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RESEARCH PAPER

A Design of Experiment Study of Nanoprecipitation and Nano Spray Drying as Processes to Prepare PLGA Nano- and Microparticles with Defined Sizes and Size Distributions

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ABSTRACT

Purpose Aim of this study was to explore the potential of a design of experiments approach to nanoprecipitation (NPR) and nano spray drying (NSD) as processes for preparing poly (lactic-co-glycolic acid, PLGA) nano- and microparticles. In particular, we determined the feasible size range, critical factors influencing particle size, size distribution or yield, and the robustness towards variations of the batch size.

Methods A fractional factorial design for response surface was applied to study the influence on continuous, categorical and discrete factors.

Results NPR yielded nanoparticles (150–200 nm) with narrow size distribution (PDI < 0.15). Polymer concentration was the main factor in this process, which was found to be very robust to varying the batch size (0.625–50.0 ml). In contrast, NSD yielded microparticles (2–163 μm). The latter process appeared, however, to be influenced by various factors and, therefore, more difficult to control and less robust towards varying the batch size (5–40 ml). By a factorial design approach to NPR, we succeeded to derive an equation, which allowed the prediction of several optimal formulations with defined particle sizes and distributions.

Conclusion DOE can help to understand innovative manufacturing processes for nano- and microparticulate drug delivery systems, as well as to optimize these processes regarding particle size, size distribution and yield. Such understanding of these processes is instrumental for their subsequent scale up and quality control as needed for preclinical and clinical test batches.

KEY WORDS Büchi Nano Spray Dryer · nanoparticles · nanoprecipitation · quality by design · submicron particles

ABBREVIATIONS

ANOVA	Analysis of variance
DCM	Dichloromethane
DDS	Drug delivery systems
DOE	Design of experiments
MP	Microparticle
NP	Nanoparticle
NPR	Nanoprecipitation method
NSD	Nano spray drying technique
PDI	Polydispersity index
PLGA	Poly (lactic-co-glycolic acid)
R	Run

INTRODUCTION

In the preparation of nano- and microscale drug delivery systems (DDS) it is highly important to understand which parameters have an influence on the process and, thus, on the formation of the nanoparticles and process yield. Size and size distribution are both critical quality properties, which may

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affect disposition, drug release and bioavailability (1). With the knowledge of process parameters and limitations, critical quality attributes and sources of variability, reproducibility and controllability of the process and consistent quality of the DDS can be achieved (2). Such knowledge can further be utilized specifically to optimize and adapt the DDS, e.g., in size for different applications. This is important for research and development and makes the production process more efficient by reducing the number of experiments and amount of material. The latter helps to reduce the costs of goods, as well as the pollution of work place and environment. In a future perspective such knowledge may also facilitate the scale-up such process towards relevant batch sizes as needed for (pre)clinical testing. The complex transfer of nano- and microscaled DDS to a larger production scale is often hindering the advancement of a promising formulation from early development to production stage and can be a bottleneck for entrance of these particle-based DDS into the pharmaceutical market (3).

In this context we pursued quality by design by applying a design of experiments (DOE) approach to a nanoprecipitation method (NPR), which represents one of the most frequently used methods for producing nanoscale DDS (4). In addition, we also evaluated a new and, therefore less examined nano spray drying technique (NSD). The goal was to produce nanoparticulate DDS from poly (lactic-co-glycolic acid) (PLGA, 50/50, *wt/wt*), which is approved in the medical field, e.g., for surgical sutures. PLGA will be degraded through hydrolysis of the ester-bonds to its two non-toxic components, lactic acid and glycolic acid, which can be metabolized in the citric acid cycle (1,5). The fastest degradation of PLGA is reached with a 50/50 ratio of lactic and glycolic acids (*wt/wt*). In this ratio PLGA is amorphous and, therefore, optimal for DDS as it decomposes within 2 months (6,7).

Quality by design in the pharmaceutical field means the systematic development of pharmaceutical products on the basis of sound scientific principles to ensure pre-defined product quality (2,8,9). Quality by design studies have increasingly been used in the field of nano- and microparticulate DDS throughout the last years, focusing mainly on the optimization of DDS loaded with active ingredients. Especially for complex processes, such as for the production of nanomedicines, DOE methods are excellent quality by design tools for identifying (a) critical process parameters, (b) to understand and (c) to optimize process conditions and, therefore, the final formulation (8–15). In these studies for example the optimization of size (10,15–18), and of drug loading for PLGA nanoparticles (15–17,19,20), Eudragit® E nanoparticles (8) and liposomes (14) were investigated. Furthermore, DOE studies have been applied to investigate colloidal stability of lipid nanoparticles (21) or the influence of the stabilizer on size and zeta potential of PLGA nanoparticles (17). Moreover, also the modification of the drug release from established DDS was investigated by a DOE (17,22). All these All these studies describe the optimization in preparation of DDS in a laboratory context. Park *et al.* (8) and Verma *et al.* (9) underlined the importance to characterize process parameters and limitations, as well as critical quality attributes to achieve high quality products and, therefore, to facilitate the entrance to further studies (8).

To investigate both methods systematically, we used a fractional factorial design based on a response surface approach with an IV (Integrated Variance)-optimal design. DOE could help to understand in detail the influence of parameters on an investigated process. Compared to classical experiments DOE has many advantages. For example DOE offers the opportunity to vary several factors at the same time and, thus, to evaluate their main effects as well as interactions of factors. In general interactions of third and fourth order are negligible (23). Multiple types of factors can be accommodated such as continuous, categorical and discrete factors. Furthermore, experimental limitations, due to safety reasons, can be taken into consideration in the design of the DOE (22), which is not possible in a classical experimental design. Hence, it appears an adequate tool for the development of DDS. The DOE can also be used to characterize new methods without knowing any limitations of the system. Moreover, it can be applied to adapt a method or process for a new application approach (24).

In the present study for both methods, NPR and NSD, the two major goals of the DOE was to (a) identify parameters controlling the respective process; and (b) identify limitations of the process with regards to fabricable batch sizes for a fixed set of (optimized) process parameters. The different batch sizes should be produced without changing the general apparatus set-up, which is relevant for example in the context of early formulation development, characterization and pre-clinical development, in particular in the field of nano- and microscaled DDS. The complexity of the process techniques can cause problems by changing the apparatus (3). Therefore, a set-up which can be used to produce first test batches up to batches for pre-clinical studies would be a benefit.

Our main focus for the NPR was to prepare PLGA based nanoparticles with a size of 150–200 nm and a narrow size distribution characterized by a polydispersity index (PDI) <0.150. For the NSD the DOE should show controllability, limitations and robustness of the process for the examined variables size, size distribution and yield larger than 50%. A nano spray dryer with vibrating mesh, instead of a nozzle for spraying feeding solutions, was used with the goal of producing PLGA particles as small as possible and with narrow size distributions for creating a novel PLGA based DDS in a single step.

Additionally, after determining the influencing factors for NPR and NSD, limitations of both processes by exploring maximal and minimal batch size were investigated. This is an indicator for the robustness of the process.

MATERIALS AND METHODS

Materials

Poly (lactic-co-glycolic acid) (PLGA; Resomer RG 503 H; inherent viscosity 0.41 dl/g) was bought from Evonik Industries AG (Darmstadt, Germany). Poloxamer 407 and polysorbate 80 were purchased form Caesar & Loretz GmbH (Hilden, Germany) and sorbitan monostearate (Span®60) was purchased from FAGRON GmbH & Co. KG (Barsbüttel, Germany). Purified water was of Milli-Q quality and prepared by a Millipore Milli-Q Synthesis system (Merck KGaA, Darmstadt, Germany).

All solvents were high performance liquid chromatography grade and all chemicals met the quality requirements of the European Pharmacopoeia 6.0–7.3.

Methods

Preparation of Nanoparticles by Nanoprecipitation (NPR)

The nanoparticles were produced by NPR as described elsewhere (25). PLGA was weighed accurately and dissolved in the organic phase (acetone; acetone/ethanol, 16/3, *v*/*v*). The solution was injected with a Hamilton® glass syringe (1005 TTL 5 ml, chromatography service GmbH, Germany) into purified water containing a stabilizer, polysorbate 80 or poloxamer 407, forming the aqueous phase. The ratio of organic to aqueous phase was kept constant in all experiments at 5 ml/10 ml. Likewise, in all experiments of the DOE the size of the magnetic stirrer (15 mm×4.5 mm, VWR, Germany), the inner diameter of the glass beaker (borosilicate glass beaker 3.3, 50 ml, VWR, Germany) and the stirring speed (500 rpm) during the injection were kept constant. After diffusion of organic solvent into the outer phase (aqueous phase), the stirring speed was reduced to 400 rpm and the organic solvent was evaporated by stirring overnight.

To accurately control the injection speed a high accuracy HARVARD® Ultra PHD pump (Hugo Sachs Elektronik, Germany) with a microprocessor controlled, small step angle motor that drives the pusher block was used. The HARV ARD® Ultra PHD pump operates at constant pressure and constant flow with a flow accuracy of ±0.25% and flow reproducibility of ±0.05%. The Hamilton® glass syringe was installed in the HARVARD® Ultra PHD pump in vertical direction to place the needle into the aqueous phase.

Preparation of PLGA Particles by Nano Spray Drying (NSD)

A Büchi Nano Spray Dryer B-90 (Büchi B-90; Büchi Labortechnik GmbH, Essen, Germany) was used for the NSD with a total drying chamber height of 150 cm (long set-up) as provided by the manufacturer (Fig. 1). To allow a safe use of organic solvent the spray drying apparatus was operated in the closed mode set-up with inert gas mixture (N_2 and CO_2 at 0.8 bar). Therefore the NSD was connected to the Inert-Loop B-295 and a cooling unit, which separates the organic solvent from the drying gas. The feeding solution was circulated in the spray head and atomized by pushing it through a 4 µm spray mesh, which vibrates at 60 kHz, and transports the feeding solution into the drying chamber. As in-process parameter the out-let temperature and the drying gas flow, which was fixed at 115 l/min, were monitored. The out-let temperature was found to be between 29 and 36°C, which is below glass-transition temperature (T_g) of the PLGA. The spray dried particles were collected by an electrostatic particle precipitator, generated by a high voltage between a grounded and a collecting electrode.

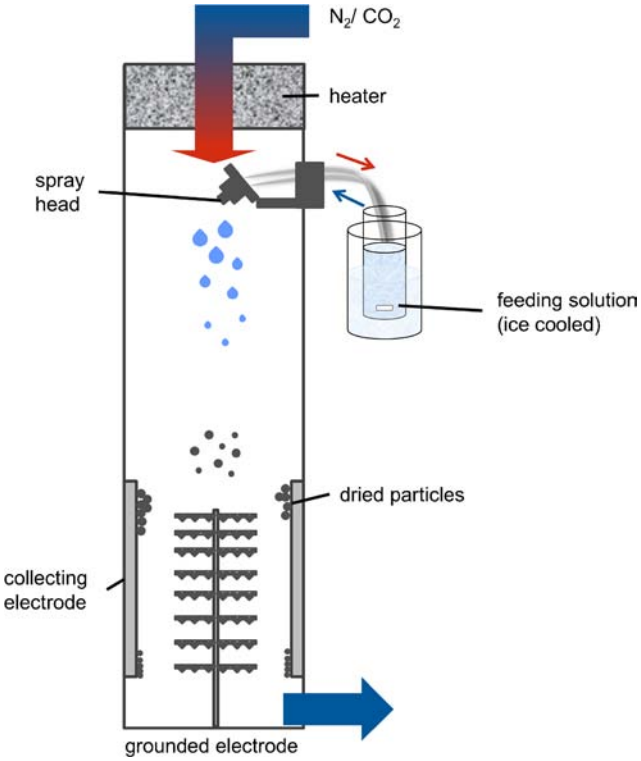

Fig. 1 Schematic set-up of the Büchi Nano Spray Dryer B-90.

For preparation of the feeding solution PLGA together with Span®60 or poloxamer 407 as stabilizer were accurately weighed and dissolved or suspended in the organic solvent (acetone, ethyl acetate or dichloromethane (DCM)). Afterwards the feeding solution was sprayed under ice cooling of the supplied dispersion to prevent heating of the circulating solution.

The particles were collected by a plastic scratcher and the amount was accurately weighed.

Design of Experiments

Design Expert software® (version 08, StatEase Minneapolis, USA) was used to perform a design of experiment study. The

design (model) was based on an IV (I = integrated; V = variance)-optimal design for surface response. Response surface designs using optimal designs provide lower average prediction variance over the region of experimentation. The design was chosen to minimize the integral of prediction variance over the region of experimentation, thereby compromising between the minimum number of experiments and maximum information on the investigated effects (23).

The selection of the experimental points for each factor was performed by the software and resulted in a table of experiments. The experiments (runs, R) were organized in a random order to minimize the effect of any drift. The results were evaluated by analysis of variance (ANOVA) and a regression analysis.

The coefficient of determination (R^2) measures the accuracy of the model and is defined as the proportion of the total variance of the experimental points explained by the model. The predicted coefficient of determination (predicted_R^2) was used to measure the reliability and robustness of the model and is defined in Eq. 1.

$$\text{predicted\_}R^2 = 1-\left(\frac{\text{residual cross validated variance}}{\text{total variance}}\right) \quad (1)$$

In Eq. 1 the residual cross-validated variance is defined by $\sum_{i=1}^{n}(y_i - y_{-i})^2$, where y_i equals the experimental response and y_{-i} equals the predicted response using a model based on n-1 other experimental points.

The ANOVA was performed with the Design Expert software® to reveal the influence of factors on total variance, expressed as effect. The influence between two factors is expressed as interaction. The results were expressed as % of total variance.

Factors Varied for the NPR

The investigated process parameters for the NPR were assigned as factors (Table I). For the NPR two continuous factors (polymer concentration and injection speed), one discrete factor (inner diameter of needle) and two level categoric factors (nature of the stabilizer and nature of the solvent) were evaluated. The investigated ranges are summarized in Table I. All factors received a coding for analyzing in the DOE. The selection of experimental points leads to the construction of initial 34 experiments including repetitions of five experiments. The investigated responses were size and size distribution. The objective was to produce particles controllable in size, expected to be between 100 and 200 nm, and narrow size distribution (PDI < 0.150). The maximum polymer concentration was fixed at 2% (*wt/v*) based on values commonly reported in the literature (26).

Table I Factors Varied for Nanoprecipitation Method and the Investigated Ranges. The Factors Were Separated in Continuous, Discrete and Level Categoric Factors and Coded (A–E) for the DOE

Coding	Factor		Investigated range
A	Continuous	Polymer concentration	0.1–2%
B		Injection speed	0.25–10 ml/min
C	Discrete	Inner diameter of needle	0.41/0.60 mm
D	Level categoric	Stabilizer	Polysorbate 80 Poloxamer 407
E		Organic solvent	Acetone Acetone/ethanol (16/3, *v/v*)

Factors Varied for the NSD

The investigated factors for the NSD and ranges are presented in Table II. For the NSD four continuous factors (temperature, polymer concentration, stabilizer concentration and spray rate) and three level categoric factors (nature of organic solvent, pumping rate and nature of stabilizer) were considered. The pumping rate was adjusted to low (level 1) or high (level 2) according to the specification of the manufacturer.

The selection of the experimental points led to the construction of initial 56 experiments including repetitions of four experiments. All factors received a coding for analyzing in the DOE.

The examined response variables were size, size distribution and yield with the objectives to produce PLGA particles as small as possible, down to the nm size range, with narrow size distributions (Eq. 2). Furthermore the yield should be above 50%.

Particles Size and Size Distribution of Nanoparticles Produced by NPR

Hydrodynamic diameter and polydispersity index (PDI) of nanoparticles were measured by dynamic light scattering

Table II Factors Varied for Nano Spray Drying and the Investigated Ranges. The Factors Were Separated in Continuous and Level Categoric Factors and Coded (A–E) for the DOE

Coding	Factor		Investigated range
A	Continuous	Temperature	50–90°C
B		Polymer concentration	0.1–5.0%
C		Stabilizer concentration	0.05–2.00%
D		Spray rate	70–100%
E	Level categoric	Organic solvent	Acetone Ethyl acetate Dichloromethane
F		Pumping rate	Low (level 1) High (level 2)
G		Stabilizer	Poloxamer 407 Sorbitan monostearate (Span®60)

(Zetasizer® Nano ZS, Malvern Instruments, UK) in purified water at 25°C and a fixed angle of 173°.

The PDI is a dimensionless value, ranging from 0 to 1, calculated by the Zetasizer® software based on ISO standard document 13321:1996 E and ISO 22412:2008. The value is calculated from a two parameter fit to the correlation data and describes the variation of individual sizes of particles in the measured sample and therefore the size distribution. Values <0.150 indicate a narrow size distribution in the field of nanoparticulate DSS (27,28).

The measurements were carried out for each batch in triplicate and the mean value and standard deviation (S.D.) was calculated.

Size, Size Distribution and Yield of the Spray Dried Particles

The spray dried microparticles were characterized by laser diffraction using a Mastersizer® 2000 equipped with a Mastersizer® 2000 µP dispersion module (Malvern Instruments, Herrenberg, Germany). The particles were redispersed in purified water. Each batch was measured in triplicate and the mean value and SD was calculated.

The volume median size (d 0.5) as well as the percentile values d 0.1 and d 0.9 were calculated by the Mastersizer® unit. D 0.1 and d 0.9 describe particle sizes, where 10 or 90% of the particles are smaller than the measured diameter for the corresponding value, respectively. The size distribution (width) is defined in Eq. 2. A narrow size distribution is indicated by a small width.

$$width = \frac{d\,0.9}{d\,0.1} \tag{2}$$

We defined an acceptable width ranges from 1, where d 0.5 is equal to d 0.1 and d 0.9, to 50. For example for a typical particle with d 0.5 of 1 µm a width of 50 would result by a d 0.1 of 0.2 µm and a d 0.9 of 10 µm.

The yield is defined in Eq. 3. The value for the starting material was corrected for the dead volume of the tubes feeding the spray head (Fig. 1). The supplied dispersion was weighed before and after the spray drying process. By subtracting both values the actually spray dried starting material could be determined.

$$yield = \frac{wt_{spray\ dried\ product}}{wt_{starting\ material}} * 100\% \tag{3}$$

Scanning Electron Microscopy

The morphology of spray dried microparticles and nanoparticles were investigated by scanning electron microscopy (SEM).

For the sample preparation of spray dried microparticles a small amount of powder was spread on adhesive tape. Surplus of powder was removed by stripping the adhesive tape twice with a second piece of adhesive tape. The remaining powder was deposited onto a double sided adhesive carbon tape, which was afterwards glued onto an aluminum stub.

The nanoparticles were re-dispersed in purified water. One drop of the suspension was pipetted onto a silica waver that was glued with double sided adhesive carbon tape onto the aluminum stub.

The samples were sputtered with gold using a Quorum Q150 RES (Quorum Technologies, UK) and examined using a Zeiss Evo HD 15 (Carl Zeiss AG, Germany).

RESULTS

Nanoprecipitation (NPR)

NPR - Results of the DOE Investigating Effect on Size and Size Distribution

Using the NPR method nanoparticles (NPs) in a size range of 50–177 nm were produced with a PDI of 0.013 to 0.294, by combination of the parameters as summarized in Table IX (supplementary material). Within the range of investigated factors, only NPs with average sizes above 110 nm met the quality criterion of PDI <0.150. Smaller NPs with average sizes down to 50 nm could be prepared, but only with slightly broader size distributions (PDI increased up to 0.294). None of the experiments failed completely and in none of the experiments aggregates were visible after evaporation of the organic solvent. Depending on the polymer concentration the NP suspensions were opalescent (polymer concentration <0.6%) to milky (polymer concentration >0.6%) in appearance.

SEM images were taken to visualize the NPs and to investigate the morphology. The batches visualized in Fig. 2 differ in particles density due to the different initial PLGA amounts used for the preparation of different batches for the purpose of a DOE. The SEM image of R 2 (Fig. 2a) confirms the size measurement and shows that the NPs are smooth and spherical in shape with a narrow size distribution. The robustness of the NPR with regards to size distribution is also highlighted when comparing R 2 to a batch prepared using calculated optimal parameters (Fig. 2d), as monodispersity is not further improved. SEM images of R 10 (Fig. 2b) and R 20 (Fig. 2c) show that in contrast to R 2 smaller NPs were generated with a wider size distribution. Also irregular shaped NPs are visible.

The statistical properties for the whole experiment were calculated: the coefficient of determination is $R^2 = 0.973$ and predicted_$R^2 = 0.950$ for size, $R^2 = 0.971$ and predicted_$R^2 =$

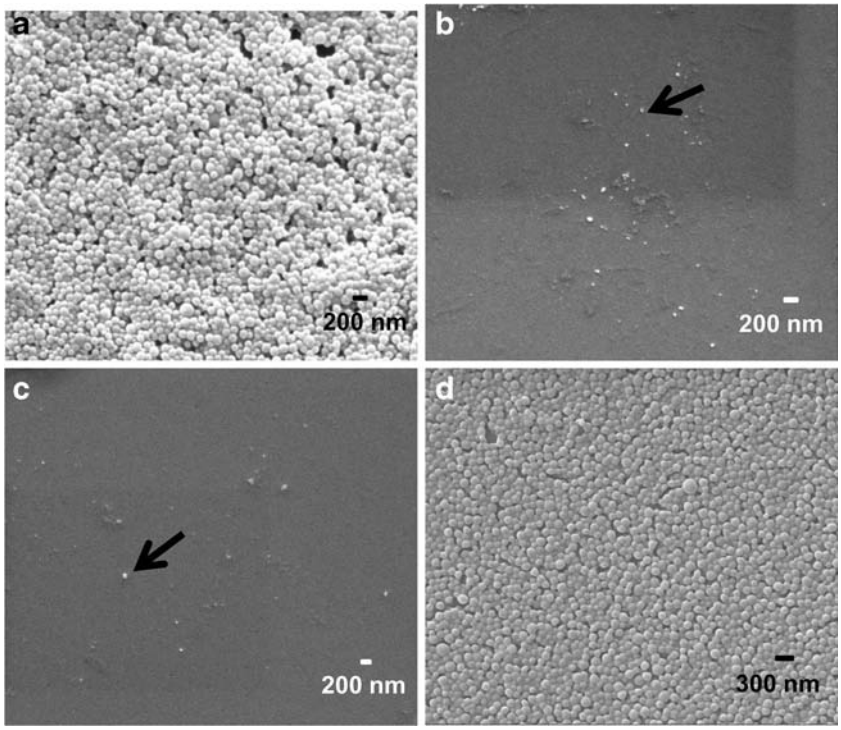

Fig. 2 SEM images of (**a**) R 2, (**b**) R 10, (**c**) R 20, and (**d**) particles produced with optimized parameters were taken to investigate and to compare the morphology. The SEM images differ in particles density due to the different initial PLGA amounts. (**a**) Nanoparticles are smooth and spherical in shape with a narrow size distribution. Monodispersity is not further improved compared to a batch prepared with optimal parameters in (**d**). Runs, which generated smaller particles, show a wider size distribution with irregular shaped nanoparticles (→) in (**c**) and (**d**).

0.928 for size distribution. This demonstrates that the model of the DOE reached high accuracy, good reliability and robustness for both, size and size distribution. These values confirm a high controllability of the NPR.

The analysis of variance (ANOVA) of the DOE showed that the polymer concentration is the main factor influencing size and size distribution. The results are summarized in Table III for size and in Table IV for size distribution. Factors have a significant contribution on the response as the *p*-value≤0.05. The ANOVA by partial sum of squares type III demonstrated that 79.5% of total variance was based on the square root of polymer concentration (Table III). The two other noticeable and significant effects were injection speed and stabilizer contributing 2.4 and 7.3% to total variance.

Table III Contribution of Process Parameters and Second Order Interactions Affecting Size in the Nanoprecipitation Method

Factor	Coding	Total variance [%]	*P*-value
Square root of polymer conc.	A	79.48	<0.0001
Inner diameter of needle	B	0.83	0.0104
Injection speed	C	2.36	<0.0001
Stabilizer	D	7.25	<0.0001
Organic solvent	E	1.50	0.001
Interaction			
Injection speed × stabilizer	CxD	0.70	0.0173
Polymer concentration	A^2	4.2	<0.0001
Square of injection speed	C^2	0.96	0.0062

$p \leq 0.05$ is considered significant

Effects equal to or less than 1.5% were contributed by the organic solvent (1.5%), the inner diameter (0.8%) and the interaction between injection speed and organic solvent (0.7%). Other interactions showed no effect and were not listed in Table III.

For the size distribution the ANOVA is shown in Table IV. All factors showed a significant effect. 58% of total variance was due to the square root of polymer

Table IV Contribution of Process Parameters and Second Order Interactions Affecting Size Distribution in the Nanoprecipitation Method

Factor	Coding	Total variance [%]	*P*-value
Square root of polymer conc.	A	58.00	<0.0001
Inner diameter of needle	B	1.04	0.0117
Injection speed	C	4.27	<0.0001
Stabilizer	D	10.43	<0.0001
Organic solvent	E	0.67	0.0369
Interaction			
Square root polymer conc. × injection speed	AxC	2.24	0.0006
Square root polymer conc. × stabilizer	AxD	2.95	0.0001
Square root polymer conc. × organic solvent	AxE	4.64	<0.0001
Injection speed × stabilizer	CxD	1.37	0.0045
Injection speed × organic solvent	CxE	1.28	0.0058
Stabilizer × organic solvent	DxE	0.49	0.0713
Polymer conc.	A^2	9.78	<0.0001

$p \leq 0.05$ is considered significant

concentration, additional 9.8% due to the polymer concentration. The two other noticeable and significant effects were stabilizer (10.4%) and injection speed (4.3%). An effect <1.5% was determined for organic solvent (0.67%) and the inner diameter (1.04%). All investigated interactions showed an influence with <3.0% of total variance except the interaction between polymer concentration and organic solvent (4.6%). Additionally the other interactions showed no effect and were not listed in Table IV.

NPR - Optimization

From the data generated for the DOE we proposed polynomial models using the Design Expert Software® to fit the experimental data for size and size distribution on factors A–E and combinations thereof. For both size and size distribution all 5 factors had a statistically significant influence. These fitting should offer the possibility to calculate the respective factors (A–E) for producing NPs with a special size or size distribution. As the size and size distribution were separate response we get separate equations. Our focus was set on a specific particle size. The results of the fitting are expressed for size and size distribution in Eqs. 4 and 5, respectively. These equations are significant and reliable, as can be seen from the good values of R^2 and predicted_R^2.

$$size = 120.79 + (50.74*A) + (3.96*B) - (8.2*C) - (11.81*D) - (5.37*E) - (4.58*C*D) - \left(23.2*A^2\right) + \left(11.73*C^2\right) \quad (4)$$

$$size\ distribution = 0.066 - (0.068*A) - \left(6.98^{-3}*B\right) + (0.018*C) + \left(0.023*D*5.76^{-3}*E\right) - (0.015*A*C) - (0.015*A*D) - (0.019*A*E) + (0.01*C*D) + \left(9.78^{-3}*C*E\right) + \left(4.87^{-3}*D*E\right) + \left(0.049*A^2\right) \quad (5)$$

The Eqs. 4 and 5 are expressed in terms of a coded factor, being a dimensionless number varying between −1 and +1 for continuous or discrete factors, and using the traditional coding for categorical factors.

Based on these equations and as an example two equations were calculated for the different stabilizer using the mixture of ethanol/acetone (3/16, *v*/*v*) as organic solvent as expressed in Eq. 6 for poloxamer 407 and Eq. 7 for polysorbate 80.

$$size_{poloxamer,\,acetone:ethanol} = -14.35 + \left(224.59*\sqrt{A}\right) + (41.73*B) - (5.80*C) - (76.41*A) + \left(0.49*C^2\right) \quad (6)$$

$$size_{polysorbate\ 80,\ acetone:ethanol} = -28.35 + \left(224.59*\sqrt{A}\right) + (41.73*B) - (7.68*C) - (76.41*A) + \left(0.49*C^2\right) \quad (7)$$

The Eqs. 6 and 7 are expressed in term of (dimensional) actual factors (instead of coded factors). With the Eqs. 6 and 7 process parameters and conditions (factors A, B, C and E) can be proposed in the investigated range of the DOE. The accuracy (trueness and precision) of these equations was therefore tested for the preparation of NPs with a size of 150 nm± 10 nm. For calculating the optimal parameters a limitation in injection speed (>1 ml/min) was set to allow reduced and feasible production times. The predicted factors were calculated and summarized in Table V.

To confirm the predicted factors particles were prepared with the parameter settings shown in Table V as calculated from Eqs. 6 and 7. Thus NPs with a size of 133.3±2.7 nm for polysorbate 80 and 135.4±2.2 nm for poloxamer 407 could be prepared. The mean size was smaller than predicted by ~17 nm (polysorbate 80) and ~15 nm (poloxamer 407), respectively. This was lower than the acceptable lower size limit of 140 nm. For both types of stabilizers we had learned from the DOE that the polymer concentration is the main influencing factor on particle size. Therefore, particles were also prepared with slightly higher polymer concentrations for both stabilizer and the results were presented in Fig. 3a and b.

Figure 3a shows that PLGA concentrations of 1.35–1.55% (compared to 1.2% assigned by the DOE, Table V) generated NPs between 147.2 and 155.2 nm and a PDI <0.058 using polysorbate 80 as stabilizer when leaving all other parameters as assigned by the DOE. PLGA concentrations between 1.20 and 1.40% (compared to 1.1% assigned by the DOE, Table V) result in particles with sizes between 147.1 and 155.7 nm and a PDI <0.040 using poloxamer 407 as stabilizer when leaving all other parameters as assigned by the DOE.

NPR - Robustness

To investigate the practical limitations of the optimized process, and therefore the robustness of the used set-up, the

Table V Optimization of Nanoprecipitation Method - Predicted Factors for Producing Nanoparticles with a Size of 150 nm± 10 nm Calculated from the Results of the DOE for Polysorbate 80 and Poloxamer 407

Factor	Coding	Polysorbate 80	Poloxamer 407
Polymer concentration	A	1.2%	1.1%
Inner diameter of needle	B	0.6 mm	0.6 mm
Injection speed	C	1.531 ml/min	4.55 ml/min
Organic solvent	E	Acetone/ethanol (16/3, *v*/*v*)	Acetone/ethanol (16/3, *v*/*v*)

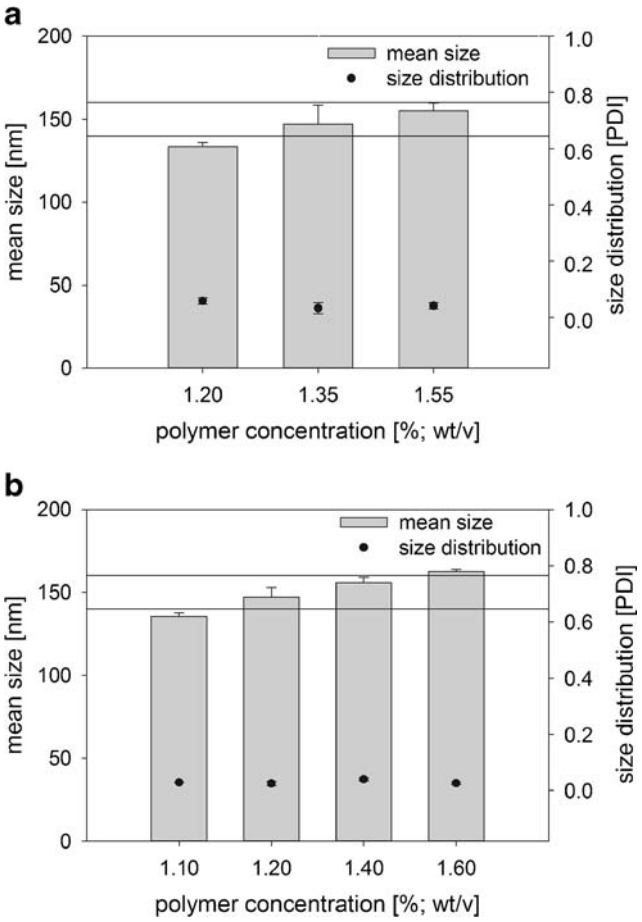

Fig. 3 Optimization of nanoprecipitation method - confirmation of predicted factors calculated from the results of the DOE. Nanoparticles were produced with different polymer concentrations using (**a**) polysorbate 80 and (**b**) poloxamer 407 as stabilizer under conditions summarized Table V. Size and size distribution were measured by dynamic light scattering. The values are presented as the mean ± S.D. ($n = 4$). - 150 nm ± 10 nm.

minimum possible batch size and the maximum possible batch size for preparing PLGA NPs with 150 nm ± 10 nm, without changing the experimental set-up, were determined. For this purpose 1.3% PLGA (*wt/v*) was dissolved in acetone/ethanol (16/3, *v/v*) for the organic phase and a needle with an inner diameter of 0.60 mm was used for the injection. For the aqueous phase poloxamer 407 at a concentration of 1% (*wt/v*) was used, which was stirred at 500 rpm during the injection. For these experiments the concentration of PLGA in the organic phase (1.3%) and poloxamer 407 (1%) in the aqueous phase was kept constant as described above to produce particles with a size of 150 nm ± 10 nm. Therefore, the volume was used as starting point for changing experimental parameter. Thus batches were prepared using 5, 2.5, 1.250 and 0.625 ml to minimize batch size, 20.0, 40.0 and 50.0 ml to determine the maximum batch size. The smallest volume (0.625 ml) was limited due to the reduced volume in the aqueous phase and due to handling reasons. 50.0 ml was set as the maximum (corresponding to 650 mg PLGA weight of polymer used for preparing one batch), which is a reasonably large batch size commonly required during formulation development, batch characterization or preclinical development.

The measured NP sizes and size distributions were summarized for the minimum and maximum batch size in Fig. 4a and b. All batches had a size of 150 ± 10 nm with a narrow size distribution, PDI < 0.042. Therefore the NPR is very robust an can be used to produce batches with minimum 0.625 ml and at least up to 50.0 ml of organic solvent without changing the experimental set-up.

Nano Spray Drying (NSD)

NSD - Results of the DOE Investigating Effect on Size and Size Distribution

All response values of size, size distribution and yield were presented Table X (supplementary material). With the NSD stable re-dispersable microparticles (MPs) could be prepared

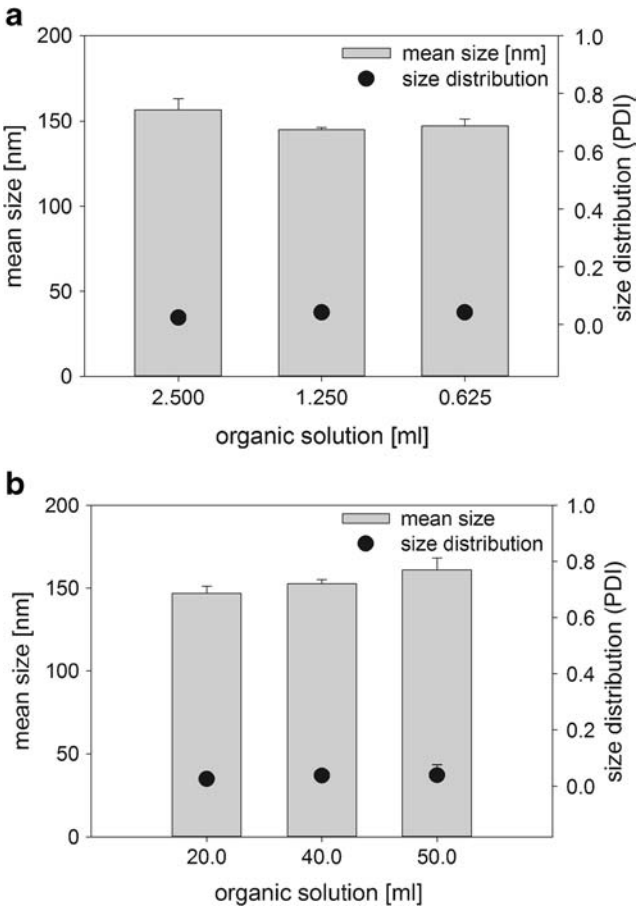

Fig. 4 Investigation of (**a**) maximum and (**b**) minimum batch size (robustness) for the nanoprecipitation method by determining size and size distribution from batches produced with different volumes. The PLGA concentration (1.3%, *v/wt*) was kept constant for all experiments and poloxamer-407 was selected as stabilizer. Size and size distribution were measured by dynamic light scattering. The values are presented as the mean ± S.D. ($n = 3$).

with the lowest mean size (d 0.5) of 2–163 μm. The smallest mean size was found in R 15 and R 4 with 2.34 and 2.38 μm respectively, both produced with Span®60 as stabilizer. The maximum mean size was found in R 21 with 163.3 μm, produced with poloxamer 407 as stabilizer.

Some experiments failed completely, which were marked in Table VII with (−) as no final product could be achieved. In these experiments the formulation was blocking the vibrating membrane (+) or a polymer film was formed on the collecting electrode (*). Looking at the response in Table X (supplementary material) using poloxamer 407 as stabilizer 15 of the 27 experiments failed, whereas only 2 out of 29 failed using Span®60. Most of the experiments with ethyl acetate as organic solvent failed (8 out of 19) With Span®60 smaller particle mean sizes (d 0.5 values), in comparison to particles prepared with poloxamer 407, were prepared, so that particle mean sizes of <8 μm were achieved in 20 runs. For poloxamer 407 only one batch had a mean size of <8 μm. Furthermore, particles prepared with poloxamer 407 and a low polymer concentration (0.1% PLGA, *wt/v*) had a mean size of <100 μm. Note that the goal was to produce particles as small in mean size as possible, if possible in the nanosize range. In the used set-up and with PLGA as polymer we achieved particles in the low micrometer size range.

The evaluation of the DOE shows that the size of the MPs is most affected by the stabilizer (Table VI). The ANOVA by partial sum of squares type II demonstrates that 38.91% of total variance is based on the stabilizer. The two other noticeable and significant effects are polymer concentration (4.39%) and stabilizer concentration (6.74%). A very weak influence was determined for the organic solvent with 1.62% of influence, which is not significant.

The ANOVA also showed that second order interactions have a strong influence on the size of the spray dried particles, but only the interaction of polymer concentration/stabilizer, contributing 21.08%, and stabilizer concentration/stabilizer, contribution 5.11%, were found to be significant interactions.

Table VI Contribution of Process Parameters and Second Order Interactions Affecting Size in the Nano Spray Drying

Factor	Coding	Total variance [%]	*P*-value
Polymer concentration	B	4.39	0.0074
Stabilizer concentration	C	6.74	0.0013
Organic solvent	E	1.62	0.2335
Stabilizer	G	38.91	<0.0001
Interaction			
Polymer concentration × stabilizer	BxG	21.08	<0.0001
Stabilizer concentration × organic solvent	CxE	6.23	0.0071
Stabilizer concentration × stabilizer	CxG	5.11	0.0042

$p \leq 0.05$ is considered significant

The remaining second order interactions showed no effect and were, therefore, not listed in Table VI. Nevertheless, the value of the coefficient of determination $R^2 = 0.899$ and predicted_$R^2 = 0.790$ demonstrated that the model is less accurate, robust and reliable compared to the NPR.

The factors most strongly influencing the size distribution, represented as width, were the polymer concentration (15.33%) and the organic solvent (19.27%) as described in Table VII. The other factors are determined to be significant, but had only weak influence (<1%). For the interactions between polymer concentration/organic solvent an influence of 6.62% was determined. The remaining second order interactions showed no effect and were not listed in Table VII.

From the ANOVA it is obvious that the formation of particles with NSD is influenced by various factors with the individual factors carrying little weight to the total variance. This is true for the particle size as well as for the width.

The shape of the prepared MPs was investigated by SEM imaging (Fig. 5). As the aim was to prepare particles as small as possible one of the batches with the lowest mean sizes (R 4) was investigated, showing that the particles were round and regular shaped (Fig. 5a). This batch was produced with a medium amount (1.18%, *wt/v*; according to the range defined in the DOE) of Span®60 as stabilizer and compared to the optimized batch with high stabilizer concentration (1.6%, *wt/v*) of Span®60 in Fig. 5d, which was used for determining maximum and minimum batch size (robustness). There was no visible change in the morphology or size distribution. In Fig. 5c particles (R 56) with lower amount (0.05%, *wt/v*) of Span®60 were visualized showing that a fraction of the particles were indeed in the submicron range <1 μm, however, the overall size distribution was relative broad with an average size of 4.9 μm. The same effect is also obvious for particles produced with a small amount (0.05%, *wt/v*) of poloxamer 407 as stabilizer (Fig. 5b).

Table VII Contribution of Process Parameters and Second Order Interactions Affecting Size Distribution (Width) in the Nano Spray Drying

Factor	Coding	Total variance [%]	*P*-value
Temperature	A	0.11	<0.0001
Polymer concentration	B	15.33	<0.0001
Stabilizer concentration	C	0.04	<0.0001
Spray rate	D	0.20	<0.0001
Organic solvent	E	19.27	<0.0001
Pumping rate	F	0.45	<0.0001
Stabilizer	G	0.30	<0.0001
Interaction			
Polymer concentration × organic solvent	BxE	6.62	<0.0001

$p \leq 0.05$ is considered significant

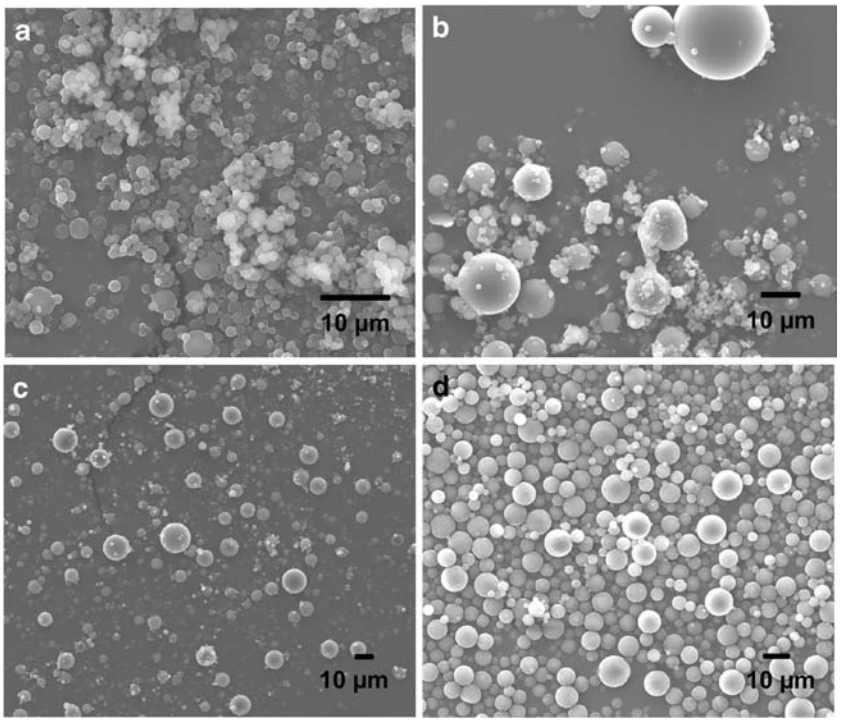

Fig. 5 SEM images of (**a**) R 4, (**b**) R 49, (**c**) R 56 and (**d**) optimal formulation used for robustness experiments. (**a**) Investigation of one of the batches with the lowest mean sizes, which contains medium amount (1.18%, *wt/v*) of Span®60, showing that the particles were round and regular shaped. Compared to the optimal batch with high stabilizer concentration (1.6%, *wt/v*) of Span®60 in (**d**) no visible change in the morphology or size distribution could be determined. In (**b**) particles with lower amount (0.05%, *wt/v*) of poloxamer 407 and in (**c**) particles produced with a small amount (0.05%, *wt/v*) of Span®60 were visualized showing a fraction of the particles in the submicron range <1 μm and a broad overall size distribution.

NSD - Results of the DOE Investigating Effect on Yield

Yields were obtained in the range of 1.16% (R 6) to 62.81% (R 4) and listed in Table X (supplementary material). In general the highest yields were achieved with Span®60 as stabilizer (R 4=62.81%, R 14=58.26%, R 45=54.14%). For 7 runs out of 56 runs the yield was greater than 40%. Furthermore, a yield greater than 30% was found for runs with mean sizes <9 µm (R 4, R 9, R 14, R 17, R 20, R 25, R 34, R 41, R 45, R 51, R 56).

The evaluation of the DOE showed that the organic solvent is the main factor influencing the yield, being responsible for 30.1% of total variance (Table VIII). The two other noticeable and significant effects were stabilizer and polymer concentration contributing 13.1 and 8.3% to total variance, respectively. The effect of temperature, stabilizer concentration, spray rate and pumping rate were revealed to be very weak with <0.1% and not significant. Interestingly the interactions of temperature/organic solvent and stabilizer/ organic solvent showed an effect of 10.19 and 7.04%, respectively. Also other interactions were tested and had an influence of 3.1–4.2%, which was significant, except the interaction polymer concentration/stabilizer. The remaining second order interactions showed no effect and were not listed in Table VIII.

Table VIII Contribution of Process Parameters and Second Order Interactions Affecting Yield in the Nano Spray Drying

Factor	Coding	Total variance [%]	*P*-value
Temperature	A	0	0.9956
Polymer concentration	B	8.33	0.0002
Stabilizer concentration	C	0.19	0.5078
Spray rate	D	0.44	0.3118
Organic solvent	E	30.09	<0.0001
Pumping rate	F	0.95	0.1418
Stabilizer	G	13.14	<0.0001
Interaction			
Temperature × stabilizer concentration	AxC	4.02	0.0047
Temperature × organic solvent	AxE	10.19	0.0002
Polymer concentration × pumping rat	BxF	4.16	0.0041
Polymer concentration × stabilizer	BxG	1.65	0.0572
Stabilizer concentration × spray rate	CxD	3.25	0.0099
Stabilizer concentration × organic solvent	CxE	7.04	0.0017
Stabilizer concentration × stabilizer	CxG	4.01	0.0048
Organic solvent × pumping rate	ExF	3.11	0.038

$p \leq 0.05$ is considered significant

The statistical properties for the whole experiment were $R^2=0.899$ and predicted_$R^2=0.666$. Therefore the model is less accurate, must be considered with caution for robustness and reliability.

NSD - Robustness

As described above by the results of the DOE size and size distribution of particles as well as the yield using NSD is influenced by various factors and therefore more difficult to control. Based on the experimental data we proposed polynomial models using the Design Expert Software® to fit the data for size and size distribution on factors A–G. The goal was to calculate values for the factors (A–G) to reach the smallest possible size and size distribution with maximum yield (optimal formulation). Several possible solutions were found which predict the composition of an optimal formulation based on the before defined criteria. The calculation was abridged as 100 solutions had been obtained.

From these solutions one formulation was selected for investigating the limitation in batch size generating a mean particles size ~2.8 µm with a narrow width (0.9). 1.1% of PLGA and 1.6% of Span®60 in acetone were used and sprayed at 71°C and 82% spray rate to generate those particles.

To investigate the practical limitations of the NSD system the possible minimum batch size and the maximum possible batch size without changing the general experimental set-up were determined. This is an indicator for the robustness of the process. The total amount (wt) of PLGA and Span®60 was calculated with respect to the used volume for each individual experiment using a concentration of 1.1 and 1.6%, respectively.

As the spray dried products always have a wider size distribution compared to particles produced with the NPR the mean particle size should be between 2 and 5 µm. Based on the dead volume, which is defined as the volume remaining in the spray head an in the feeding tubes, which was experimentally determined to be 2.9 ml, 5 ml was fixed as minimum batch size. To find the maximum batch size 10.0, 20.0, 40.0 and 50.0 ml batches were sprayed. Additionally also the yield was determined. All results of size, size distribution and yield were presented in Fig. 6a and b. Figure 6a shows a slight increase in size from 10.0 to 20.0 ml and in parallel also the PDI increases. From 40.0 to 50.0 ml the mean size doubles, but the width kept constant.

The lowest yield was determined for 5 ml with 60%. For other volumes the yield ranges from 71.1 to 84.8%. From 5.0 to 40.0 ml the yield increased, whereas with 50.0 ml the yield decreased slightly. The results show that between 5.0 and 40.0 ml particles with a mean size of 2.6 to 4.3 µm, a mean width of 0.91 to 2.50 and yield >60% could be produced.

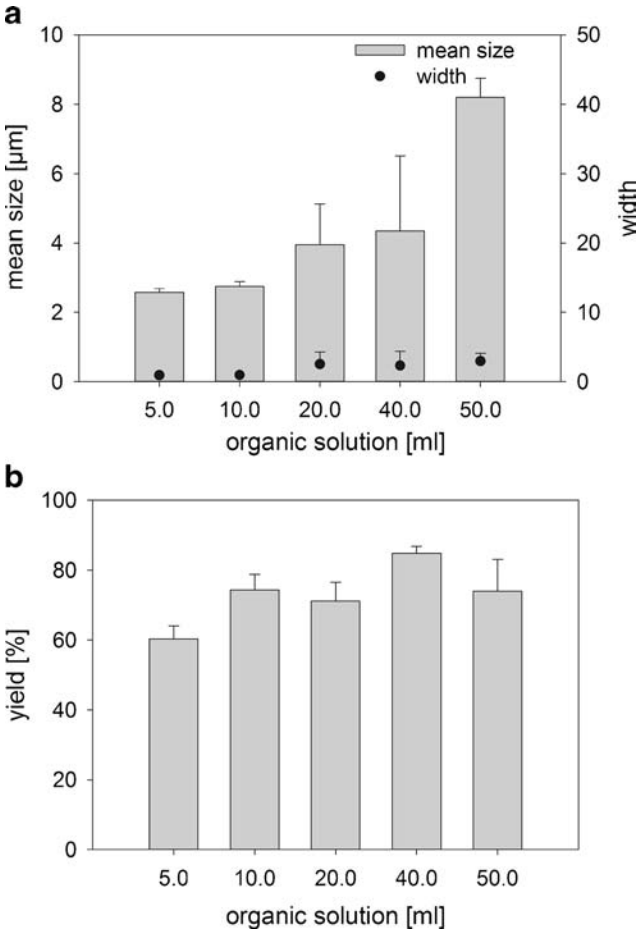

Fig. 6 Investigation of maximum and minimum batch size (robustness) for the nano spray drying by determining (**a**) mean size and size distribution (width) and (**b**) yield from batches produced with different volumes. The concentration of PLGA and Span®60 was kept constant. Mean size and size distribution (width) were measured by laser light diffraction. The values are presented as the mean ± S.D. ($n=3$).

DISCUSSION

The absence of information regarding the influence of parameters on the production of nanoparticle-based DDS is hindering the scale up and the more widespread production of such systems in the pharmaceutical industry (3). To enter in the pharmaceutical production and thereafter the pharmaceutical market a transfer from bench top to pharmaceutical production (scale-up) has to be achieved. It is known that process limitations can become significant in a larger production process, which were not apparent before. At the moment only one albumin based nanoparticulate DDS (Abraxane®) is approved by the Food and Drug Administration and European Medicines Agency and a second, which is a polyalkylcyanoacrylate based nanoparticulate DDS (Livatag®, doxorubicin Transdrug™), is currently in phase III clinical trial (29).

To evaluate the influence of process parameters, a DOE was applied on a nanoprecipitation method (NPR) and a nano

spray drying (NSD) technique. Choosing the optimal design of the DOE a full factorial design requires a large number of experiments. Therefore, fractional factorial designs, e.g., response surface designs, are appropriate for series varying several factors. Furthermore, a fractional factorial design can reduce costs and material due to the reduced number of experiments (22).

NPR is one of the most frequently used physicochemical methods (4) for producing NPs as DDS (30,25). Compared for example to emulsion-diffusion-evaporation methods, it is less time consuming, more reproducible and controllable due to less production steps and was, therefore, investigated in this study (3). One disadvantage of the NPR is the low polymer concentration at the end of the process in the aqueous phase (0.65%), which is generally known (31). For NPR, DOE studies have been carried out before, mostly focusing on drug loading (8,19). So far no study was carried out using a DOE as a tool to understand this process regarding parameters that might critical for size and size distribution. In general the formation of particles involves complex interfacial hydrodynamic phenomena, and approaches to explain them have for example been based on interfacial turbulence (32) or the Gibbs–Marangoni effect (33).

The second method we used in the present work was a spray drying technique, which are generally used to transform liquids into solid powders with the objective to conserve and stabilize the product for storage. Apart from this, the here investigated nano spray drying technique (NSD) can also be used to formulate DDS in one step without extra washing or drying steps (single step process) (34). For the NSD, the Büchi B-90 apparatus was used, which has been especially designed to produce and to collect spay dried products with a particle size in the submicron size range (35). This is realized by using a vibrating mesh for generating the spray and an electrostatic particle precipitator, which allows the particle collection (35). As of now only a few studies have been done with this NSD, mainly investigating the preparation of particles using polymeric wall materials, the drying of pharmaceutical excipients (36) and drying of nano-emulsions (34). First studies were carried out focusing on the encapsulation of proteins (10) and model drugs in biodegradable polymers (35,37). Bürki *et al.* and Lee *et al.* were the first to apply a DOE, full factorial design and Taguchi method respectively, focusing on spray drying of a protein (10,18). A recent study used a 2^3 factorial design to develop inhalable capreomycin powders (38).

For both methods, NPR and NSD, the major goal of the DOE was to identify parameters controlling the respective process as well as limitations of the process with regards to producing different batch sizes for a fixed set of (optimized) process parameters and without changing the experimental set-up. This is relevant for example in the context of early formulation development, characterization and preclinical development.

It is obvious that a DOE may generate some more objective view on an unknown or poorly characterized process. However, the gain of information of a DOE could be little or enormous compared to the investment of practical work. The main challenge is to precisely ask the questions, which should be answered by the DOE. A DOE is useless/inefficient answering yes-no questions, such as for example: Can my drug be encapsulated in NP at all? Instead, a DOE will answer the following questions: Do different stabilizers have an effect on the encapsulation efficiency? Does a given stabilizer has a different effect when combined with another solvent?, etc. Depending on the influence of the factors in question, the DOE would reveal a statistical correlation.

Before starting the DOE it is important to perform some preliminary studies to decrease the range of investigated factors and hence to increase the accuracy of the DOE model. For example it will not make sense to use an amount of polymer which is insoluble in the organic phase or at so high contents that an injection is impossible due to the increased viscosity. Such limitations can be taken into account in the DOE. A DOE could be a benefit and save time for frequently used processes for example production processes of solid dosage forms, mixing processes, coating processes improving quality, accuracy and reliability for the respective process in development as well as in research.

Within such framework we have observed that a DOE has the following advantages in the investigated processes.

Advantage of the DOE Approach for the Nanoprecipitation Process

To use the DOE as a tool to understand process parameter was successful for the NPR. The results of the DOE revealed that the size is mostly influenced by the polymer concentration (79.5%), which is, thus, easy to control. This is in accordance with a meta-analysis performed by Mora-Huertas *et al.* on published and experimental data showing that the size of submicron particle size can be changed by the polymer concentration, regardless of the nature of polymer, nature of stabilizer or the operating conditions (30). In the present study we achieved stable NPs in the range of 110–180 nm with polymer concentrations of 1–2% (*wt/v*) and none of the experiments failed completely. Moreover, in none of the experiments aggregates were visible after evaporation of the organic solvent. Increasing polymer concentrations lead to the formation of larger particles which is in agreement with the observations of Thioune *et al.* (39) on the preparation of NPs from hydroxypropyl methylcellulose phthalate and Galindo-Rodriguez *et al.* (33) using polymethacrylic acid copolymers. High polymer concentrations of 2% in our study invariably lead to the largest particle sizes (>170 nm) with narrow size distributions (PDI

< 0.070) independent of used inner needle diameter, injection speed, stabilizer or organic solvent. At high polymer concentrations the theoretical concentration of polymer chains is higher and a more even distribution of polymer chains among all solvent droplets is expected resulting in particles with the same size. Low concentrations increase the probability to have less or different concentration of polymer chains in the droplets. Below a critical polymer concentration hence a wider size distribution results (33). For the investigated set-up the critical polymer concentration could be proposed to be at ~0.1% (*wt/v*) (lowest polymer concentrations) or below, which resulted in a dramatic increase in the PDI (0.132–0.294).

In our study the stirring speed was set to 500 rpm in order to improve distribution of the organic phase into the aqueous phase and largely prevent unstirred layers. Unstirred layers hinder diffusion, which lead to a time-dependent particle growth as the droplets of the organic solvents will not be split evenly at fast injection speeds (30). A high stirring speed reduces this influence of injection speed, demonstrated in the results of the DOE (Table III), organic/aqueous phase ratios and stirring speed, both were fixed in the DOE, on the complex fluid dynamics and therefore particle formation (30,40). Furthermore, in our study we fixed the stabilizer concentrations. In the NPR the stabilizer is in general needed to avoid aggregation during particles formation and known to have independent of the concentration an influence on particle mean sizes, which was also demonstrated by the DOE (Table III) with 7.3% of total variance (30). It was reported that poloxamer 407 generates larger particles in comparison to polysorbate 80 (30). A tendency could be seen in the optimization of the NPR as both stabilizers need different parameter to generate 150 nm particles.

Indeed, that the influence on size and size distribution was dominated by the polymer concentration might appear relatively obvious. However, the DOE allowed to define a quantitative, statistically reliable correlation, as expressed in Eqs. 6 and 7. Using these equations, the process can be easily adapted to generate other particles sizes, between 110 and 180 nm with narrow size distributions, within the constraints of the dataset used for the development of the equations. We demonstrated furthermore that the used system based on these equations was robust and accurate within a range between some minimally needed and maximally possible volume-based batch size. The maximal batch size using 50 ml of organic solvent allows to fabricate particles based on 650 mg PLGA, which is likely to suffice for first preclinical studies. Very small batch sizes (0.625 ml) allow to prepare particles for first physicochemical characterization studies. Therefore, the used set-up can be adapted in the particle quantity for different experimental purposes.

Advantage of the DOE Approach for the Nano Spray Drying Process

Significance for Particle Size and Size Distribution

For the NSD the DOE was designed to investigate controllability, limitations and robustness regarding particle size, size distribution and yield of this so far less characterized process (34). Therefore, seven factors were screened which were expected to have an influence on the outcome of the spray drying process. Combining rather a high number of factors is also a limit for a DOE, but necessary to thoroughly screen an unknown system. A reduced number of factors allows investigating more values in the defined ranges, but would not necessarily reduce the number of initial experiments. By reducing the factors of a totally unknown process effects could be missed. Furthermore, the outcome could be so weak that the other factors had to be investigated in a second DOE, further increasing the number of experiments. The results show clearly, that NSD is influenced by various factors and hence more difficult to control than the NPR. Not only one optimal formulation could be calculated from the results. The aim was to produce the smallest possible size using PLGA and within the technical constraints of the Büchi B-90 with a spraying tower of 150 cm in length. Our results show that the smallest particles which were prepared were still MPs with an average size of ~2 µm. In the NSD the formation of the particles is complex and is governed in a first step by an interplay of polymer concentration in the droplet, pore size of the vibrating membrane used for evaporating the spraying solution and the frequency of membrane. The frequency defines the volume of the droplet, which is injected in the drying gas flow. The droplet size is mostly influenced by the pore size of the membrane. The membrane with the smallest pore size which is currently available has a pore size of 4 µm and generates droplets ~8 µm (41). It would be interesting to see the effect on the size using a membrane pore size ~1 µm, while changing the frequency of vibration (at the moment fixed at 60 kHz). A smaller pore size will however increase spraying times. In a second step the droplets are dried in the gas flow forming the particles depending on surface tension and density. Therefore the feeding material, as well as density and viscosity of the feeding solution, containing in our study the polymer and the stabilizer, influence the transport through the membrane and the successful particle formation. In comparison to NPR some experiments failed completely (Table X, supplementary material) in particular if poloxamer 407 was used as stabilizer or if ethyl acetate was used as organic solvent. Especially high viscosity and density of the feeding solution can block the vibrating membrane and droplet coalescence can lead to the formation of a polymer film on the collecting electrode.

In our study polymer and stabilizer were found to have an effect on the particles size (Table VIII, table IX). The

identified interaction between polymer concentration and stabilizer underscores this aspect. This is not unexpected as recent studies using polyvinyl alcohol, maltodextrine, arabic gum and modified starch, have also shown that polymer concentration influences particle size (10,34,42) and, furthermore, that the particle size depends on the type of material used (34). Beck-Broichsitter *et al.* investigated that also the molecular weight (MW) of PLGA can influence the particles size (28). As in our study the stabilizer is part of the DDS, the different MW of poloxamer 407 (MW 12,785 g/mol) and Span®60 (MW 430 g/mol) could therefore influence the particles size, as can be seen from our data (Table X supplementary material). Smaller mean sizes were in general achieved with Span®60, and the mean size increased strongly by increasing the concentration of poloxamer 407.

For the size distribution, there was a significant an effect of the polymer concentration and of the organic solvent, and there was a significant interaction of these two factors. This reflects that intuitively quicker drying of the spray droplets in a gas flow would avoids droplet coalescence. The generated particles had a smooth surfaced as visualized in SEM images in Fig. 5 and reported before by Vehring *et al.* More hollow particles were normally obtained with fast drying processes (37,43).

Due to the complexity interplay of influencing factors on the NSD no single mathematical solution for an optimal formulation could be expressed. Nevertheless, a selected formulation was robust and accurate to a volume-based evaluation of the minimum (5.0 ml) and the maximally possible (40.0 ml) batch size. Both the maximally possible and the minimum batch size were limited by technical constrains and characterized by a smaller range compared to the nanoprecipitation method.

Significance for Yield

Yields achieved with the Büchi B-90 ranged between 43 and 95% as reported in the literature (35,44), depending for example on spray dried material (34) and spray rates (18). The yields we obtained were in the range of 1.16 to 62.81%. We found in our DOE that yield was mostly influenced by organic solvent, stabilizer and polymer concentration and the interaction between stabilizer/organic solvent. But in contrast to Bürik *et al.* no effect of the spray rates were determined (18). The spray rate in the NSD can only be adjusted indirectly by the relative volume flow, which passes through the membrane. This volume flow is influenced by the viscosity of the feeding solution as well as of the pore size of the spray cap and the inlet temperature of the spray head. Thus, the spray rate can indirectly be influenced by the choice of organic solvent, polymer concentration and stabilizer, which is obvious in the DOE, as the different combinations of organic solvent, polymer concentration and stabilizer resulted in different viscosities of the feeding solution. Therefore, no direct influence on the spray rate could be determined.

In the NSD an electrostatic precipitator is used for the particle collection, which has in general an efficacy of 90–99% depending on the sprayed material, mass and the used gas (45). A low precipitation efficiency of submicron particles in electrostatic particle precipitators is due to the difficulty in charging the spray dried material, which is depending on the electrical resistivity (45). Low charge results in low particle mobility and low collection efficiency due to fast discharging (46). We observed an effect of the interaction temperature/organic solvent on yield (Table VIII) which may indicate that inefficiently dried particles have a different ability to be charged than fully dried particles. We assumed that due to the solvent residues in the sprayed particles the electrical resistivity could be changed. Electrostatic precipitators are normally constructed with a system to remove the deposited particles from the collector electrode before the saturation is reached for example a mechanical rapping device. In the NSD no such auto-cleaning system is installed and we observed a saturation of the collector electrode which limits the robustness of the method when spraying large batch sizes. The yield decreases 84.8% to 74.0% when using 40 and 50 ml initial volume yielding an effective amount of particles 1080 and 1350 mg, respectively.

CONCLUSION

The DOE approach as performed in this study revealed that particle size and particle size distribution in nanoprecipitation (NPR) are dominantly controlled by the polymer concentration. Moreover, the DOE allowed for the NPR to define a mathematical model and statistically reliable correlation, which can be used for the optimization of the process and prediction of optimal formulation. It was possible to distinguish between important and unimportant factors based on the unambiguous statistical criteria, providing a sound basis for discarding unimportant parameters and focusing on the essential ones. The model built by the DOE for NPR method for producing particles with a size of 150 ± 10 nm was accurate, robust and reliable so that the predicted optimized parameters were in excellent agreement with experimental data.

In case of nano spray drying (NSD), the DOE revealed that the formation of particles is more complex because it is influenced by various process parameters and, therefore, more difficult to control. The use of the DOE approach allowed discerning the interaction of factors and helped in explaining the indirect influence of factors on the process. We could demonstrate that each of the seven investigated process parameters in the DOE were of significant influence and necessary to be thoroughly addressed. With NSD, the smallest possible particle sizes were in the lower micrometer size range.

Several possible solutions were found which predict the composition of an optimal formulation based on the DOE.

Both NPR and NSD were robust and accurate to a volume-based evaluation of the minimally necessary and the maximally possible batch size using optimized set-ups.

We conclude that DOE can help to optimize well known processes and, furthermore, to understand and optimize innovative manufacturing processes, which is urgently needed for the quality by design preparation of nano- and micrometer sized drug delivery systems

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