyme concentration. These calibration curves were used to calculate the enzyme concentration, which the prepared sample solutions would contain, if the enzyme was still fully active. To determine the residual enzyme activity, the calculated concentration was then compared to the concentration, which had in fact been prepared (Eq. (1)).

$$\text{residual activity}\,[\%] = \frac{C_{\text{calc.}}[\text{mg/mL}]}{C_{\text{prep.}}[\text{mg/mL}]} \times 100 \quad (1)$$

where $c_{\text{calc.}}$, concentration that was calculated; $c_{\text{prep.}}$, concentration that was prepared.

2.2.5. Particle size distribution

Particle size distribution was measured by means of laser diffractometry using a Mastersizer X® (Malvern Instruments, United Kingdom). Wet dispersion method was applied because it allows working with small sample amounts (approximately 10–30 mg). Acetonitrile was used as the dispersant and the samples were stirred for 10 minutes. The measurement was carried out in triplicate. The volume median particle size (d_{50}) was calculated by the Mastersizer unit. Span was taken as the indicator of the width of size distribution. A smaller span value indicates a narrower size distribution (Eq. (2)).

$$\text{span} = \frac{d_{90} - d_{10}}{d_{50}} \quad (2)$$

where d_{50}, volume median size; d_{90}, 90% of the volume has a size smaller than d_{90}, d_{10}, 10% of the volume has a size smaller than d_{10}.

2.2.6. Particle size and morphology

Powder samples sputtered with gold were examined with a scanning electron microscope (ESEM Philips XL-30, Philips, The Netherlands) with regard to morphology and particle size.

2.2.7. Shelf life

To compare the shelf life, the samples were stored in an oven at 70 °C and the activity was measured after 1, 2 and 3 weeks.

3. Results and discussion

3.1. Effects of inlet temperature, ethanol content and cap size

The results of the experiments are shown in Table 2. The significances of the effects of process parameters and their interactions on the response variables are presented in Table 3.

3.1.1. Effects on residual enzyme activity

The enzyme activity was fully preserved in runs R1 and R3. Also for runs R11, R19, R22, R26 and R27 a residual enzyme activity greater than 95% was measured. The lowest residual activity was found for run R9 (78%).

The statistical analysis revealed that higher inlet temperatures had a negative effect on the residual enzyme activity. Higher inlet temperatures led to an increased outlet temperature which is

Table 2
Properties of spray dried powders.

Run	Process parameters				Response variables			
	T	*E*	*C*	Yield [%]	d_{50} [μm][a]	Span	Activity [%] (day 0)	Activity [%] (3 weeks)
R01	80	0	4.0	87	1.93 ± 0.02	3.0	103	79
R02	100	0	4.0	85	1.64 ± 0.00	3.1	87	77
R03	120	0	4.0	79	2.21 ± 0.08	3.0	86	69
R04	80	10	4.0	94	1.65 ± 0.01	2.9	107	62
R05	100	10	4.0	86	2.02 ± 0.10	2.9	80	59
R06	120	10	4.0	76	1.76 ± 0.04	2.9	87	55
R07	80	20	4.0	90	1.50 ± 0.01	2.6	102	58
R08	100	20	4.0	89	1.55 ± 0.01	3.3	88	64
R09	120	20	4.0	90	1.52 ± 0.01	2.7	78	61
R10	80	0	5.5	74	5.82 ± 0.03	2.6	92	87
R11	100	0	5.5	74	5.48 ± 0.06	2.9	96	92
R12	120	0	5.5	77	6.00 ± 0.02	3.0	91	91
R13	80	10	5.5	86	4.91 ± 0.11	3.5	90	84
R14	100	10	5.5	82	4.52 ± 0.04	3.7	83	73
R15	120	10	5.5	70	4.61 ± 0.06	3.4	89	74
R16	80	20	5.5	86	2.81 ± 0.03	4.5	88	76
R17	100	20	5.5	86	2.66 ± 0.02	4.4	93	78
R18	120	20	5.5	84	3.29 ± 0.04	4.4	91	68
R19	80	0	7.0	68	5.91 ± 0.12	2.8	95	91
R20	100	0	7.0	60	5.29 ± 0.37	3.3	94	87
R21	120	0	7.0	66	5.79 ± 0.09	3.4	98	89
R22	80	10	7.0	87	4.96 ± 0.09	3.4	97	84
R23	100	10	7.0	71	5.43 ± 0.47	3.5	91	82
R24	120	10	7.0	76	5.20 ± 0.03	3.4	91	83
R25	80	20	7.0	87	3.62 ± 0.03	3.9	89	75
R26	100	20	7.0	87	4.48 ± 0.11	4.1	97	80
R27	120	20	7.0	87	3.09 ± 0.14	3.7	97	78
C01	26	0	4.0	–	–	–	106	–
C02	26	0	5.5	–	–	–	101	–
C03	26	0	7.0	–	–	–	100	–
S01	140	0	–	79	–	–	100	–
S02	160	0	–	80	–	–	100	–

R##: spray dried using a Nano Spray Dryer B-90; C##: sprayed using a Nano Spray Dryer B-90 with sample collection directly after atomization; S##: spray dried using a Mini Spray Dryer B-290; *T*: inlet temperature [°C]; *E*: ethanol content [%]; *C*: cap size [μm]; d_{50}: volume median particle size.
[a] Mean ± S.D., $n = 3$.

Table 3
Statistical significance of effects and interactions.

			Response variables				
			Enzyme activity	d_{50}	Span	Yield	Activity loss after storage[a]
Main effects							
	T	*E*	−3.0500	4.6441	0.0500	−2.9444	−0.5000
		S	**	–	–	**	–
	E	*E*	−1.1444	−0.8639	0.3489	6.5444	6.5000
		S	–	***	***	***	***
	C	*E*	1.7278	1.5550	0.2861	−4.8222	−8.222
		S	–	–	–	–	–
Interactions							
	$T \times E$	*E*	0.2750	−0.0308	−0.1711	0.4250	3.6111
		S	–	–	–	–	–
	$T \times C$	*E*	5.5833	−0.0683	0.0922	1.1083	−3.3889
		S	**	–	–	–	–
	$E \times C$	*E*	0.3500	−0.3825	−0.3794	4.2417	8.7778
		S	–	**	**	***	***
Fit (R^2)			0.6331	0.9321	0.7293	0.8125	0.8082

E: effect; *S*: significance; –: not significant; *T*: inlet temperature; *E*: ethanol content; *C*: cap size; $T \times E$: interaction between inlet temperature and ethanol content; $T \times C$: interaction between inlet temperature and cap size; $E \times C$: interaction between ethanol content and cap size; d_{50}: volume median particle size.
* $p \leq 0.1$.
[a] Activity loss after 3 weeks of storage at 70 °C.
** $p \leq 0.05$.
*** $p \leq 0.01$.

reported to have a major impact on the degradation of proteins during spray drying (Broadhead et al., 1994; Ståhl et al., 2002; Yoshii et al., 2008). Although the effect of the inlet temperature is significant, the fit of the model equation, is only poor ($R^2 = 0.6331$). This can be explained by the fact that the inlet temperature is only one factor influencing the outlet temperature. The other factor is the spray rate. With the Nano Spray Dryer B-90, the spray rate cannot be controlled directly and it was, therefore, different for each run.

Furthermore, the following results support the assumption that the activity losses occurring during the spray drying process with the Nano Spray Dryer B-90 are not due to thermal degradation alone: Samples of β-galactosidase and trehalose, which were spray

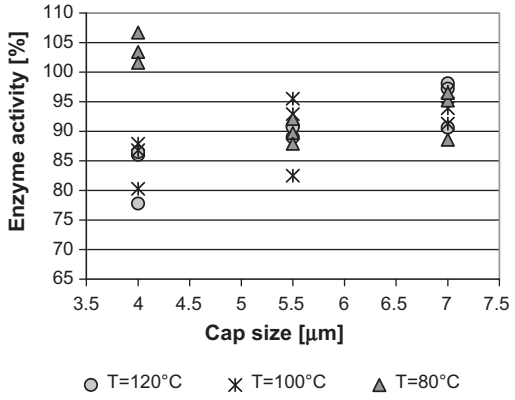

Fig. 2. Residual enzyme activity vs. cap size.

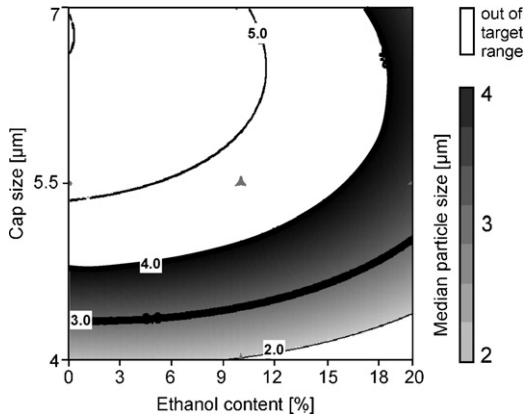

Fig. 3. Contour plot of median particle size vs. cap size and ethanol content.

dried using a Mini Spray Dryer B-290, showed full enzyme activity even though the outlet temperatures were much higher (70 °C and 80 °C) than the outlet temperatures reached with the Nano Spray Dryer B-90 (36–53 °C). Likewise, Broadhead et al. (1994) found that samples of β-galactosidase and trehalose showed full residual enzyme activity up to outlet temperatures of about 100 °C when they were spray dried with a Mini Spray Dryer B-190 (older model, BÜCHI Labortechnik AG, Flawil, Switzerland). The possible mechanisms to prevent protein degradation described in literature are glassy immobilization and/or water replacement (Crowe et al., 1996; Anhorn et al., 2008; Maltesen et al., 2008; Maury et al., 2005a,b; Schüle et al., 2008).

The main differences between the two spray dryer types are the use of different atomization techniques and different particle collectors. It is unlikely that the enzyme degradation during spray drying with the Nano Spray Dryer B-90 was caused by the electrostatic particle collector because it was possible to retain full enzyme activity with several of the investigated process parameter settings. However, the statistical analysis showed a significant interaction of the spray cap size and the inlet temperature. Fig. 2 shows that the residual activity was much more sensitive to changes of the inlet temperature when the smallest spray cap was used. This leads to the assumption that protein degradation during spray drying with the Nano Spray Dryer B-90 could be connected with the step of atomization. However, at room temperature, no activity loss was observed when the enzyme activity was measured directly after atomization. Further experiments would be necessary to clarify the cause of enzyme degradation.

The addition of trehalose did not reduce the activity loss during the spray drying process with the Nano Spray Dryer B-90. Samples of β-galactosidase were spray dried using the same process settings as in Runs R3, R11 and R12. The residual enzyme activities measured for the corresponding experiments were 86% (R3) and 81%, 96% (R11) and 93% as well as 91% (R12) and 91%. These results show that the addition of trehalose did not have an influence on the residual enzyme activity. This is contradictory to the results of several research groups who used the Mini Spray Dryers B-190, B-191 and B-290. They found trehalose to be a good stabilizer during drying (Broadhead et al., 1994; Maury et al., 2005a; Schüle et al., 2008). It appears that the dominant path of β-galactosidase degradation during the spray drying process with the Nano Spray Dryer B-90 is different from the one occurring when the Mini Spray Dryer is used and trehalose was not able to prevent this degradation.

Nevertheless, the process conditions of the Nano Spray Dryer B-90 appear to be mild and well suited for the drying of proteins. The residual enzyme activities of β-galactosidase samples spray dried in the absence of any excipients were higher than reported by other

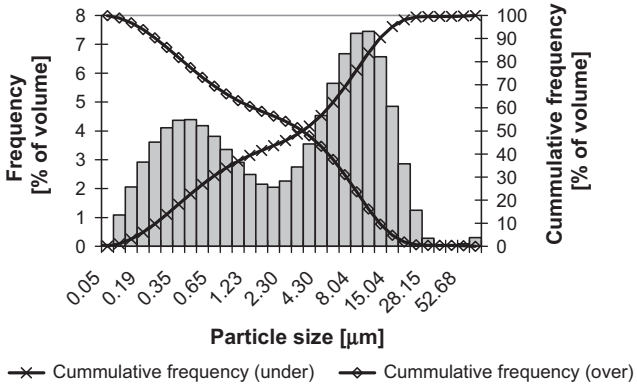

Fig. 4. Particle size distribution of a sample spray dried with spray cap size 7.0 µm and 20% ethanol.

research groups that used a Mini Spray Dryer B-190 (Broadhead et al., 1994; Vasiljevic and Jelen, 2003).

3.1.2. *Effects on particle size and size distribution*

Results for median particle size d_{50} were in the range between 1.5 µm (run R7) and 6 µm (run R12). The target range for d_{50} of 2–4 µm was achieved in the following runs: R3, R5, R16, R17, R18, R25, R27.

As anticipated, the statistical analysis showed that the hole size of the spray cap membrane had a significant impact on the particle size of the spray dried powder. Using a smaller cap size resulted in smaller particles. It was also revealed that the ethanol content, as well as the interaction between cap size and ethanol content had a significant influence. The decrease in particle size with increasing ethanol content could either be due to the generation of smaller droplets because of the reduced surface tension of the feed solution or it might be due to lower particle porosity.

The contour plot (Fig. 3) shows that the target range for d_{50} – represented by the gray area – can be attained with different settings. Within the investigated range, an ethanol concentration of 20% led to the best results for the 5.5 µm and the 7.0 µm spray caps. No ethanol in the feed formulation was best when the 4.0 µm cap was used. Analysis of the particle size distribution showed that the samples spray dried with the bigger spray caps and with 20% ethanol in the solution (T_{in} 100 °C) had a polydisperse size distribution (Figs. 4 and 5). The fractions of the volume with a respirable particle size (1.00–5.29 µm) were 26% for the 7.0 µm cap and 34% for the 5.5 µm cap. The sample which was spray dried with the 4.0 µm cap and no ethanol (T_{in} 80 °C) had a monodisperse size distribution (Fig. 6). A fraction of 52% of this sample was within the

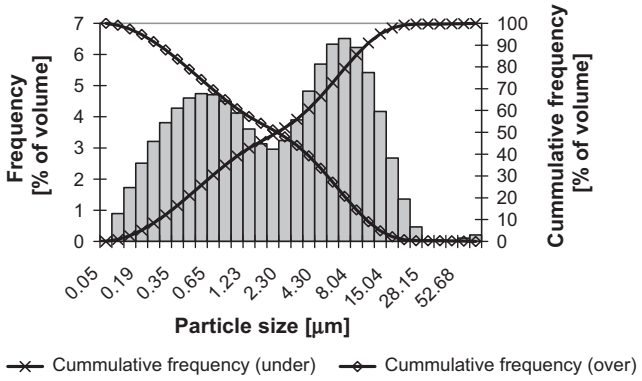

Fig. 5. Particle size distribution of a sample spray dried with spray cap size 5.5 μm and 20% ethanol.

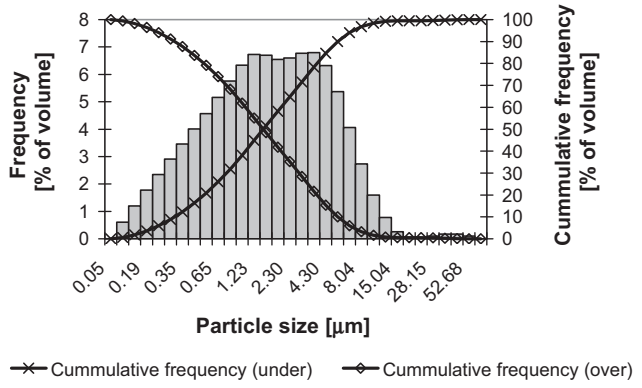

Fig. 6. Particle size distribution of a sample spray dried with spray cap size 4.0 μm and 0% ethanol.

desired range of particle size. Therefore, this setting appears to be better suited for the preparation of respirable powders.

Results for span lay between 2.6 (run R7) and 4.5 (run R16). Span increased with higher ethanol contents and larger spray cap sizes. The results for span are much higher than the span values for droplets reported by the supplier of the spray dryer: <1.4 for the 4.0 μm cap and <1.6 for the 5.5 μm and the 7.0 μm cap (Nano Spray Dryer B-90 Brochure). The high values could be due to clogging of the spray cap membrane, which was observed during the spraying. The clogging might be caused by the trehalose as it is known that stickiness is a commonly occurring problem in the spray drying of sugar-containing solutions (Bhandari et al., 1997; Sollohub and Cal, 2010; Truong et al., 2005).

3.1.3. Effects on yield

Yields obtained with the different settings ranged from 60.1% (R20) to 93.7% (R4) for powder amounts of 500 mg. For 13 out of the 27 runs, recovery was greater than 85%.

Yields were high, compared to the results (50–70%) reported for optimized runs using other laboratory scale spray dryers (Mini Spray Dryer B-190, B-191 and B-290) (Broadhead et al., 1994; Jensen et al., 2010; Maury et al., 2005b). Loss of product occurred mainly through wall deposit and losses during the manual collection from the collecting electrode.

The statistical analysis revealed that lower inlet temperatures, higher ethanol contents and a smaller cap size resulted in higher yields. The influence of the parameters on the yield could be related with their effect on the spray rate. With the Nano Spray Dryer B-90, the spray rate cannot be controlled directly; only by the setting of the relative spray rate. The spray rate depends on the hole size of the spray cap membrane, the formulation, the inlet temperature and the setting of the relative spray rate. Higher spray rates have been reported to have a negative influence on the yield (Broadhead et al., 1994; Maury et al., 2005b). Fig. 7 shows the tendency of the yield to be higher when the spray rate was low. The correlation coefficient was low (R^2 = 0.4432), but it has to be kept in mind that the spray rate was not the only parameter that was varied. Additionally, the manual collection of the powder led to a variation of the results which is not related to any of the process parameters.

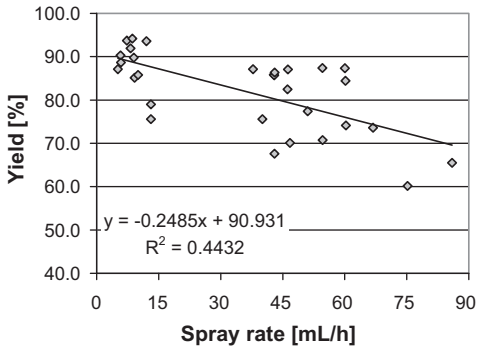

Fig. 7. Correlation between yield and spray rate.

3.1.4. Effects on particle morphology

The particles produced with spray cap size 4.0 μm were spherical and they generally had a smooth surface (Fig. 8A). Shriveled particles were very rarely observed. In contrast, powders obtained with spray cap size 5.5 μm and 7.0 μm consisted of a mixture of smooth and shriveled spheres (Fig. 8B and C). Fig. 8D shows that the spheres – or at least some of them – were hollow.

The shriveled morphology is typical for proteins spray dried together with low molecular excipients (Adler et al., 2000; Broadhead et al., 1994) but also smooth particles are not uncommon (Maa et al., 2007). However, it is exceptional to find a mixture of smooth and shriveled particles in one batch.

Neither different ethanol concentrations, nor different outlet temperatures led to a modified particle morphology. Possibly, the investigated ranges of these parameters were too narrow. Furthermore, an influence of the formulation or the outlet temperature could not explain why different morphologies were found in one batch. However, it seems that the surface properties are related to the particle size as almost no shriveled particles were found with a size smaller than approximately 6 μm. Possibly, a different distribution of the compounds within the crust is responsible for the varying appearances. Small particles dry faster and the critical point of crust formation is reached shortly after atomization. For larger particles, however, it takes longer to form a crust, which gives the protein time to accumulate at the surface, resulting in shriveled particles. This assumption is supported by the research of Adler et al. (2000). It showed that spray drying bovine serum albumin together with trehalose led to shriveled particles with a large excess concentration of the protein on the particle surface. However, the smooth texture could be restored by adding a surfactant, which displaced the protein from the surface. The same phenomenon was observed by Maa et al. (2007) for mixtures of humanized anti-IgE monoclonal antibody and lactose. The particles changed from a deep hole morphology to spherical particles as a surfactant was added. However, further investigations of the crust's composition would be necessary to ascertain if the different morphologies in this study are due to different surface compositions.

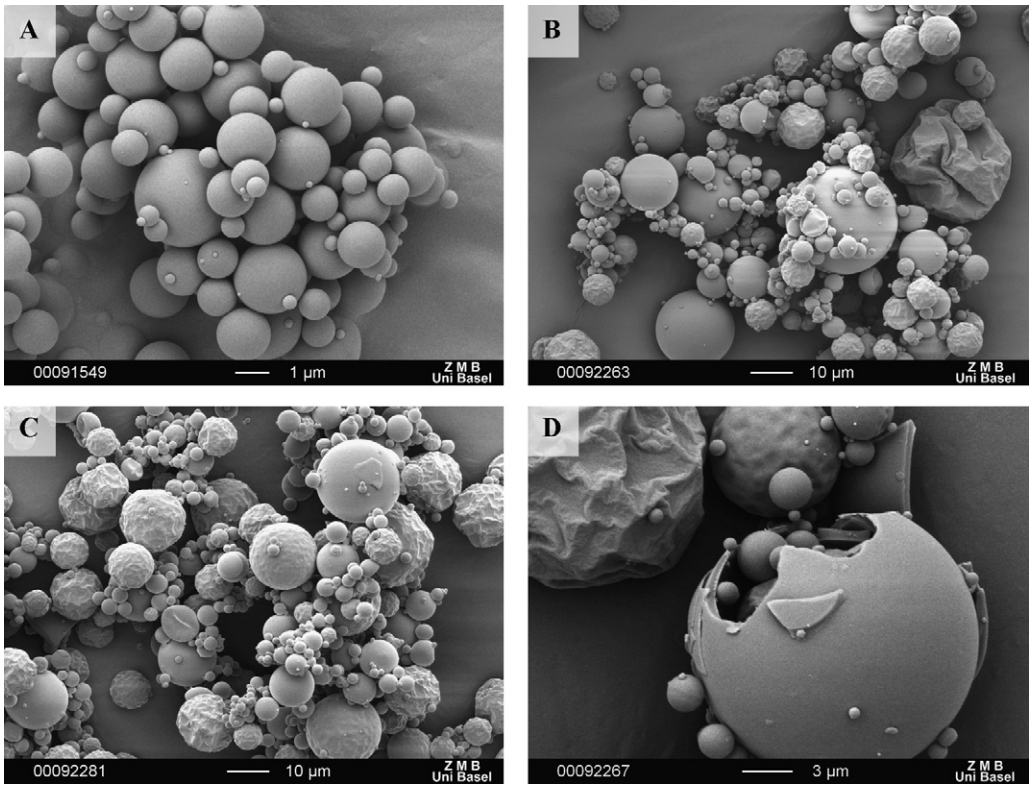

Fig. 8. Representative scanning electron microscopy pictures of samples produced with cap sizes 4 µm (A), 5.5 µm (B) and 7 µm (C) and a picture of a disintegrated particle (D).

3.1.5. Effects on shelf life

During the 3 weeks of storage at 70 °C the samples lost up to 43% of their activity (Fig. 9). This is in contradiction to the results of several research groups, which showed that trehalose is an excellent stabilizing agent during storage (Broadhead et al., 1994; O'Brien, 1996; Schebor et al., 1999; Schüle et al., 2008; Sun and Davidson, 1998). However, the addition of trehalose appears to improve the shelf life. A sample of spray dried β-galactosidase lost 34% of its activity during storage. In contrast, the sample that was spray dried together with trehalose using the same process settings (R11) lost only 3% of its activity.

Even though the composition of the powder and the storage conditions were the same for all samples, they exhibited big differences in shelf life. Powders produced with bigger cap sizes and lower ethanol contents in the spray solution exhibited smaller losses of activity (Figs. 10 and 11). Also, there was a tendency for powders with larger particles to have a higher residual enzyme activity after storage than powders with small particles (Fig. 12).

The influence of the process parameters on shelf life might be due to differences in the amorphous structure. Moran and Buckton (2007) found that, depending on the process conditions, the amorphous state of spray dried trehalose had a different short range order. This led to different water sorption and crystallization behaviour of the dry material. These characteristics are of importance for the ability of trehalose to stabilize proteins during storage (Sun and Davidson, 1998). Another explanation for the impact of the process parameters could be a difference in the porosity of the powders. Burin et al. (2004) found that lower porosity had a negative impact on the stability of β-galactosidase in dry powders. As mentioned in Section 3.1.2, the decrease in particle size with increased

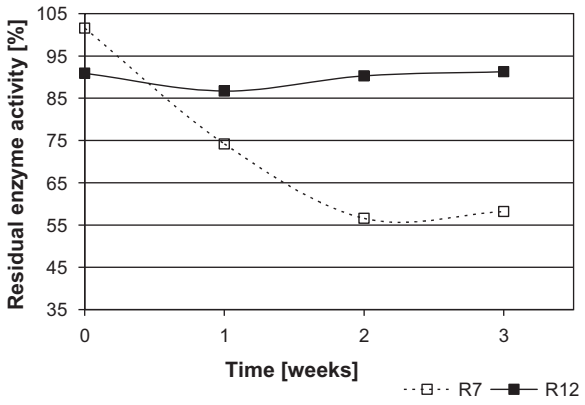

Fig. 9. Shelf life (run R7 showed the greatest loss of activity (43%); run R12 showed no loss of activity).

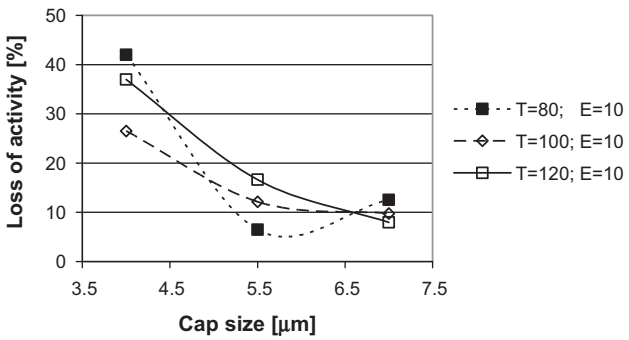

Fig. 10. Correlation between loss of activity and cap size.

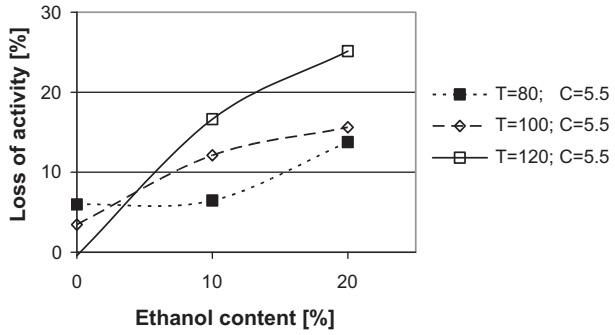

Fig. 11. Correlation between loss of activity and ethanol content.

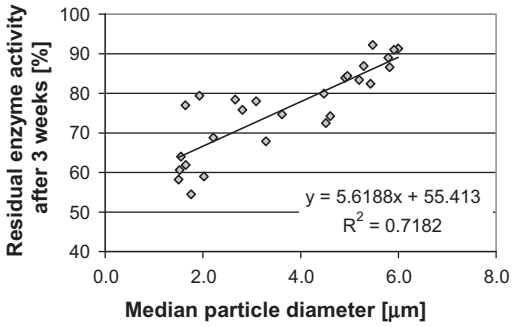

Fig. 12. Correlation between residual enzyme activity after 3 weeks of storage at 70 °C and particle size.

ethanol content could be due to lower porosity of the particles. Further experiments would be necessary to determine the powder characteristics, which are related with the influence of the process parameters on shelf life.

4. Conclusions

This study showed, that β-galactosidase could be spray dried without activity loss using the Nano Spray Dryer B-90 when the optimized process setting was applied (inlet temperature: 80 °C, spray cap size: 4.0 μm, ethanol content of the spray solution: 0%). Furthermore, this setting allowed producing particles of respirable size and it resulted in high yields (approximately 90%).

Further studies are suggested to identify stabilizers that are better suited than trehalose to protect β-galactosidase or other proteins during the spray drying process with the Nano Spray Dryer B-90. Further optimization would also be necessary with respect to the shelf life of the final formulation. Identifying the product properties that affect the storage stability could facilitate the optimization. Additionally, detailed investigations of the aerodynamic behaviour by means of cascade impactor analysis would be useful to improve the powder's inhalation properties.

Acknowledgements

The authors are grateful to BÜCHI Labortechnik AG for the kind offer of equipments and materials.

References

Adler, M., Unger, M., Lee, G., 2000. Surface composition of spray-dried particles of bovine serum albumin/trehalose/surfacteant. Pharm. Res. 17, 863–870.
Agu, R.U., Ugwoke, M.I., Armand, M., Kinget, R., Verbeke, N., 2001. The lung as a route for systemic delivery of therapeutic proteins and peptides. Respir. Res. 2, 198–209.
Anhorn, M.G., Mahler, H., Langer, K., 2008. Freeze drying of human serum albumin (HSA) nanoparticles with different excipients. Int. J. Pharm. 363, 162–169.
Bhandari, B.R., Datta, N., Howes, T., 1997. Problems associated with spray drying of sugar-rich foods. Dry. Technol. 15, 671–684.
Branchu, S., Forbes, R.T., York, P., Petrén, S., Nyqvist, H., Camber, O., 1999. Hydroxypropyl-beta-cyclodextrin inhibits spray-drying-induced inactivation of beta-galactosidase. J. Pharm. Sci. 88, 905–911.
Broadhead, J., Edmon Rouan, S.K., Hau, I., Rhodes, C.T., 1994. The effect of process and formulation variables on the properties of spray-dried β-galactosidase. J. Pharm. Pharmacol. 46, 458–467.
Burin, L., Jouppila, K., Roos, Y.H., Kansikas, J., Buera, M.P., 2004. Retention of β-galactosidase activity as related to Maillard reaction, lactose crystallization, collapse and glass transition in low moisture whey systems. Int. Dairy J. 14, 517–525.
Cabral-Marques, H., Almeida, R., 2009. Optimisation of spray-drying process variables for dry powder inhalation (DPI) formulations of corticosteroid/cyclodextrin inclusion complexes. Eur. J. Pharm. Biopharm. 73, 121–129.
Chan, H., Clark, A., Gonda, I., Mumenthaler, M., Hsu, C., 1997. Spray dried powders and powder blends of recombinant human deoxyribonuclease (rhDNase) for aerosol delivery. Pharm. Res. 14, 431–437.
Chan, H., Chew, N.Y.K., 2007. Dry powder aerosols: Emerging Technologies. In: Swarbrick, J. (Ed.), Encyclopedia of Pharmaceutical Technology, vol. 3. Informa Healthcare USA Inc., New York, pp. 1428–1434.
Crowe, L.M., Reid, D.S., Crowe, J.H., 1996. Is trehalose special for preserving dry biomaterials? Biophys. J. 71, 2087–2093.
DeFelippis, M.R., Sukumar, M., Rajagopalan, N., 2007. Peptides and proteins: non-invasive delivery. In: Swarbrick, J. (Ed.), Encyclopedia of Pharmaceutical Technology, vol. 4, 3rd ed. Informa Healthcare USA Inc., New York, pp. 2692–2712.
Gonda, I., 2004. Targeting by Deposition. In: Hickey, A.T. (Ed.), Pharmaceutical Inhalation Aerosol Technology (134 Drugs and the Pharmaceutical Sciences). Marcel Dekker Inc., New York, pp. 65–88.
Jalalipour, M., Gilani, K., Tajerzadeh, H., Najafabadi, A.R., Barghi, M., 2008. Characterization and aerodynamic evaluation of spray dried recombinant human growth hormone using protein stabilizing agents. Int. J. Pharm. 352, 209–216.
Jensen, D.M., Cun, D., Maltesen, M.J., Frokjaer, S., Nielsen, H.M., Foged, C., 2010. Spray drying of siRNA-containing PLGA nanoparticles intended for inhalation. J. Control. Release 142, 138–145.
Kuny, T., Leuenberger, H., 2003. Compression behaviour of the enzyme β-galactosidase and its mixture with microcrystalline cellulose. Int. J. Pharmacol. 260, 137–147.
Lee, S., Heng, D., Kiong Ng, W., Chan, H., Tan, H., 2011. Nano spray drying: a novel method for preparing protein nanoparticles for protein therapy. Int. J. Pharm. 403, 192–200.
Li, X., Anton, N., Arpagaus, C., Belleteix, F., Vandamme, T.F., 2010. Nanoparticles by spray drying using innovative new technology: The Büchi Nano Spray Dryer B-90. Journal of Controlled Release 147 (2), 304–310.
Maa, Y.F., Costantino, H.R., Nguyen, P., Hsu, C.C., 2007. The effect of operating and formulatin variables on the morphology of spray-dried protein particles. Pharm. Dev. Technol. 2, 213–223.
Maltesen, M.J., van de Weert, M., 2008. Drying methods for protein pharmaceuticals. Drug Discov. Today: Technol 5, e81–e88.
Masters, K., 1972a. Spray Drying Handbook, 3rd ed. George Godwin Limited, London, pp. 1–17.
Masters, K., 1972b. Spray Drying Handbook, 3rd ed. George Godwin Limited, London, pp. 21–33.
Maury, M., Murphy, K., Kumar, S., Mauerer, A., Lee, G., 2005a. Spray-drying of proteins: effects of sorbitol and trehalose on aggregation and FT-IR amide I spectrum of an immunoglobulin G. Eur. J. Pharm. Biopharm. 59, 251–261.
Maury, M., Murphy, K., Kumar, S., Shi, L., Lee, G., 2005b. Effects of process variables on the powder yield of spray-dried trehalose on a laboratory spray-dryer. Eur. J. Pharm. Biopharm. 59, 565–573.
Moran, A., Buckton, G., 2007. Adjusting and understanding the properties and crystallisation behaviour of amorphous trehalose as a function of spray drying feed concentration. Int. J. Pharm. 343, 12–17.
Nano Spray Dryer B-90 Brochure: 11592236 en 09 07, BÜCHI Labortechnik AG, Flawil., 2010. Available on website www.buchi.com.
O'Brien, J., 1996. Stability of trehalose sucrose and glucose to nonenzymatic Browning in Model Systems. J. Food Sci. 61, 679–682.
Prinn, K.B., Costantino, H.R., Tracy, M., 2002. Statistical modeling of protein spray drying at the Lab Scale. AAPS Pharm. Sci. Tech. 3, article 4.
Schebor, C., Burin, L., del Pilar Buer, M., Chirife, J., 1999. Stability to hydrolysis and browning of trehalose sucrose and raffinose in low-moisture systems in relation to their use as protectants of dry biomaterials. Lebensm. Wiss. Technol. 2, 481–485.
Schmid, K., Arpagaus, C., Friess, W., 2010. Evaluation of the Nano Spray Dryer B-90 for pharmaceutical applications. Pharm. Dev. Technol., 1–8, Early Online. Doi:10.3109/10837450.2010.485320.
Schüle, S., Schulz-Fademrecht, T., Garidel, P., Bechtold-Peters, K., Frieß, W., 2008. Stabilization of IgG1 in spray-dried powders for inhalation. Eur. J. Pharm. Biopharm. 69, 793–807.
Sollohub, K., Cal, K., 2010. Spray drying technique II: current applications in pharmaceutical technology. J. Pharm. Sci. 99, 587–597.
Ståhl, K., Claesson, M., Lilliehorn, P., Lindén, H., Bäckström, K., 2002. The effect of process variables on the degradation and physical properties of spray dried insulin intended for inhalation. Int. J. Pharm. 233, 227–237.

Sun, W.Q., Davidson, P., 1998. Protein inactivation in amorphous sucrose and trehalose matrices: effects of phase separation and crystallization. Biochim. Biophys. Acta (BBA): Gen. Sub. 1425, 235–244.
Truong, V., Bhandari, B.R., Howes, T., 2005. Optimization of co-current spray drying process of sugar-rich foods Part I—moisture and glass transition temperature profile during drying. J. Food Eng. 71, 55–65.
Vasiljevic, T., Jelen, P., 2003. Drying and storage of crude β-galactosidase extracts from Lactobacillus delbrueckii ssp. bulgaricus 11842. Innov. Food Sci. Emerg. Technol. 4, 319–329.
Yoshii, H., Buche, F., Takeuchi, N., Terrol, C., Ohgawara, M., Furuta, T., 2008. Effects of protein on retention of ADH enzyme activity encapsulated in trehalose matrices by spray drying. J. Food Eng. 87, 34–39.