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Process optimization of a novel production method for nanosuspensions using design of experiments (DoE)

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ABSTRACT

Particle size reduction is a suitable method to enhance the bioavailability of poorly soluble drugs. The reduction effectiveness depends on compound properties like crystallinity, hardness and morphology. Sometimes, it is difficult to obtain small particles. To solve this problem a combinative method was developed: a combination of freeze drying with high pressure homogenization (so-called H 96 process). The freeze drying modifies the drug structure to obtain a brittle, fragile starting material for the subsequent homogenization step. Screening experiments with glibenclamide have shown a relation between the lyophilization conditions and the final particle size. Systematic investigations using design of experiment (DoE) were conducted to identify optimal process parameters. The influence of the independent variables drug concentration and organic solvent composition during freeze drying were tested by conducting a two factorial design of experiment. The model drug was dissolved in mixtures of dimethyl sulfoxide (DMSO) and tert-butanol (TBA) in different concentrations, freeze dried and subsequently homogenized at high pressure. Using optimized process conditions the particle size after 20 cycles was very small: 164 nm (*z*-average) and 0.114 μm (d50%). On the contrary, with unmodified drug the results were 772 nm (*z*-average) and 2.686 μm (d50%). It was shown, that the structure modification of the drug by means of freeze drying can significantly improve the particle size reduction effectiveness of high pressure homogenization. The study confirmed also the usefulness of DoE for nanocrystal production.

1. Introduction

New developed drugs show very often poor water solubility, associated with an inadequate dissolution rate that results consequently in a low oral bioavailability. Particle size reduction is a viable way to formulate poorly soluble compounds showing dissolution rate dependent oral bioavailability (Lipinski, 2002). Particle size and particle size distribution are parameters of high importance. Small drug nanocrystals possess an increased surface area, which leads to better dissolution rates of poorly soluble compounds (Yoncheva et al., 2003). The particle size can also affect the interaction between the particles and the cells, e.g. by enabling an improved endocytotic uptake (Zimmer et al., 1991). In recent years the nanosuspension formulation approach has become a promising and serious development tool in the pharmaceutical industry (Müller et al., 2001). The term nanosuspension refers to a sub-micron colloidal dispersion of drug particles in a dispersion medium, which contains often polymers and/or surfactants as stabilizing agents. The small size of the nanoparticles offers an enhanced surface area and increased dissolution rates of poorly water soluble compounds. These properties lead to an improved bioavailability, a rapid onset of the pharmacological effect, reduced food effect, and further desirable effects (Rabinow, 2004).

Currently four different production principles for drug nanoparticles can be distinguished: chemical reactions, bottom-up or precipitation techniques, top-down or comminution techniques and combinative approaches. The latter ones combine bottom-up with top-down steps for an enhanced particle size reduction effectiveness (Shegokar and Müller, 2010).

Bottom-up technologies start with a molecular dispersion of an active pharmaceutical ingredient. Particles are formed by a constructive assembling of the molecules to larger structures (Möschwitzer and Müller, 2006). The oldest way to produce drug nanoparticles is based on precipitation techniques, also known as "via humida paratum" (v.h.p.). A poorly water soluble active pharmaceutical ingredient (API) is dissolved in an organic solvent, which is normally water-miscible. By pouring this solution into water, finely dispersed drug nanocrystals are obtained as result of a precipitation. Advantage of the precipitation technique is the use of relatively simple, low-cost equipment. Up-scaling can be achieved by using static blenders (Müller and Böhm, 2001). This technique

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requires the drug to be soluble in at least one solvent, which must also be miscible with a non-solvent. This is a limiting factor, as it excludes drugs being poorly soluble in aqueous and in non-aqueous media (Müller et al., 2001). The difficulty of this precipitation principle is to control the process and to stop the particle growth at a given time point, when the desired particle size is reached. Disperse systems have the tendency to attain a thermodynamically stable state. The solvents used during the precipitation process can lead to a relatively high solubility due to cosolvent effects and therefore higher molecular mobility of the API in the system. This can eventually lead to particle growth up to the micrometer range as a result of Ostwald ripening (de Waard et al., 2008). Furthermore, the solvents need to be removed completely from the nanosuspension before it can be used for most pharmaceutical applications. These challenges and disadvantages prevented the broad usage of bottom-up systems so far. They have shown potential but failed to reach the market so far (Van Eerdenbrugh et al., 2008).

Typical bottom-up techniques are the solvent–antisolvent method, supercritical fluid processes, spray drying and freeze drying. The last two are promising technologies for the pharmaceutical development. Spray drying has been widely employed as a formulation technique for flowable powders production, although relative low yields are potential disadvantages of spray-drying processes, especially on small scale equipment (Rogers et al., 2002). Other issues of the spray drying technology are for example related to the selection of a suitable solvent for the spray drying process. Another drawback is that in general thermolabile drugs are difficult to be processed by spray drying because of the elevated temperatures generated during the process (Kondo et al., 2009). Freeze drying is a bottom-up process full of potential. It can also be combined with a top-down step to produce ultrafine drug nanoparticles, making it a very diverse process. Interestingly, a freeze drying process as such can be performed to produce drug nanoparticles. Previous experiments have shown, that the size of the drug crystals can be controlled through a bottom-up process like freeze drying by adjusting process parameters, like freezing rate and solvent ratios (de Waard et al., 2009). Most important disadvantages of freeze drying processes are the relatively high costs due to extensive energy consumption and long cycle times. The process costs are further increased when poorly water soluble APIs are freeze dried from organic solutions.

Currently, top-down technologies are clearly the most important particle size reduction methods. At the moment all marketed drug products containing drug nanocrystals are produced by using top-down techniques. Therefore these techniques could be considered as being already accepted by the industry (Van Eerdenbrugh et al., 2008). Typical examples are wet ball milling and high pressure homogenization. Micronized drug powders are usually used as starting material. They are suspended into a dispersion medium containing surfactants and/or other stabilizers. In the case of high pressure homogenization (HPH), the suspension is further processed either through a jet-stream homogenizer (Khan and Pace, 2002) or a piston gap homogenizer (Müller et al., 2000). When the suspension passes the tiny homogenization gap under pressures up to 2000 bar in case of the piston-gap homogenizers, the high energy input leads to cavitation forces, shear forces and particle collision. Larger drug crystals are reduced in size to very small drug nanoparticles. Depending on factors like homogenization parameters and drug hardness, a different number of homogenization cycles are needed to produce a nanosuspension. In contrast to the bottom-up technologies, almost any poorly soluble drug can be processed with top-down processes, despite of being poorly soluble in aqueous and simultaneously in non-aqueous media (Müller et al., 2001). However, the use of standard high pressure homogenization is associated with some drawbacks. In case of very hard drug material the standard high pressure homogenization requires an extended number of homogenization cycles (typically 20 cycles), causing long production times, machine wearing and higher costs (Keck and Müller, 2006). Wet ball milling is another suitable top-down technique. A technology platform based on this principle, known as Nanocrystal® technology, was developed in the early nineties (Liversidge et al., 1992). This technique utilizes milling beads to grind the APIs in water containing surfactant and/or steric-stabilizers which are used to impede the agglomeration of the nanocrystals (Merisko-Liversidge et al., 2003). General advantages of all top-down processes are their lack of harsh chemicals or co-solvents, formulation simplicity, high drug loading capability and ease of scale up. Issues related to these processes are amongst others long processing times and difficulties to achieve a uniform size distribution in case of very hard materials (de Waard et al., 2008).

From an industrial and economical point of view it is highly desirable to minimize the milling times or homogenization cycles. To overcome the limitations of the standard bottom-up as well as top-down techniques new combinational methods have been developed for the production of ultrafine suspensions. Combinative technologies, which combine bottom-up with top-down steps are a relatively new approach to improve the particle size reduction effectiveness. The technology described in this paper combines freeze drying (FD) as bottom-up step for organic solvent elimination and API structure modification with high pressure homogenization as classical top-down technique for particle size diminution (Möschwitzer and Lemke, 2006). This technology is also referred to as H 96 technology, the code name for this process during the development. It belongs to the smartCrystal® technology platform of Abbott (Shegokar and Müller, 2010).

As already mentioned above, freeze drying, especially from aqueous solutions, is a well-established pharmaceutical unit operation. Freeze drying is a promising technique for developing pharmaceutical powders with improved solubility properties, although the freeze drying process could be relatively slow (Kondo et al., 2009). Due to a continuously increasing number of new chemical entities (NCEs) with poor water solubility, freeze drying from organic solutions has attracted more attention over the past years. Advantages of using organic solvent systems for a lyophilization process are for instance the enhanced wetting and solubility of the hydrophobic substances, the reduced solvent amount that has to be removed during the drying process and the shortened cycle times. However, the use of organic solvents is also related to a number of challenges as proper and safe handling of flammable, toxic and/or explosive solvents which requires special facilities and equipment. Furthermore a proper control of residual solvent levels are required as a consequence of the potential toxicity of organic solvents (Teagarden and Baker, 2002).

The freeze drying process can also influence the structure and crystal behaviour of drugs, making them porous and bulky. These properties are interesting in the case of applying a combinative particle size reduction method. The efficiency of the secondary top-down step can be significantly increased when more fragile material is used (Salazar et al., submitted for publication). The challenge is to identify optimal process parameters of the freeze drying step which result in the desired material properties of the modified API.

Design of experiments (DoE) is a very useful tool for the identification of critical process parameters and to optimize the respective process conditions (Verma et al., 2009). Critical factors of the freeze drying process are amongst others: organic solvent/solvent mixtures, API concentration, freezing rate/technique, container system and the used freeze dryer. The critical factors to analyze with respect to the high pressure homogenization step are: homogenization pressure, stabilizing system, number of homogenization cycles, batch size and the used homogenizer. DoE helps to identify and classify critical and non-critical parameters affecting product

quality. Furthermore it is possible to quantify the interactions between different input variables and the responses (Lionberger et al., 2008).

The presented research was conducted in order to establish a better understanding of the factors influencing the particle size reduction effectiveness of this novel combinative process. One objective was to identify the influence of the intermediate's attributes powder morphology and solid state characteristics on the minimal achievable particle size. Another objective was the identification of process conditions leading to the smallest particle size after the high pressure homogenization process. The interaction between the two factors solvent composition and API concentration in the solvent during the freeze drying step were therefore systematically examined by applying a two factorial customized design. The particle size as *z*-average and the polydispersity index (PDI) were investigated as responses describing the quality of the resulting nanosuspensions.

2. Materials and methods

2.1. Materials

Micronized (jet-milled) glibenclamide and the surfactant docusate sodium salt (DSS) were purchased from Sigma Aldrich, Germany. The freeze drying solvents dimethyl sulfoxide (DMSO) and tert-butyl alcohol (TBA) were both purchased from Merck KGaA, Germany. Liquid nitrogen was used for freezing. Demineralized water was supplied by a Millipore Milli Q-Plus system.

2.2. Methods

2.2.1. Design of experiment

Previous research in nanocrystal production with the H 96 process (Salazar et al., submitted for publication) revealed the importance of two main factors on the particle size reduction effectiveness. The first factor is the API concentration in the organic solution, which is prepared as first step of the freeze drying process. The second factor is the solvent composition of the organic solvent. Based on these screening results the experimental limits for the intended optimization were established for the critical parameters API concentration and solvent composition during FD. The effect of both parameters on the morphological appearance and solid state of the modified powders were investigated with a customized two-factor, five-level DoE. The levels for the API concentration were varied between 7 mg/ml and 27 mg/ml, the solvent composition was varied in its relative DMSO content between 90 and 10 (v/v) of the used DMSO-TBA mixture. It was intended to establish a response surface model with respect to the responses particle size and PDI. A schematic overview on the experiments carried out is shown in Fig. 1. Design-Expert® (Stat-Ease Inc., USA) was used as software for the DoE modeling.

2.2.2. Freeze drying

Freeze drying was performed using a lab scale freeze dryer Christ Alpha 2-4 (Martin Christ GmbH, Germany). API solutions were prepared according to the two-factorial design at five glibenclamide concentrations and at five DMSO:TBA solvent ratios. Therefore, different glibenclamide amounts, i.e. 0.5, 0.875, 1.25, 1.625 and 2 g were dissolved in 75 ml solvent comprising different DMSO:TBA volume ratios (v/v): 90:10, 70:30, 50:50, 30:70 and 10:90, respectively (Fig. 1). Consequently, the API concentrations for the FD process ranged from 7 mg/ml (low) to 27 mg/ml (high). Because of the space capacity of the freeze dryer only 6 HDPE containers could be processed at the same time. The complete sequence was freeze dried in several individual runs under the same freeze drying conditions. The glibenclamide solutions were transferred to high density polyethylene (HDPE) containers and the content was snap-frozen by pouring a sufficient amount of liquid nitrogen into the container. The frozen matrix was directly transferred to the freeze dryer. The freeze drying time was 4 days at a shelf temperature of approximately −20 °C and a condenser temperature of −80 °C, the pressure was below 0.5 mbar.

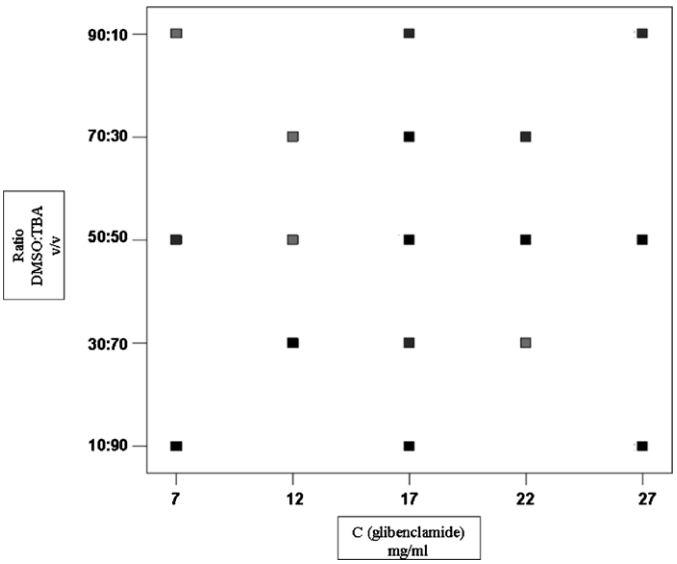

Fig. 1. Design of experiment matrix for the applied freeze drying process with solvent mixtures of DMSO:TBA (v/v) on the *y*-axis (factor 1: solvent composition) and glibenclamide concentrations (mg/ml) on the *x*-axis (factor 2: API concentration).

2.2.3. Scanning electron microscopy (SEM)

SEM was used to characterize the particle morphology of the jet milled starting material as well as the freeze dried API powders. A small fraction of each API powder sample was fixed on a double-sided conductive carbon tape and sputter-coated with 5 nm of a Pt–Pd alloy. Micrographs were obtained on a Zeiss DSM 982 Field Emission Gun Scanning Electron Microscope (Carl Zeiss AG, Germany). Powders were examined at 2 kV accelerating voltage in the secondary electron (SE) mode.

2.2.4. Differential scanning calorimetry (DSC)

A computer-interfaced differential scanning calorimeter DSC 821e (Mettler Toledo AG, Germany) was used to determine the crystallinity of the various API powders. The samples were accurately weighed (approx. 1–2 mg) and heated from 25 °C to 200 °C at a rate of 5 K/min.

2.2.5. Powder X-ray diffraction (PXRD)

PXRD (wide-angle X-ray scattering, WAXS) was used to study the crystallinity of pure micronized drug powders and of the freeze-dried modified drug powders. Diffraction patterns were measured by using a Philips X-ray generator PW 1830 equipped with a copper cathode (λ = 1.5418 Å, 40 kV, 20 mA) coupled to a computer-interfaced Philips PW 1710 diffractometer control unit (Philips Industrial & Electro-Acoustic Systems Division, The Netherlands). The scattered radiation was measured with a vertical goniometer (Phillips PW 1820). The scans were performed with a scanning rate of 0.5° per minute and steps of 0.04° from 1–40° 2 theta.

2.2.6. High pressure homogenization (HPH)

The unmodified, micronized glibenclamide and the different lyophilized glibenclamide powders were further processed to nanosuspensions. The API concentration was kept constant at 1% (w/w) (i.e. 0.4 g API in 40 g suspension). 0.2% (w/w) DSS was used

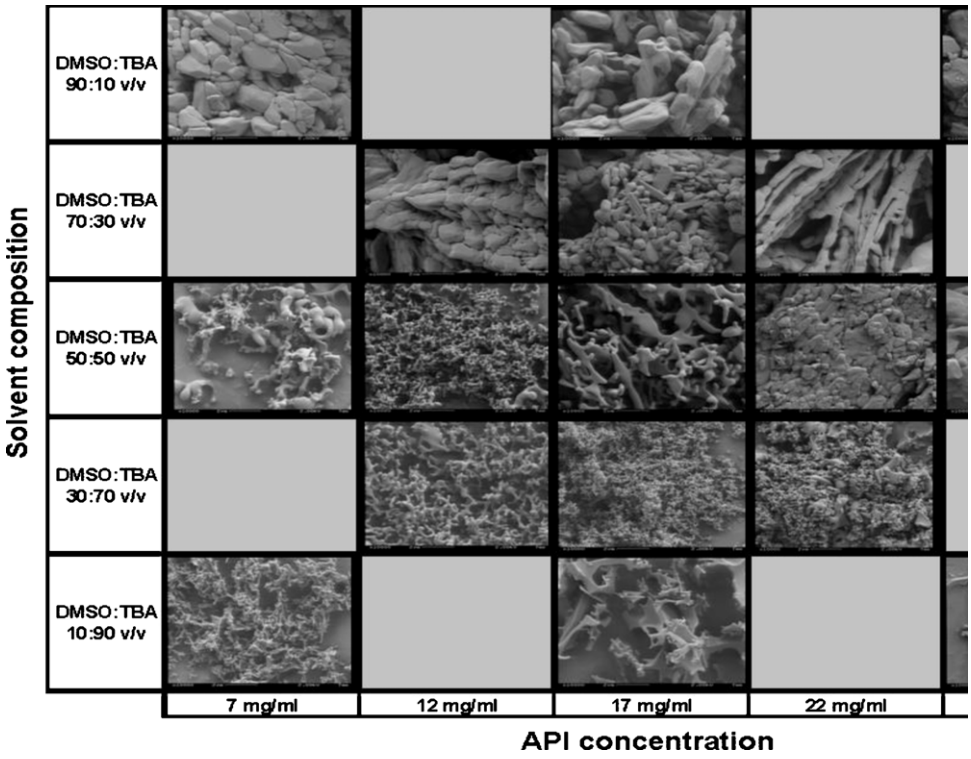

Fig. 2. SEM pictures of the freeze dried powders, magnification: 10,000×, 2 μm scale bar.

as electrostatic surfactant. The API was suspended in the surfactant solution and pre-dispersed by using an Ultra Turrax T-25 (IKA® Werke GmbH & Co. KG, Germany) for 1 min at 9000 rpm. The pre-mixes were further processed to nanosuspensions by using an APV Micron LAB 40 homogenizer (APV Systems GmbH, Germany). The different suspensions were first homogenized at low pressure (500 bar) for 2 cycles, then at high pressure (1500 bar) for 20 cycles. Samples were taken after Ultra Turrax, 1, 5, 10, 15 and 20 cycles of homogenization. The nanosuspension samples were further analyzed by photon correlation spectroscopy (PCS) and laser diffraction (LD).

2.2.7. Photon correlation spectroscopy (PCS)

PCS using a Zetasizer Nano ZS (Malvern Instruments, Germany) was performed to determine the *z*-average of the nanosuspensions. Simultaneously, the polydispersity index (PDI), as measure for the width of the particle size distribution, was calculated by the equipment. 4 μL of each suspension were diluted with 2 ml of demineralized water before being measured. Each sample was analyzed by ten consecutive runs and the result was calculated as the mean value of these 10 runs.

2.2.8. Laser diffraction (LD)

LD was performed with a Mastersizer 2000 (Malvern Instruments, Germany) to examine the particle size and particle size distribution of the nanosuspensions. The samples were given directly into the equipment filled with dematerialized water until the measurement-level (obscuration range) was reached. That means the medium in the cell was saturated with API from the added nanosuspension. For each sample five runs were made to determine the volume based diameters d50% and d90%, and finally the average was calculated as the mean value of the individual runs. The real refractive index was 1.616 and the imaginary refractive index was 0.001.

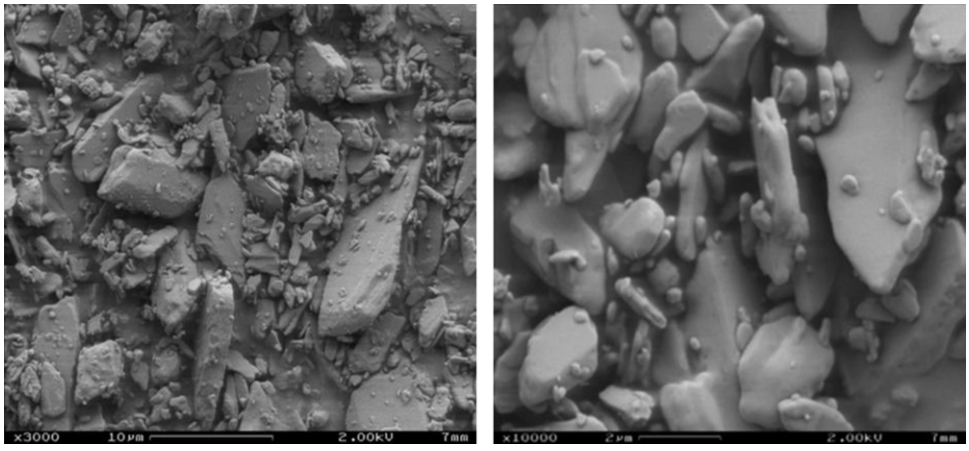

Fig. 3. SEM pictures of unmodified glibenclamide (starting material). Left: 3000× magnification, 10 μm scale bar. Right: 10,000× magnification, 2 μm scale bar.

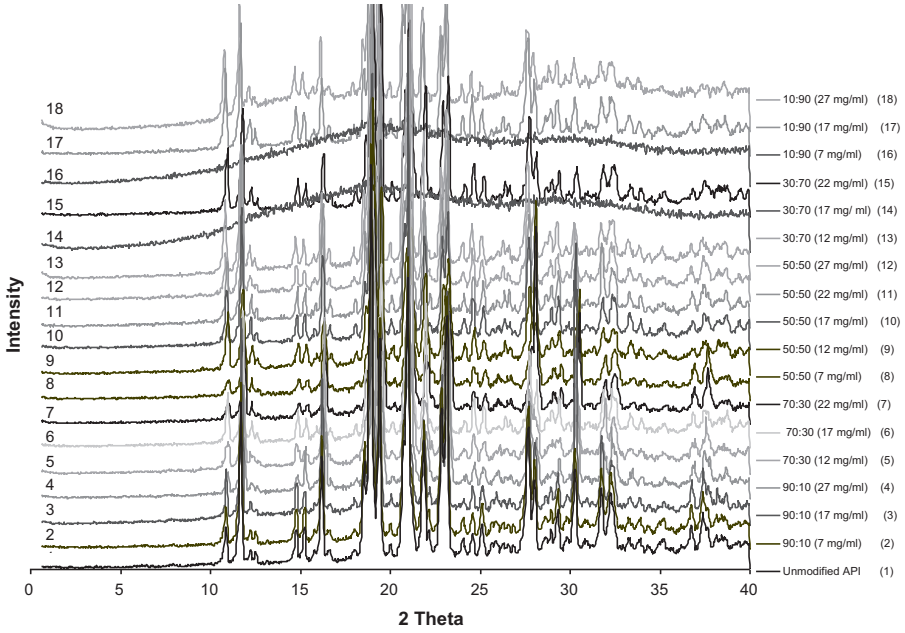

Fig. 4. X-ray diffraction patterns of glibenclamide as unmodified API and modified by freeze drying from DMSO:TBA mixtures with different solvent mixtures and API concentrations.

3. Results and discussion

3.1. Bottom-up: freeze drying

3.1.1. Freeze drying process induced morphology

In preliminary experiments, it was found that both the solvent composition and API concentration were key variables for the preparation of nanosuspensions according to the novel combinative method. They determined dominantly the macroscopic appearance of the freeze dried intermediates and appeared to be critical to obtain certain quality attributes of the nanosuspension, like a certain particle size and polydispersity index (PDI) (Salazar et al., submitted for publication). Fig. 2 shows the influence of the two key process parameters on the powder morphology of freeze dried glibenclamide. It can be seen that the fragility of the intermediates can be influenced by changing the solvent composition and the API concentration during the FD process step. The figure shows the trend that higher TBA amounts in the solvent and low API concentrations in the solution enhance the porous appearance of the modified API. A very porous intermediate was e.g. formed with 7 mg/ml API concentration in 10:90 DMSO:TBA, shown in the bottom-left corner of Fig. 2. In contrast the powders freeze dried from solutions containing high DMSO levels (90:10, v/v) showed no increase in porosity. Their powder morphology was comparable with that of unmodified, micronized glibenclamide (Fig. 3) irrespectively of the API concentrations in the solutions. The crystal like drug structure starts to change to a more fragile, porous material at the point of DMSO:TBA 50:50 and a medium drug concentration.

3.1.2. Solid state characterization of freeze dried intermediates

Besides the investigations regarding the powder morphology also the solid state of all unmodified and modified API powders, respectively, was investigated. In contrast to the powder morphology no clear trend could be found regarding the resulting crystallinity. Depending on the setting of the parameters API concentration and solvent composition, freeze dried, modified drug powders can be obtained either in crystalline, partially crystalline or amorphous state. Fig. 4 shows the PXRD patterns of the obtained freeze dried intermediates in comparison to the unmodified API. Interestingly, in most cases crystalline or predominantly crystalline glibenclamide was found. That means that despite the ultra rapid freezing rate crystalline API powders can be obtained. This could be beneficial with regard to long term physical stability of the resulting nanosuspensions. Only two samples were identified as being amorphous. These samples were freeze dried from DMSO:TBA solvents at 30:70 and 10:90 ratios at concentrations of 17 mg/ml or 7 mg/ml, respectively (no. 14 and no. 16 in Fig. 4). These results could also be confirmed by DSC as shown in the thermograms in Fig. 5 (no. 14 and no. 16, respectively). Both amorphous samples show a dramatically decreased endothermic melting peak. As characteristical feature of amorphous substances these samples show also a step height change at the glass transition, followed by an endothermic peak due to enthalpy relaxation and by an exotherm related to cold crystallization (or devitrification).

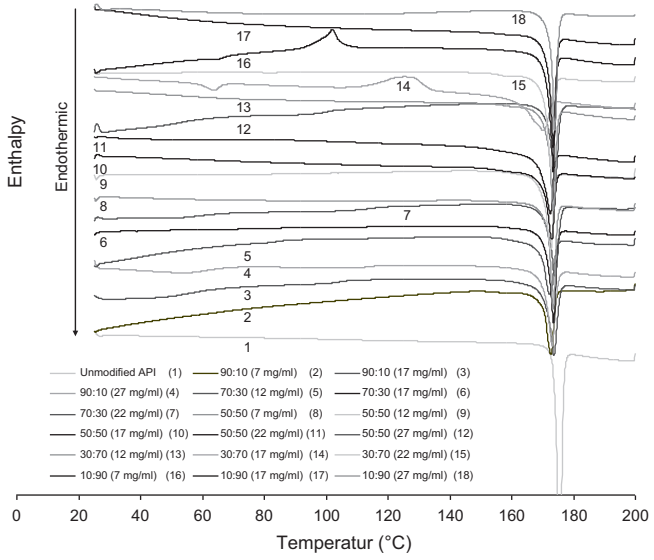

Fig. 5. DSC thermographs of glibenclamide as unmodified API and modified by freeze drying from DMSO:TBA mixtures with different solvent mixtures and API concentrations.

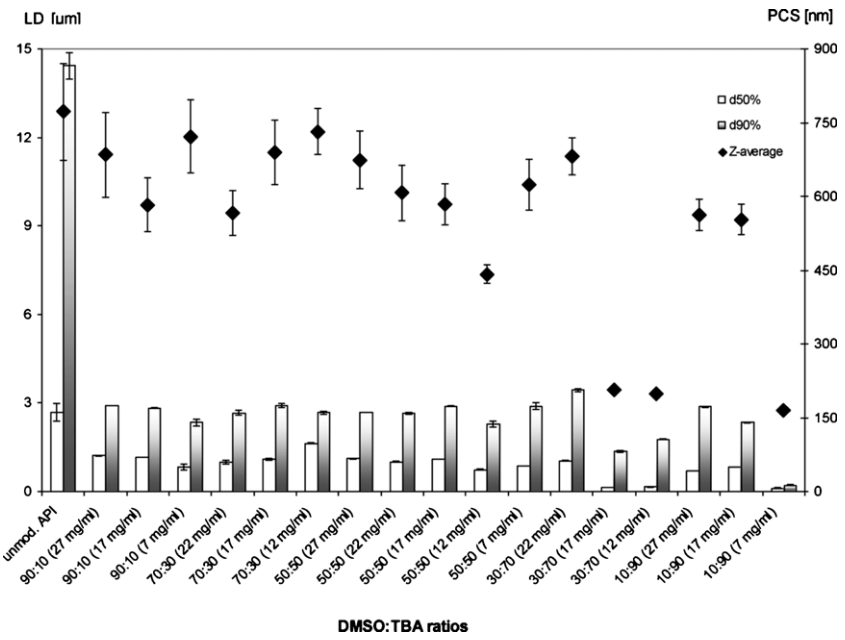

Fig. 6. PCS (*z*-average) and LD analysis (d50% and d90%) after 20 cycles of HPH at 1500 bar of glibenclamide as unmodified API and modified by freeze drying from DMSO:TBA mixtures with different solvent mixtures and API concentrations.

3.2. Top-down: high pressure homogenization and nanosuspension characterization

Nanosuspensions have subsequently been prepared from all modified and unmodified glibenclamide powders. Fig. 6 shows the results of the particle size determination by PCS and LD. All nanosuspensions prepared from modified API powders had a smaller d90% diameter as a measure for the largest particles in the suspension compared to unmodified material. This exemplifies the improved particle size reduction efficiency of the novel process. However, the mean particle size of most nanosuspensions was comparable with the result obtained from the unmodified powder. The majority of them have a relatively similar particle size, between 500 and 750 nm. The d50% and d90% particle diameters are also similar. There were three remarkable exceptions from the general trend. First, the nanosuspensions produced from amorphous powders obtained from the DMSO:TBA 30:70 (17 mg/ml) and 10:90 (7 mg/ml) points. The *z*-average was 207 nm and 164 nm respectively. The d50% and d90% values were 0.142/1.353 μm and 0.114/0.209 μm, respectively. Their PDI values were 0.208 and 0.133, both showing a very narrow particle distribution. Another interesting result was found for the nanosuspension produced from

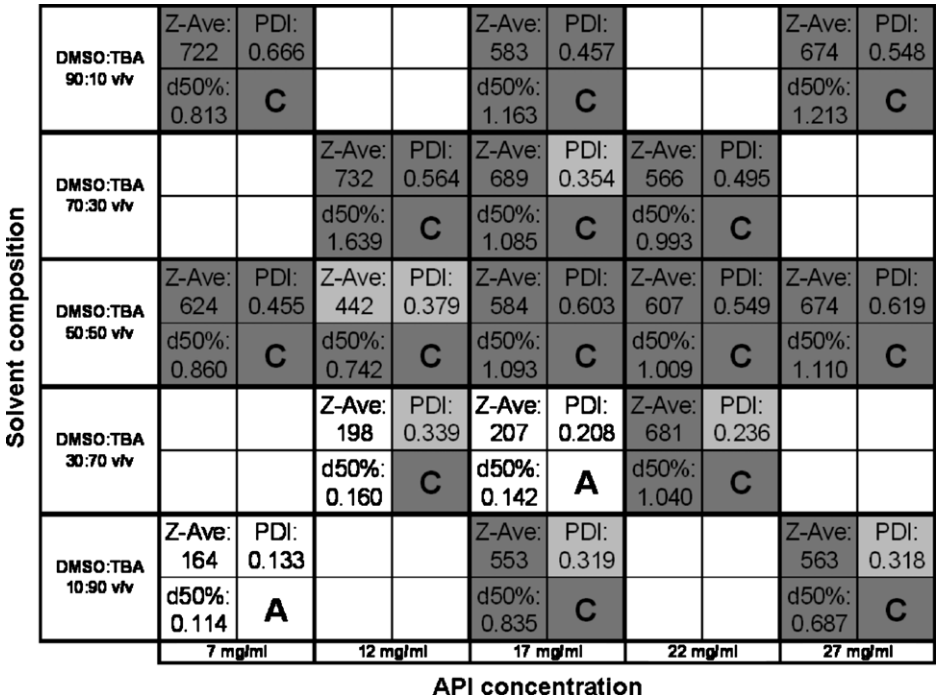

Fig. 7. DoE matrix results. *z*-average and d50% values: dark grey > 500 nm, bright grey 250–500 nm, white < 250 nm. Polydispersity index (PDI) values: dark grey > 0.4, bright grey 0.22-0.4, white < 0.22. Crystal behaviour: crystalline (C) dark grey, amorphous (A) white.

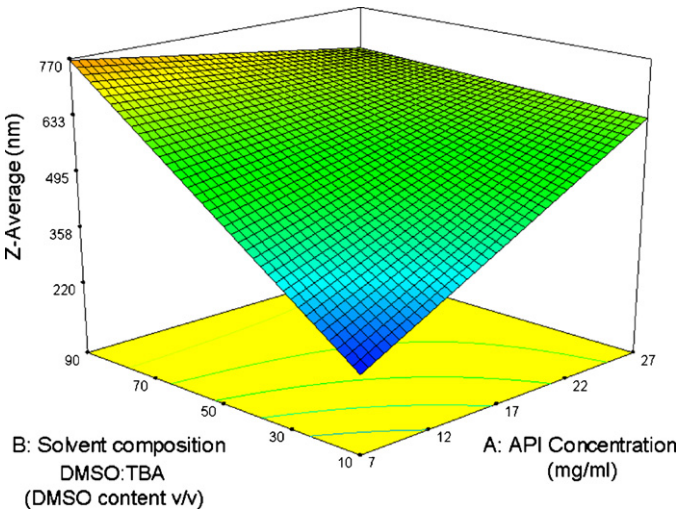

Fig. 8. Response surface model (RSM) showing the influence of the independent variables solvent composition and drug concentration during freeze drying on the quality attribute *z*-average particle size.

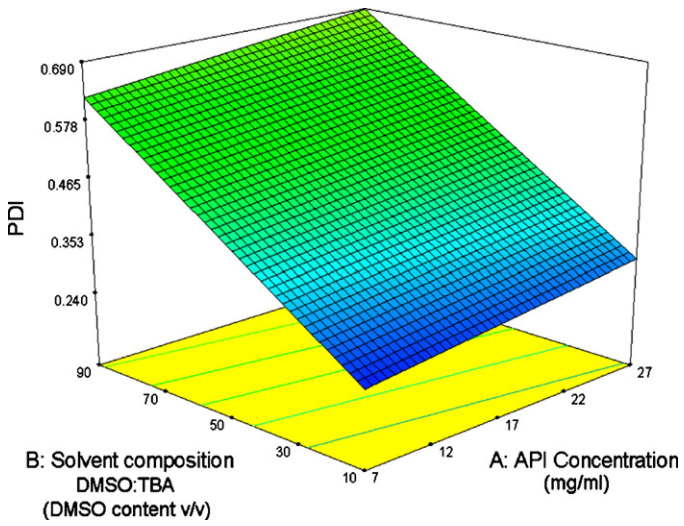

Fig. 9. Response surface model (RSM) showing the influence of the independent variables solvent composition and drug concentration during freeze drying on the quality attribute polydispersity index.

the DMSO:TBA 30:70 (12 mg/ml) sample. Although it showed a predominantly crystalline solid state, the *z*-average was 198 nm, and hereby comparable to the two suspensions produced from amorphous API. An increased d90% diameter (1.761 μm) and an increased PDI value (0.339) support the hypothesis that it is more difficult to obtain homogeneously dispersed nanosuspension of highly crystalline compounds in high pressure homogenization processes. Fig. 7 shows an overview of all experimental results in order to correlate it with the design outline of the two-factorial design as depicted in Fig. 1.

3.3. DoE aspects

The usage of DoE has allowed the identification of settings for API concentration (factor 1) and solvent composition (factor 2) that result in small particle sizes and narrow particle size distributions. Additionally interactions between the independent factors were investigated. Particle size and PDI data shown in Figs. 6 and 7 were taken as input for the calculation of the respective response surface models (RSM).

3.3.1. RSM for z-average

Fig. 8 shows the response surface model for *z*-average in response to the investigated factors solvent composition and API concentration. It can be seen that both factors affect the *z*-average. The area of a high TBA concentration and a low API concentration shows overall the smallest particle size. Whereas the influence on particle size at low API concentrations is highly variable with solvent composition it remains rather constant at high API concentrations. A similar situation occurs for the dependence on the API concentration, which is more variable at high TBA ratios. The latter two statements are based on the presence of a significant interaction of the two factors as suggested by DesignExpert®.

3.3.2. RSM for PDI

Fig. 9 shows the response surface model of the PDI as measure for the width of the particle size distribution in response to the investigated factors. In contrast to the *z*-average the PDI shows almost exclusive dependence on the solvent composition. Higher TBA ratios in the solvent mixtures led to a narrower particle size distribution resulting in a smaller PDI. The factor API concentration shows only a limited influence on the PDI. There is no significant interaction suggested between the factors by DesignExpert®, i.e. the extent of the solvent composition's influence on the PDI is independent from the API concentration.

3.3.3. Confirmation experiments

The results of the two RSMs were challenged in a confirmatory experiment. The "lower left" quadrant of the design (medium-high TBA content, low-medium API concentration) showed the most variation in the responses. Therefore three settings of this quadrant were processed in threefold. The resulting data is shown in Tables 1–3. It can be stated that all three repetitions led to equal results as previously obtained for the respective setting. The optimum for the investigated process as it was indicated by the model could hereby be confirmed. This demonstrates the reproducibility of the process even though the three settings result in intermediates with different solid state characteristics of the modified API powders.

Table 1
Results of confirmation experiments (runs 1–3) in comparison with the results from the first experiments (DoE) performed with a DMSO:TBA ratio of 10:90 at a drug concentration of 7 mg/ml.

DMSO:TBA 10:90 7 mg/ml	DoE	1	2	3
z-average (nm)	164	184	187	164
d50% (μm)	0.114	0.125	0.132	0.113
d90% (μm)	0.209	0.242	0.255	0.208
PDI	0.133	0.125	0.180	0.151
Crystal behaviour	Amorphous	Amorphous	Amorphous	Amorphous

Table 2
Results of confirmation experiments (runs 1–3) in comparison with the results from the first experiments (DoE) performed with a DMSO:TBA ratio of 30:70 at a drug concentration of 12 mg/ml.

DMSO:TBA 30:70 12 mg/ml	DoE	1	2	3
z-average (nm)	198	207	194	203
d50% (μm)	0.160	0.220	0.146	0.245
d90% (μm)	1.761	1.988	1.633	1.915
PDI	0.230	0.301	0.217	0.339
Crystal behaviour	Crystalline	Crystalline	Crystalline	Crystalline

Table 3
Results of confirmation experiments (runs 1–3) in comparison with the results from the first experiments (DoE) performed with a DMSO:TBA ratio of 50:50 at a drug concentration of 17 mg/ml.

DMSO:TBA 50:50 17 mg/ml	DoE	1	2	3
z-average (nm)	584	675	685	620
d50% (μm)	1.093	1.205	1.229	1.234
d90% (μm)	2.894	5.243	3.736	3.123
PDI	0.603	0.314	0.558	0.591
Crystal behaviour	Crystalline	Crystalline	Crystalline	Crystalline

3.4. Comparison of the novel combinative method with standard HPH

Fig. 10 shows the particle size evolution as a function of the number of homogenization cycles for nanosuspensions produced with standard HPH using unmodified API and FD-HPH using modified API produced according to the optimized conditions with regard to the minimal achievable particle size. In this figure the superior diminution effectiveness of the combinative method FD-HPH can be seen. Already after 5 homogenization cycles a very small particle size of 182 nm was obtained compared to 1377 nm obtained with the unmodified material. Furthermore, the minimum achievable particle size after 20 homogenization cycles was much smaller when modified API was used as starting material. Actually, the size after 5 cycles (182 nm) and 20 cycles (164 nm) is almost identical. This suggests that the maximum dispersity was already reached after 5 cycles when modified drug was used.

4. Discussion

The experiments described in this paper were conducted in order to establish a deeper understanding of the factors influencing the particle size reduction effectiveness of this novel H 96 process. It was intended to identify whether the increased porosity of the modified API or the change of the solid state properties during the freeze drying process are contributing to a better process efficiency. The DoE principles were applied to plan all experiments and to conduct the different steps in a controlled and efficient manner. The systematic investigations were beneficial in terms of time and informative value of the results.

The particle size and the particle size distribution were used as qualitative and quantitative measure for the quality of the resulting nanosuspensions. It was not in the scope of these experiments to derive a quantitative measure for the porosity. Therefore electron microscopic pictures were used to visualize the different appearances of the freeze dried intermediates. The morphological differences were so obvious, that it was not attempted to measure them quantitatively.

Both, the solvent composition and the API concentration have influenced the morphological appearance of the intermediates. Though it appears that more porous structures are present toward lower API concentrations and DMSO contents, this does not seem to directly reflect an influence on the mean measured particle size. The morphological appearance of samples freeze dried from highly concentrated glibenclamide solutions (27 mg/ml) at various solvent compositions is rather different. All samples have a comparable mean particle size. However, the influence of the increased porosity is also not negligible. For all modified samples a better particle size reduction in terms of a decreased d90% diameter and a decreased PDI was obtained irrespectively of potential changes in the solid state properties. This shows that the modification of the porosity could be beneficial for improved particle size reduction effectiveness.

The systematic experiments of the DoE enabled the identification of some points which showed surprisingly tremendously improved particle size reduction effectiveness. Solid state investigations have revealed that the API in these experiments was amorphous as result of the modification step. In confirmatory experiments it could be shown that this change of the solid state properties and the resulting particle size after the high pressure homogenization step was reproducible. Therefore it is very important to keep the process conditions constant. A deviation from the process conditions could result in more crystalline material which would result eventually in a larger particle size after the homogenization process.

Finally it can be stated that the influence of the porous appearance of the modified API powders seems to be less important than the change in the solid state properties of the freeze dried API. The apparently obvious interaction between amorphous solid state of the modified API and the minimal achievable particle size could only be investigated separately by applying DoE. The usage of DoE resulted also in the identification of process conditions which lead to an improved particle size reduction efficiency despite a crystalline character of the modified API powders. This is important with regard to the broad acceptance of this novel combinative technology. It is possible to obtain tailor-made solutions for poorly soluble APIs without the need to use fully amorphous systems, which could potentially recrystallize.

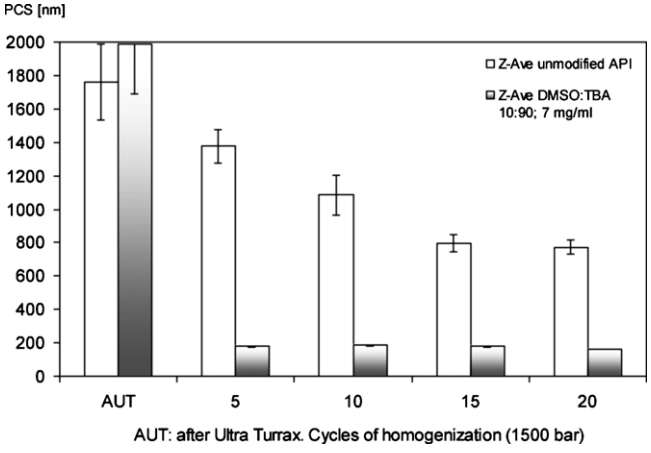

Fig. 10. PCS particle size decrease as a function of the processed type of drug and homogenization cycles: comparison of standard HPH (unmodified API) and FD-HPH (modified API using optimized FD conditions).

5. Conclusion

A novel combinative particle size reduction method, the combination of freeze drying and high pressure homogenization, was investigated in detail. Systematical investigations according to DoE principles helped to conduct the experiments in a controlled and efficient manner. The investigations have revealed that both, the morphological appearance and the solid state properties are influenced by the solvent composition and the API concentration as a consequence of the freeze drying step. The best results in terms of particle size reduction and particle size distribution after high pressure homogenization could be obtained when the freeze drying step yielded amorphous, highly porous API powder. However, surprisingly acceptable particle size results could also be obtained when the modification step yielded crystalline, highly porous glibenclamide. Therefore, porosity seems to be an important parameter. However, why also crystalline drug yields so small sizes cannot yet be theoretically explained and requires further mechanistic studies. Since the result of the particle size reduction step

depends clearly on the freeze drying process, a proper control of all process conditions is required to obtain reproducible results. DoE has clearly proved to be helpful in identifying the optimal settings for the successful processing of glibenclamide and can therefore be considered as an indispensable tool in optimization of process parameters.

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