ASIAN JOURNAL OF PHARMACEUTICAL SCIENCES 11 (2016) 507–515

HOSTED BY

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

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

Original Research Paper

Spray drying of fenofibrate loaded nanostructured lipid carriers

Dengning Xia [a,b,1], *Neha Shrestha* [a,1], *Jacco van de Streek* [a], *Huiling Mu* [a], *Mingshi Yang* [a,c,d,*]

[a] *Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, Copenhagen 2100, Denmark*

[b] *Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China*

[c] *Department of Pharmaceutics, School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China*

[d] *Key Laboratory of Smart Drug Delivery, Ministry of Education; Fudan University, Shanghai 201203, China*

ARTICLE INFO

Article history:
Received 17 September 2015
Received in revised form 28 December 2015
Accepted 8 January 2016
Available online 25 January 2016

Keywords:
Fenofibrate
Nanostructured lipid carriers
Spray drying
Design of experiments
Central composite face-centered design

ABSTRACT

The conversion of aqueous dispersion of nanostructured lipid carriers (NLCs) into dry powder by spray drying could be a useful approach to render NLCs with better physical chemical stability than the aqueous dispersion. In this study, aqueous NLC dispersion containing fenofibrate was converted into dry, easily reconstitutable powder using spray drying. A central composite face centered design (CCFD) was used to investigate the influence of the ratio of lipid to protectant (mannitol and trehalose) and crystallinity of spray-dried powder on the particle size, yield and residual moisture content of the dried powder. A linear relationship ($R^2 = 0.9915$) was established between the crystalline content of the spray-dried powders against the ratio of mannitol to trehalose from 3:7 to 10:0 (w/w). Spray drying of NLC aqueous dispersion using a mannitol and trehalose mixture resulted in an increase in particle size of the NLCs after reconstitution in water as compared to that in the initial aqueous dispersion. The decrease in crystallinity of the dry powder by reducing the ratio of mannitol to trehalose could improve the reconstitution of the NLCs in water. However the yield and residual moisture content of dry powder decreased with an increase in the ratio of mannitol to trehalose. Lipid nanoparticles were able to retain the drug incorporation and the prolonged drug release profile after spray drying. The experimental model was robust, and suggested that spray drying is a viable technique for the conversion of NLCs into dry powder.

* *Corresponding author.* Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, Copenhagen 2100, Denmark. Tel.: +45 35 33 61 41; fax: +45 35 33 60 01.
E-mail address: mingshi.yang@sund.ku.dk (M. Yang).

[1] Co-first author.

Peer review under responsibility of Shenyang Pharmaceutical University.
http://dx.doi.org/10.1016/j.ajps.2016.01.001

1. Introduction

Lipid nanoparticles, solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs), are innovative drug carrier systems which are composed of biocompatible and biodegradable solid/liquid lipids as a matrix. These lipid nanoparticles have a broad field of application[s] including improving oral bioavailability [1,2] and dermal drug delivery [3].

The lipid nanoparticles are often prepared in aqueous phase. The lipids and drug are first melted and then dispersed in hot water containing surfactants to form an emulsion by high-pressure homogenization or the sonication method. The hot emulsion is finally cooled down to solidify the melted lipid particles. During this process, the supercooled melts of lipid-containing drugs are usually first formed and subsequent crystallization of lipids will take place during storage [4,5]. The major drawback related to the SLNs and NLCs are their physico-chemical instability, such as particle growth, unpredictable gelation, drug expulsion during storage or unexpected dynamic polymorphic transitions of the lipid particles [6–13].

The conversion of the aqueous dispersion of lipid nanoparticles into a dry powder is very useful and necessary for improving stability. The aqueous dispersions can be transformed by spray drying into a dry, fine, reconstitutable powder that can be stored over a long period [14]. The dried powder can be easily further converted into final products, such as tablets or capsules. Spray-drying is widely used in the pharmaceutical industry. The feed, *e.g.* aqueous solution or suspension, is atomized in a hot gas stream and dried fast to form solid microparticles. Carbohydrate excipients, such as mannitol or trehalose, are often added and serve as protectants or bulking agents that enable production of stable microparticles with nanoparticles entrapped in the microparticle matrix to provide the nanoparticles some degree of protection [15,16]. These carbohydrates are dissolvable in water, thus the nanoparticles can be reconstituted [17,18].

The properties of the spray-dried product, such as yield, moisture content and particle size after redispersion in water, may be affected by the process parameters including the concentrations of the lipid nanoparticles and the carbohydrates. The crystallization tendency of the carbohydrates also has potential influence on the particle formation [19], redispersity [20] of the spray dried particles. Optimization of these parameters involves evaluation of a large number of variables, which is often time-consuming and expensive. The use of design of experiments (DOE) in such scenarios can dramatically reduce the number of experiments without compromising the quality of the final product. The central composite face centered design (CCFD) is one of the most widely used designs that efficiently evaluates the influence of several variables on investigated responses. CCFD is a flexible design which analyzes all the factors at three levels and has a high-quality prediction in the entire design space [21–23]. Several studies have been performed for NLCs and their solidification using freeze drying; however, limited research is available regarding the transformation of NLCs into solid powder using spray drying.

In this study fenofibrate was used as a model drug. Fenofibrate is mainly used for primary hypercholesterolemia or mixed dyslipidemia. The oral absorption of fenofibrate is poor due to poor water solubility [24]. Our previous study showed that the oral absorption of fenofibrate could be improved by NLC [25]. However, the NLC suspension is physically unstable for long-term storage [10]. To improve the storage stability, the fenofibrate loaded NLCs were transformed into dry powder by spray drying. Mannitol and trehalose were selected to formulate with NLCs as they have been commonly used as drying protectants for nanoparticles or proteins in the freeze drying process [26] and spray drying process [16,22]. Mannitol is known to result in a crystalline material after the spray drying process, while trehalose is an amorphous material after spray drying. However, to which degree the crystallinity of these protectants in a drying formulation will influence the reconstitution of nanoparticles has not been fully understood. In the present study, we adjusted the crystallinity of spray dry powder by varying the ratio of mannitol to trehalose in the formulation. The effects of the crystallinity of the powder and lipid content on the reconstitution of NLCs after the spray drying process were investigated by using a DOE approach.

2. Materials and methods

2.1. *Materials*

Fenofibrate (purity ≥ 99%) was purchased from Sigma-Aldrich (Germany). Glycerol Monostearate (40~55) (GMS), Sodium Lauryl Sulfate (SLS) and Medium-Chain Triglyceride (MCT) were obtained from Unikem (Copenhagen, Denmark). Poloxamer 407 (Lutrol F127) was obtained from BASF Aktiengesellschaft (Germany). D(–)-Mannitol was obtained from VWR International Ltd. (England) and Trehalose was obtained from VVW BDH Prolabo (Belgium).

2.2. *Preparation of the NLCs*

The NLCs were prepared according to our previous publication [10]. Briefly, weighed lipid mixture of GMS/MCT (1.25 g/0.625 g) and drug (0.125 g) were melted in a water bath at 75 to 80 °C. The melting point of lipid component is about 60 °C [10]. The drug-containing lipid melt was dispersed under continuous stirring in 20 ml hot aqueous surfactant solution of the same temperature containing 3% Poloxamer 407 and 0.1% SLS (w/v) under magnetic stirring to form an o/w emulsion. The o/w pre-emulsion was treated with an ultrasonic probe (50/60 KHz, 230V, Chemical Instruments AB, Sweden) at 80% amplitude for 10 min with ultrasound burst of 2 s followed by a 2 s pause. Finally, the formed o/w nanoemulsion was allowed to cool down to room temperature to form nanostructured lipid particles.

2.3. *Conversion of the NLCs liquid dispersion into dry product via spray drying*

The lipid nanoparticles suspension was solidified using a BÜCHI B-290 mini spray dryer (BÜCHI, Switzerland). Two-fluid nozzles

were used with co-current flow and air as the drying medium in an open cycle mode. The inlet temperature was maintained at 100 °C; the outlet temperature was maintained at 50 to 55 °C by adjusting the peristaltic pump rate (%). The drying gas flow was maintained at 33 m^3/h with an atomization gas flow rate of 667 L/h. Sample solutions with 20% (w/w) solid content were prepared with varying lipid-to-carbohydrate ratios and mannitol-to-trehalose ratios. The nanoparticles suspension was feed in spray dryer at rate of 4~5 ml/min.

2.4. *Characterization of NLC suspension and spray dried powder*

2.4.1. *Particle size, particle size distribution and zeta potential*

The particle size, particle size distribution and zeta potential of the NLCs in suspension were determined using a Zetasizer Nano-ZS (Malvern Instruments, UK). The particle size was determined as the mean hydrodynamic diameter (*z-average*) and the particle size dispersion as the polydispersity index (PDI). Measurements were performed at 25 °C. Samples were diluted by Milli-Q water for 25 times prior to analysis. The Malvern DTS v5.2 software was used for data acquisition and analysis. The spray-dried powders were re-dispersed in Milli-Q water in a concentration of 1% (w/v) by using a vortex for 30 s. The reconstituted dispersion was again diluted 25 times before analysis in a Zetasizer to obtain the particle size, polydispersity index and zeta potential.

2.4.2. *Entrapment efficiency and total drug content*

Drug entrapment efficiency was determined indirectly by measuring the total drug in the formulation and the free drug in the aqueous phase of the nanoparticles dispersion [10]. The free drug was separated from the formulation by using ultrafiltration units along with centrifugation. Ultracentrifugal filter (Amicon Ultra-0.5, Millipore) with a molecular weight cutoff of 50 kDa was used. A 0.5ml lipid nanoparticles suspension was accurately taken and placed into the upper chamber of the centrifuge filter, which was centrifuged at 7000 rpm for 15 min at room temperature. The ultrafiltrate was then analyzed using HPLC as described later in this section. For the total drug content, 0.5 ml of lipid nanoparticle suspension was accurately taken and 4.5 ml acetone was added to it. The solution was heated to 50 °C to ensure that the lipid and the drug were completely dissolved in acetone. The solution was then cooled down to room temperature and filtered using a 0.45 μm filter. The filtrate was diluted 100 times with the mobile phase and analyzed in HPLC as described later in this section. The drug entrapment efficiency (EE %) can be calculated using equation (1). The experiments were performed in triplicate ($n = 3$).

$$EE(\%) = \frac{W_{total} - W_{free}}{W_{total}} \times 100 \quad (1)$$

where W_{free}: free drug in suspension; W_{total}: total drug amount in suspension.

To measure the total drug content in the spray-dried NLCs, 0.260 mg of NLC powder, which contains fenofibrate equivalent to the amount in 0.5 ml NLC dispersion, was weighed, and 0.5 ml of water and 4.5 ml of acetone were added to it. The dispersion was heated to 50 °C to extract fenofibrate from the lipid nanoparticles. The dispersion was allowed to cool down to room temperature and filtered using 0.45 μm filter. The filtrate was diluted 100 times with the mobile phase and analyzed with HPLC. The HPLC analysis was carried out on a Dionex HPLC system (California, USA) and was analyzed using the software Chromeleon 7.1. The analytical column used was the Thermo analysis hypersil gold C_{18} column (5 μm, 150 × 4.6 mm). The mobile phase was composed of methanol and water (80:20). The flow rate was fixed at 1.0 ml/min and the detection was performed at 288 nm using a UV-VIS detector.

2.4.3. *In vitro drug release study*

The drug-release study was performed using USP type 2 paddle dissolution apparatus. 500 ml of 0.5% SLS was used as dissolution medium. The dissolution experiments were performed at 37 ± 0.5 °C, with a paddle speed of 100 rpm. 2 ml of lipid nanoparticles suspension, which is equivalent to 12.5 mg of fenofibrate, was dispersed in dissolution medium. Samples of 0.4 ml were withdrawn at 5, 15, 30 min, 1, 2, 4, 6 and 8 h and placed in a centrifugal tube with a 50 kDa molecular weight cutoff, then centrifuged at 9000 rpm for 3 min. After every withdrawal, the sample volume was replaced with 0.4 ml fresh dissolution medium. Quantification of the ultrafiltrate samples was done using the aforementioned HPLC method. The experiments were performed in triplicate ($n = 3$).

The drug-release study from spray-dried NLC powders was performed with the amount of spray-dried NLCs which contains an amount of fenofibrate equivalent to the amount present in 2 ml of an NLC dispersion. The experimental setup for the drug-release study from spray-dried NLC particles was similar to the drug-release study from NLC dispersion.

2.4.4. *X-ray powder diffraction*

The X-ray powder diffraction patterns were recorded using a PANalytical X'Pert Pro diffractometer equipped with a PIXcel detector (PANalytical X'Pert PRO MPD system, PW3040/60, The Netherlands). Samples were placed on zero-background silicon plates, and measured at ambient condition in reflection mode. A continuous 2θ scan was performed in the range 5° to 45° with a step size of 0.026° using Cu Kα radiation (λ = 1.5418 Å). The voltage and current applied were 45 kV and 40 mA, respectively. The scanning speed was 0.067° 2θ/s. Data were collected using X'pert Data Collector software and analyzed using X'Pert HighScore.

For the calculation of the degree of crystallinity a whole-pattern approach rather than a single-peak approach was used to eliminate the effect of preferred orientation. The calculation relies on the division of the powder diffraction pattern into three contributions: the crystalline part, the amorphous part and the background. The background was determined by measuring three empty zero-background silicon plates and averaging the results; the three background patterns were essentially identical and consisted of weak intensity uniform across the 2θ range. This averaged background was subtracted from all powder patterns before further calculations, leaving powder patterns consisting of only the crystalline and the amorphous signals. The amorphous part was distinguished from the crystalline part by means of the Brückner algorithm [27],

Table 1 – CCFD of experiments and its responses.

Exp. name	Run order	Lipid content (%)	Mannitol content (%)	Particle size (nm)	Moisture content (%)	Yield (%, w/w)
N1	9	5	50	1255	3.7	15.7
N2	8	20	50	2219	2.8	26.6
N3	4	5	100	1417	0.4	9.4
N4	5	20	100	3588	0.7	5.4
N5	10	5	75	1942	2.0	3.9
N6	7	20	75	5123	2.0	0.9
N7	1	12.5	50	4945	2.9	15.7
N8	11	12.5	100	2022	0.8	3.1
N9	3	12.5	75	2088	1.9	6.3
N10	2	12.5	75	2417	2.7	6.8
N11	6	12.5	75	2256	2.6	6.4

Note: Central point repetition tests = 3; The variation of these repetition test results are: standard deviation for Particle size = 164 nm; standard deviation for Moisture content = 0.4 %; standard deviation for Yield = 0.3%.

essentially a high-pass filter that separates the slowly fluctuating background from the higher-frequency changes associated with Bragg peaks and noise; we note that application of the Brückner algorithm does not require the crystal structures of the crystalline phases to be known. Because mannitol and trehalose have the same chemical composition, no further corrections are needed and the degree of crystallinity can be calculated directly as the ratio of the crystalline contribution divided by the sum of the crystalline and the amorphous contributions. The calculations were carried out in the Materials Studio software [28].

2.4.5. *Residual moisture content and yield*

The residual moisture content in the spray-dried samples was determined by Perkin Elmer TGA 7 (Perkin Elmer, USA) and Pyris software. The samples were heated from 25 °C to 180 °C at 10 °C/min in a dry nitrogen atmosphere at a nitrogen flow rate of 20 ml/min. The moisture content was then calculated by the sample weight loss after heating using equation (2).

$$\text{Moisture content}\,(\%) = \frac{(SWb - SWa)}{SWb} \times 100\% \quad (2)$$

where SW_b is the sample weight before heating and SW_a is the sample weight after heating. The yield was determined by calculating the percentage of spray dried powder as compared to the total amount of feed materials including drug, lipid, mannitol and trehalose that were used in the formulation.

2.4.6. *Scanning electron microscope*

The spray-dried samples were analyzed using a JEOL, JSM 5200 scanning electron microscope (JEOL Inc., MA, USA). The samples were placed on aluminum probes and sputter coated with gold using an Auto Sputter Coater E5200, Bio-Rad Polaron, and observed under SEM at an accelerating voltage of 1 kV. The Atomic Force Microscope (AFM) image was acquired by Multimode NanoScope V system (Veeco Instruments Inc., Plainview, NY). Samples were prepared by deposition of a 2 µl drop of a NLC suspension onto freshly cleaved mica. Commercial silicon nitride cantilevers, 100 µm in length, with a normal spring constant 0.5 Nm^{-1}, with integrated sharpened tips, were used. The topographic profile was obtained by tipping mode in liquid with a scan rate of 1–2 Hz.

2.5. *Experimental design and statistical evaluation*

A central composite face-centered design (CCFD) with 11 experiments was developed for the optimization of solidification of NLCs using spray drying, as shown in Table 1. The influence of variables such as ratio of lipid to total carbohydrate content and ratio of mannitol to trehalose was investigated. The responses were yield of powder (%), moisture content (%) and particle size after re-dispersion of spray dried NLC. The experiments were generated and analyzed by the software MODDE 9.1 (Umetrics AB, Sweden). The significance of the model was determined by analysis of variation (ANOVA). The responses of the model, Y_1 = Particle size, Y_2 = Yield and Y_3 = Moisture content, were modeled using the following polynomial quadratic equation.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1X_1 + b_4X_2X_2 + b_5X_1X_2 \quad (3)$$

where b_1 and b_2 are the linear coefficients, b_3 and b_4 are the quadratic coefficients and b_5 is the interaction coefficient between the two factors X_1 and X_2. The results were analyzed by ANOVA, which determined if the factors and the interactions were significant or not on a 95% significance level. The dissolution profiles were compared using two-way ANOVA and post test Bonferroni with multiple comparisons using the software GraphPad Prism 5 (GraphPad Software, Inc.).

3. Results and discussion

3.1. *Crystallinity content of carbohydrates in the spray-dried powder*

The crystallinity content of the protectant in the spray-dried powder may affect the particle size and moisture content of the sprayed powder. Thus in this section, the relationship between degree of crystallinity and the content of mannitol was established. This degree of crystallinity was determined experimentally by co-spray drying mannitol and trehalose solutions with six different ratios, and calculating the degree of crystallinity from the XRPD pattern. As shown in Fig. 1, a good linear relationship (R^2 = 0.9915) could be established between the crystalline content (%) against the mannitol content (%) from 30% to 100%.

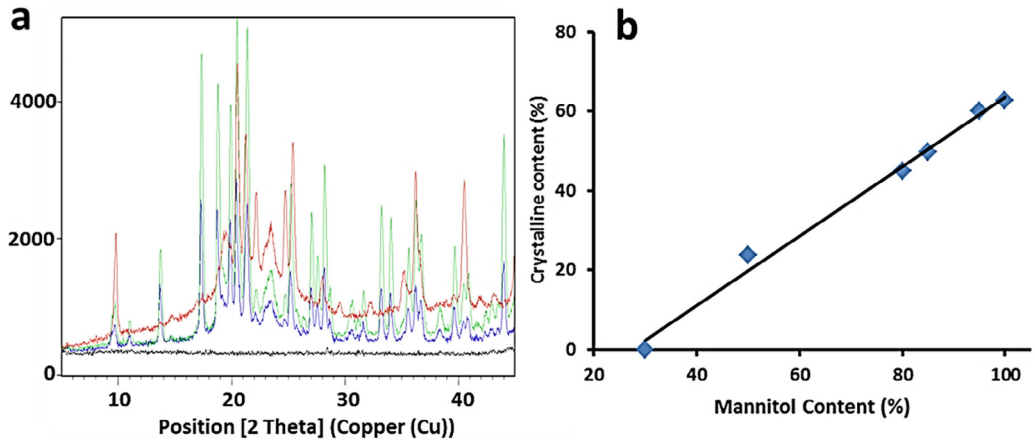

Fig. 1 – (a) X-ray powder diffraction patterns showing an increase in crystalline phase with an increase in the mannitol content in the formulation: red line: 50% mannitol; blue line: 75% mannitol; green line: 100% mannitol; (b) Linear relationship between mannitol content (%) and crystalline content (%) (R^2 = 0.9915).

3.2. *Optimization of spray drying using CCFD*

3.2.1. *Statistical analysis and fitted polynomial equation of CCFD*

Based on the linear relationship between degree of crystallinity against mannitol content, approximately 75% mannitol (which corresponds to a 75:25 mannitol to trehalose ratio) was used as the midpoint to design a set of experiments in which the mannitol content was varied from 50% to 100%. The lipid-to-carbohydrate ratio was varied from 5:95 to 20:80 maintaining the total solid concentration at 20% (w/v). A CCFD with 11 experiments was set up to investigate the influence of crystalline content and lipid content on yield, moisture content and the particle size after redispersion of the spray-dried NLC. The CCFD of experiments and its responses are shown in Table 1.

ANOVA was performed on the experimental data to evaluate the significance and validity of the model. As shown in Table 2, the model was significant for all responses with good regression coefficients. The predictive power (Q^2) was very good (>0.5) for particle size and moisture content, but was below the reference for yield (0.3545). The particle size and moisture content fitted well with a significance level of 95%, but the yield showed a lack of fit on a confidence level of 95%. The fitted polynomial equations for all the responses were constructed by generating the scaled and centered coefficients in MODDE 9.1, which are shown in Table 3. The contour plots for particle size, moisture content and yield are shown in Fig. 2. These polynomial equations allow us to evaluate the effect of the investigated factors and predict the responses to obtain the optimal process parameters.

3.2.2. *Particle size after redispersion in water*

The coefficients (b_1 to b_4) were statistically significant (p value <0.05). The significant and positive values of the coefficients indicate that the particle size of the redispersed NLC system significantly increases with an increase in lipid content and mannitol content (Fig. 2). The potential explanation for this is that carbohydrates can form a thick protective layer around the lipid nanoparticles which protects them against the mechanical stress and heat stress during spray drying. When the lipid content increases, the corresponding amount of carbohydrate present decreases and is insufficient to protect the lipid nanoparticles against the stress during spray drying, which could increase the tendency of particle aggregation, thus causing an increase in particle size [14,29]. It can also be observed that when the mannitol content is increased from 50% to 100%, the particle size also increases significantly. Similar results have been reported before where trehalose showed better redispersion and better retention of the particle size of original dispersion than mannitol [14]. From the powder diffraction patterns in our study, the crystalline content of the formulations increased significantly up to 62% when increasing the

Table 2 – Model performance indicators and ANOVA analysis results for the investigated responses for spray drying NLC.

Model performance indicator		Reference value for good model	Investigated responses		
			Particle size (nm)	Moisture Content (%)	Yield (%, w/w)
Regression coefficients(R^2)		Close to 1	0.9850	0.9466	0.8811
Difference (R^2–Q^2)		<(0.2–0.3)	0.0428	0.199	0.5266
Goodness of prediction (Q^2)		>0.5	0.9422	0.7470	0.3545
Model validity		>0.25	0.8978	0.9292	–0.2000
Reproducibility		>0.5	0.9601	0.8379	0.9987
ANOVA	Significance of regression model (p-value)	<0.05	0.001	0.001	0.006
	Lack of fit test (p-value)	>0.05	0.665	0.754	0.004[a]

[a] Non-significant coefficient contributions.

Table 3 – Scaled and centered coefficients of the investigated responses for the polynomial equation (3).

Polynomial equation for the selected model

$Y_i = \text{constant} + b_1 \times X_1 + b_2 \times X_2 + b_3 \times X_1^2 + b_4 \times X_2^2 + b_5 \times X_1 \times X_2$.

X_1, Lipid:Carbohydrates ratio; X_2, Mannitol:Trehalose ratio

Coefficients		Responses		
		Y_1, Particle size	Y_2, Moisture content	Y_3, Yield
	Constant	2281.15	2.30226	4.864
b_1	x_1	1053.86	–0.113833[a]	0.680833[a]
b_2	x_2	703.504	–1.25817	–6.69917
b_3	$x_1 \times x_1$	632.431	–0.174658[a]	7.78183
b_4	$x_2 \times x_2$	–1063.94	–0.295657[a]	[b]
b_5	$x_1 \times x_2$	[b]	0.30725[a]	–3.71625[a]

[a] Non-significant coefficient contributions.
[b] Missing values indicate removal of the term during model refinement.

mannitol content from 50% to 100%. The increase in particle size could be due to the poor wetting ability of the crystalline phase compared to the amorphous phase [30]. With increasing mannitol content, the particles were dispersed with more difficulty in water, leading to insufficient breakdown of particle aggregates. In addition, the higher amount of mannitol increased the crystallization tendency of the spray dried powder. The mechanical forces exerted in the crystallization of mannitol may lead to fusion of NLCs as a result of failure to protect nanoparticle from agglomeration.

Freeze-drying is another technique to improve stability of colloidal nanoparticles. The high concentration of particulate system and the crystallization of ice may also induce aggregation and in some cases irreversible fusion of nanoparticles, leading to its destabilization. For these reasons, cryoprotectants and/or lyoprotectant must be added to the suspension of nanoparticles before freezing drying to protect nanoparticles [26]. Trehalose is also proved to be most effective in preventing nanoparticle growth during freeze-drying process. Changes in particle size during lyophilization could be minimized by optimizing the parameters of the lyophilization process [31], whereas some solid lipid nanoparticles (SLN), such as SLN containing hydrocortisone and progesterone complexes with β-cyclodextrins, could be freeze-dried without the addition of cryoprotectants [32]. The freeze-drying is cost intensive and time-consuming compared with spray drying.

The powder obtained by freeze-drying has a loose structure, while spray drying generates powder with better flow property. It was observed in this study that the spray-dried product flowed more freely when the proportion of trehalose in the feed solution was increased. However, we failed to explain the mechanism. The difference in particle size of spray dried powder would be an explanation, as flow properties of powder are known to be influenced by particle size. We speculate that when trehalose was used as a protectant, the particles would have relative larger particle size than that of mannitol used, because the spray dried trehalose particle is in amorphous state and has a lower density than that of crystalline material. However, we did not observe a distinct difference in particle sizes of spray dried powder containing mannitol or trehalose under SEM. The difference in flow properties could also be attributed to other factors such as electrostatic interactions and surface chemistry. However they were not investigated in the current study. Nevertheless we speculate there was more lipid presenting on the surface of the particles made of mannitol. It could be due to the fact that crystallization of mannitol 'squeezed' lipid to the surface of the particles, resulting in fusion of particles and making the particles sticky.

3.2.3. *Moisture content*

After investigation of the fitted polynomial equation for response variable Y_2 (moisture content), only linear coefficient b_2 is significant with a high regression coefficient ($R^2 = 0.9466$). The significance of coefficient b_2 and the high predictive power ($Q^2 = 0.7470$) suggest that the moisture content is only significantly influenced by the ratio of mannitol to trehalose, as shown in Fig. 2, while the effect of lipid content on the moisture content is not significant. When the mannitol content is increased from 50% to 100%, the residual moisture content in

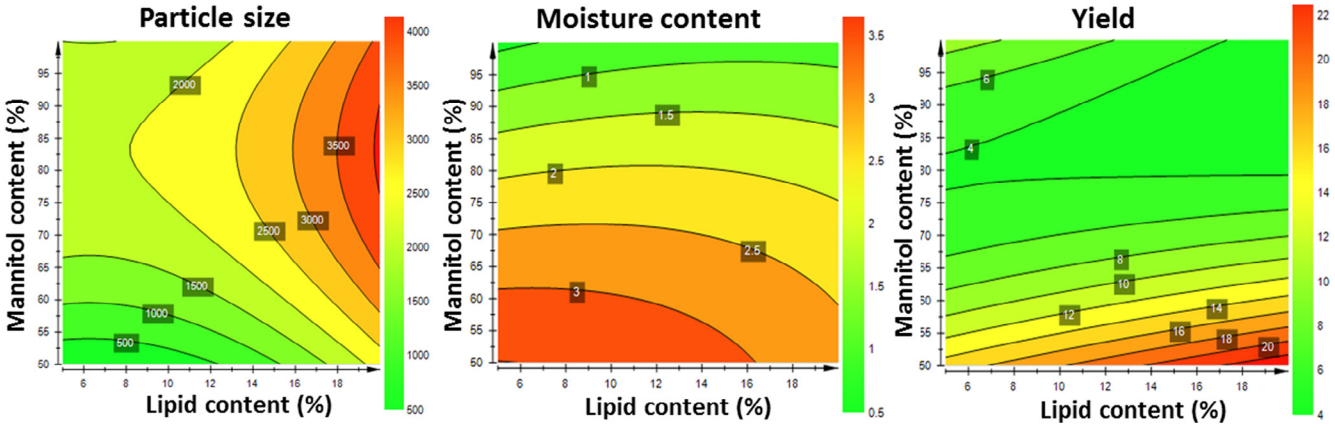

Fig. 2 – Contour plots for particle size, moisture content and yield. The particle size of the redispersed NLC system significantly increases with an increase in lipid content and mannitol content. The moisture content is only significantly influenced by the ratio of mannitol to trehalose. The yield decreases with an increase in the mannitol content.

the spray-dried product decreased significantly from 3.7% to 0.4%. This could be explained by the lower hygroscopicity of the crystalline phase of mannitol compared to the amorphous phase [33]. Therefore, it can be concluded that a low moisture content can be maintained by using a sufficient amount of mannitol.

3.2.4. *Yield*

The model fitted for the response yield (Y_3) was found to be significant with a regression coefficient value of 0.8811, as shown in Table 2. The predictive power of the model is very low (0.3545), showing lack of fit on a significance level of 95%. Further investigation of the fitted polynomial equation for the yield showed that the only statistically significant regression coefficients were b_2 and b_3 (Table 3). The negative value for the linear regression coefficient b_2 indicates that with the increase in the mannitol content, the yield decreases; this can also be observed in the contour plots of Fig. 2. A possible reason behind this could be the formation of sticky and less free flowing powder with increasing content of mannitol probably due to electrostatic interactions. A spray-dried formulation with 100% mannitol yielded particles that were sticking to the walls of the drying chamber, the cyclone and the collection vessel because of van der Waals force or electrostatic adherence. The results indicate there is no significant influence of the lipid content on the yield, which may be attributed to the large percentage of carbohydrate in the formulation. The material property of the spray dried powder is dominated by carbohydrate.

3.3. *Optimized formulation and characterization of optimized spray dried NLC*

After analyzing and validating the design, an optimizer was used to predict a combination of investigated factors that would result in an optimal product [34]. Here, the responses were specified according to the experimental goals i.e., maximum yield (>50%), minimal particle size (500 nm) and minimal moisture content (<1.0%). Different combinations of factors were generated by the optimizer, but none of them could meet the desirable values for all the responses. So, considering particle size and yield as the primary concern, factor combinations from the optimizer were selected that would meet optimal responses for yield and particle size which is shown in a sweet spot plot in Fig. 3, where the green region indicates the sweet spot.

To validate the experimental model, experiments in the sweet spot with 13% lipid content and 50% mannitol content were performed in triplicate ($n = 3$), and were compared with the predicted value. The predicted response and the obtained experimental results are shown in Table 4. Two sample t-tests ($\alpha = 0.05$) showed that there was no significant difference between the observed and the predicted values for all the responses, confirming that the model is robust. The results from this optimization study showed the possibility of regulating the characteristics of a spray-dried powder by altering the investigated factors.

The particle size (z-average, nm) of freshly prepared NLCs was 98 ± 1 nm. The entrapment efficiency of NLC was determined about 99.8% due to the high affinity of drug to α-form

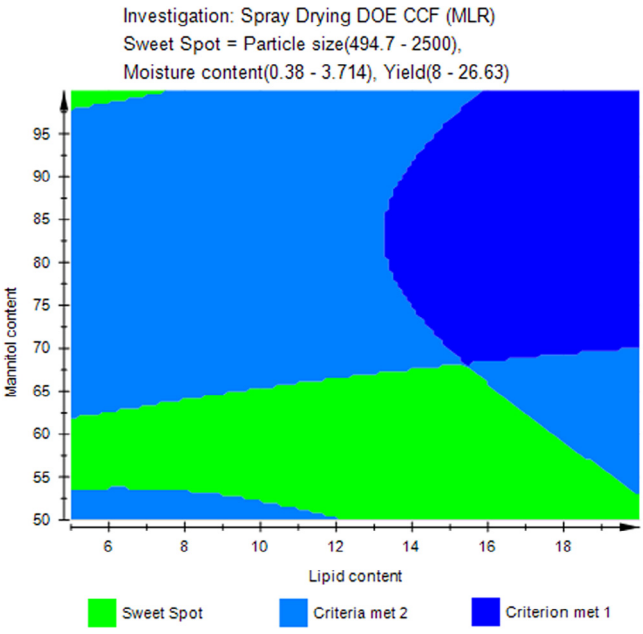

Fig. 3 – Sweet spot plot showing the optimal region where the green region indicates the sweet spot.

of lipid [10,25]. The total fenofibrate content in 260 mg of spray dried NLC was measured to be 5.52 mg. A two sample Student's t-test showed that the difference between amount of fenofibrate present in 260 mg of spray dried NLC and 0.5 ml of NLC dispersion was insignificant ($p = 0.60$). This indicates that spray drying NLC is able to retain the incorporated drug in it. This is probably because of the lipophilic nature of the drug which facilitates the retention of the drug in the lipid matrix even during spray drying. The NLCs have a spherical shape observed by atomic force microscopy, as shown in Fig. 4a. The spray-dried powder shows irregular shapes with particle sizes ranging from 10 to 100 μm (Fig. 4b).

The *in vitro* drug-release profile of NLC dispersion before and after spray drying is shown in Fig. 5. Although a two way ANOVA showed that after spray drying the release profile has been slightly changed ($p = 0.0235$), a prolonged drug release was still observed, showing a possibility of maintaining sustained drug release from NLCs even after conversion to dried powder. Our previous study showed that the dominant factors affecting the drug release profiles could be the nature of lipid matrix, which was influenced by the transformation of lipid crystalline structure. The crystalline form of freshly prepared lipid particles is α-form with drug well entrapped [10]. The crystalline form of lipid may be partly changed from α-form to a more rigid β-form by heat stress during spray drying, leading to expulsion of drug

Table 4 – Predicted and observed results for the optimized spray-dried NLC (observed value ± SD, $n = 3$).

Formulations (lipid content 13%, mannitol content 50%)	Moisture content (%)	Particle size (nm)	Yield (%)
Predicted results	3.2 ± 0.6	586 ± 332	19.6 ± 4.7
Spray dried NLC with drug	3.3 ± 0.5	736 ± 105	16.7 ± 1.5

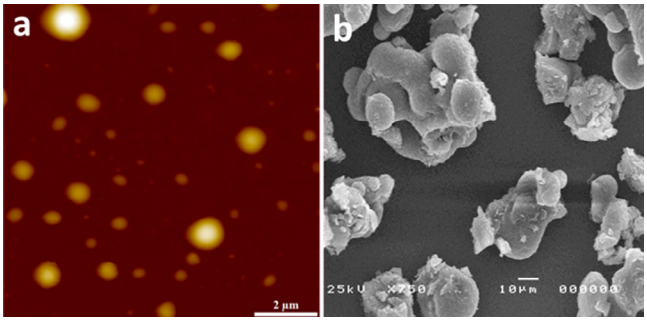

Fig. 4 – AFM pictures showed a spherical shape of the NLCs before spray drying (a), and SEM pictures showed irregular shape of the spray-dried NLC powder (b).

out of lipid nanoparticles during this crystalline transformation [10]; however, our XRPD result was not able to prove this speculation due to the shielding by mannitol crystals. Another possible reason could be due to the fact the fusion of lipid particles during the spray drying process may expel some drug to the surface of the particles.

4. Conclusion and future perspective

This study demonstrated spray drying as a viable method to solidify NLCs to a powder form. A CCFD was constructed to investigate the influence of lipid-to-carbohydrate ratio and mannitol-to-trehalose ratio on the characteristics of the resultant product such as yield, residual moisture content and the particle size after redispersion. The results from the statistical analysis and mathematical modeling of the experimental design indicated that both lipid and mannitol content significantly influenced the particle size after redispersion. However, the residual moisture and the yield were only significantly influenced by the mannitol-to-trehalose ratio. The experimental model is robust as no significant difference was seen between observed and predicted responses for the optimal formulation. These results suggest that spray drying is a suitable technique for conversion of NLCs into dry powder, and that manipulation of some crucial factors can result in the desired formulation. Moreover, the statistical design has shown usefulness in the optimization process, and this study might serve as groundwork for the further understanding of the solidification of NLCs.

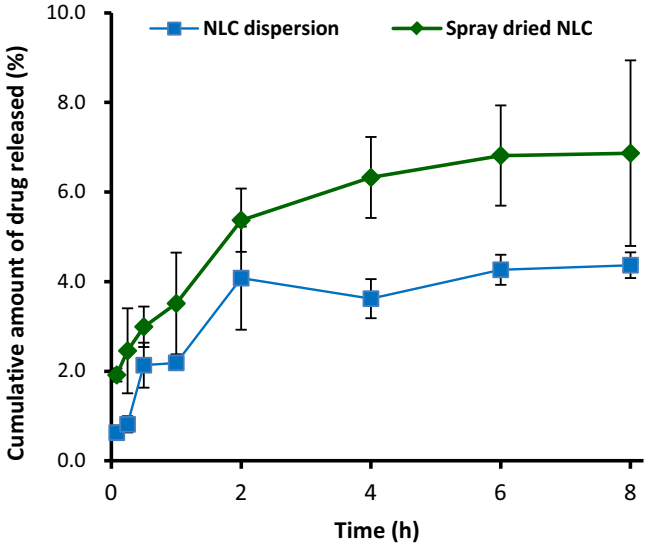

Fig. 5 – *In vitro* drug-release profile from NLCs before and after spray drying over a period of 8 hours (n = 3, error bars = standard deviation).

Acknowledgements

Jacco van de Streek gratefully acknowledges the Lundbeck Foundation (Denmark) (grant No. R49-A5604) for financial support. Mingshi Yang acknowledges the support from the National Nature Science Foundation of China (No. 81573380).

REFERENCES

[1] Porter CJ, Trevaskis NL, Charman WN. Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. Nat Rev Drug Discov 2007;6:231–248.
[2] Mu H, Holm R, Müllertz A. Lipid-based formulations for oral administration of poorly water-soluble drugs. Int J Pharm 2013;453:215–224.
[3] Vaghasiya H, Kumar A, Sawant K. Development of solid lipid nanoparticles based controlled release system for topical delivery of terbinafine hydrochloride. Eur J Pharm Sci 2013;49:311–322.
[4] Bunjes H, Drechsler M, Koch MHJ, et al. Incorporation of the model drug ubidecarenone into solid lipid nanoparticles. Pharm Res 2001;18:287–293.
[5] Bunjes H, Westesen K, Koch MHJ. Crystallization tendency and polymorphic transitions in triglyceride nanoparticles. Int J Pharm 1996;129:159–173.
[6] Heurtault B, Saulnier P, Pech B, et al. Physico-chemical stability of colloidal lipid particles. Biomaterials 2003;24:4283–4300.
[7] Freitas C, Müller RH. Correlation between long-term stability of solid lipid nanoparticles (SLN(TM)) and crystallinity of the lipid phase. Eur J Pharm Biopharm 1999;47:125–132.
[8] Westesen K, Bunjes H, Koch MHJ. Physicochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential. J Control Release 1997;48:223–236.
[9] Westesen K, Siekmann B. Investigation of the gel formation of phospholipid-stabilized solid lipid nanoparticles. Int J Pharm 1997;151:35–45.
[10] Xia D, Cui F, Gan Y, et al. Design of lipid matrix particles for fenofibrate: effect of polymorphism of glycerol monostearate on drug incorporation and release. J Pharm Sci 2014;103:697–705.
[11] Shidhaye SS, Vaidya R, Sutar S, et al. Solid lipid nanoparticles and nanostructured lipid carriers—innovative generations of solid lipid carriers. Curr Drug Deliv 2008;5:324–331.
[12] Müller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Deliv Rev 2002;54(Suppl. 1):S131–S155.
[13] Müller RH, Mader K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery—a review of the state of the art. Eur J Pharm Biopharm 2000;50:161–177.

[14] Freitas C, Müller RH. Spray-drying of solid lipid nanoparticles (SLN TM). Eur J Pharm Biopharm 1998;46:145–151.
[15] Ingvarsson PT, Yang M, Nielsen HM, et al. Stabilization of liposomes during drying. Expert Opin Drug Deliv 2011;8:375–388.
[16] Lee YY, Wu JX, Yang M, et al. Particle size dependence of polymorphism in spray-dried mannitol. Eur J Pharm Sci 2011;44:41–48.
[17] Yang M, Yamamoto H, Kurashima H, et al. Design and evaluation of inhalable chitosan-modified poly (DL-lactic-co-glycolic acid) nanocomposite particles. Eur J Pharm Sci 2012;47:235–243.
[18] Yang M, Yamamoto H, Kurashima H, et al. Design and evaluation of poly(DL-lactic-co-glycolic acid) nanocomposite particles containing salmon calcitonin for inhalation. Eur J Pharm Sci 2012;46:374–380.
[19] Grohganz H, Lee YY, Rantanen J, et al. The influence of lysozyme on mannitol polymorphism in freeze-dried and spray-dried formulations depends on the selection of the drying process. Int J Pharm 2013;447:224–230.
[20] Cun D, Wan F, Yang M. Formulation strategies and particle engineering technologies for pulmonary delivery of biopharmaceuticals. Curr Pharm Des 2015;21:2599–2610.
[21] Ingvarsson PT, Yang M, Mulvad H, et al. Engineering of an inhalable DDA/TDB liposomal adjuvant: a quality-by-design approach towards optimization of the spray drying process. Pharm Res 2013;30:2772–2784.
[22] Baldinger A, Clerdent L, Rantanen J, et al. Quality by design approach in the optimization of the spray-drying process. Pharm Dev Technol 2012;17:389–397.
[23] Lebrun P, Krier F, Mantanus J, et al. Design space approach in the optimization of the spray-drying process. Eur J Pharm Biopharm 2012;80:226–234.
[24] Ren S, Mu H, Alchaer F, et al. Optimization of self nanoemulsifying drug delivery system for poorly water-soluble drug using response surface methodology. Drug Dev Ind Pharm 2013;2013:799–806.
[25] Borkar N, Xia D, Holm R, et al. Investigating the correlation between in vivo absorption and in vitro release of fenofibrate from lipid matrix particles in biorelevant medium. Eur J Pharm Sci 2014;51:204–210.
[26] Abdelwahed W, Degobert G, Stainmesse S, et al. Freeze-drying of nanoparticles: formulation, process and storage considerations. Adv Drug Deliv Rev 2006;58:1688–1713.
[27] Brückner S. Estimation of the background in powder diffraction patterns through a robust smoothing procedure. J Appl Cryst 2000;33:977–979.
[28] Materials Studio version 6.0. Accelrys Inc., San Diego, California, USA; 2011.
[29] Reddy LH, Murthy RSR. Etoposide-loaded nanoparticles made from glyceride lipids: formulation, characterization, in vitro drug release, and stability evaluation. AAPS PharmSciTech 2005;6:9.
[30] Puri V, Dantuluri AK, Kumar M, et al. Wettability and surface chemistry of crystalline and amorphous forms of a poorly water soluble drug. Eur J Pharm Sci 2010;40:84–93.
[31] Schwarz C, Mehnert W. Freeze-drying of drug-free and drug-loaded solid lipid nanoparticles (SLN). Int J Pharm 1997;157:171–179.
[32] Cavalli R, Peira E, Caputo O, et al. Solid lipid nanoparticles as carriers of hydrocortisone and progesterone complexes with beta-cyclodextrins. Int J Pharm 1999;182:59–69.
[33] Yoshinari T, Forbes RT, York P, et al. Moisture induced polymorphic transition of mannitol and its morphological transformation. Int J Pharm 2002;247:69–77.
[34] Statistical notes. In: Eriksson L, Johansson E, Kettaneh-Wold N, et al., editors. Design of experiments: principles and applications. Umeå, Sweden: Umetrics AB; 2008. p. 430.