COMBINATIVE PARTICLE SIZE REDUCTION TECHNOLOGIES FOR THE FORMULATION OF POORLY SOLUBLE DRUGS

Dissertation zur Erlangung des akademischen Grades des

Doktors der Naturwissenschaften (Dr. rer. nat.)

eingereicht im Fachbereich Biologie, Chemie, Pharmazie

der Freien Universität Berlin

vorgelegt von

Jaime Salazar

aus Osorno, Chile

März 2013

Die vorliegende Arbeit wurde von April 2009 bis Dezember 2012 unter der Leitung von Prof. Dr. Rainer H. Müller am Institut für Pharmazie der Freien Universität Berlin, Abteilung Pharmazeutische Technologie, Biopharmazie und NutriCosmetics, angefertigt.

1. Gutachter: Prof. Dr. Rainer Müller

2. Gutachter: Prof. Dr. Michael Dittgen

Disputation am 14. März 2013

To my family

Table of Contents

I

1. INTRODUCTION

An increasing problem for pharmaceutical development is the large number of poorly water-soluble drugs emerging from drug discovery that have an aqueous solubility lower than 0.1 mg/ml [1, 2]. The drug candidates emerging from high-throughput screenings (HTS) are modeled to have a high target-receptor affinity. This feature is desirable from a pharmacological point of view. Yet this characteristic results in drug compounds with high molecular mass and high log P, factors that both contribute to poor water solubility [3].

This situation currently affects about 40% of the drugs in the development pipelines and 70% of the new chemical entities (NCEs) coming from organic synthesis or HTS. Many of these drugs are also insoluble in organic solvents as well, which increases the formulation problems [4-6]. Poorly soluble compounds have a wide variety of issues, which include poor oral bioavailability, fed/fasted variation, slower onset of action, erratic absorption and high patient variability [5, 7]. Problematic poorly soluble compounds need to be formulated with new technologies in order to save time and resources during the formulation efforts [1, 8].

Standard formulation approaches are salt formation, pH adjustment, solubilization with co-solvents, emulsions and molecular complexation (e.g., cyclodextrins) [9]. Their drawbacks include, among others, excessive amounts of solvent and toxicity-related issues in the case of the solubilization with co-solvents, difficulty in finding a suitable salt in the case of the salt formation, extreme and non-physiological pHs in the case of the pH adjustment, and a high molar ratio of excipients causing toxicity issues in the case of the molecular complexation with cyclodextrins [10, 11].

Although these approaches can be successfully applied for a variety of drugs, they are not sufficient to formulate new, challenging compounds, such as the poorly water-soluble drugs, especially those presenting with high log P, a high melting point and a high dose [9].

These problems can be avoided by employing new formulation technologies, the so-called "enabling technologies". Some examples thereof are solid dispersions [12-14], lipid-based complexation such as liposomes, solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) [15-17], and particle size reduction into the nanometer range [18, 19]. The latter approach is an established non-specific technique for the production of drug nanocrystals [20, 21]. Standard techniques employed for the production of drug nanocrystals are high pressure homogenization and wet bead milling (NanoCrystal® technology). These

processes have been already employed to successfully formulate problematic compounds to bring them to market [22]. Examples thereof are Rapamune® (sirolimus) by Wyeth Pharmaceuticals, Emend® (aprepitant) by Merck & Co., Tricor® (fenofibrate) by Abbott Laboratories and Megace® ES (megestrol acetate) by Par Pharmaceuticals, all formulated employing the NanoCrystal® technology. Another product containing drug nanocrystals is Triglide® (fenofibrate) by SkyePharma, which was developed employing the IDD-P™ high pressure homogenization technique [6]. All these products are solid oral dosage forms, with the only exception being Megace® ES, which is presented in nanosuspension form [22]. However, the standard techniques still present drawbacks, such as long processing times and the necessity of employing a micronized drug as the starting material [23]. Next-generation technologies involve combinative particle size reduction methods to improve the particle size reduction effectiveness of the standard processes [24].

1.1 Drug nanocrystals for the formulation of poorly soluble drugs

Drug nanocrystals are defined as particles consisting of a pure drug stabilized with ionic (e.g., sodium dodecyl sulfate, docusate sodium salt, sodium cholate) or non-ionic (e.g., poloxamer, Tweens) surfactants. The higher drug loads of the drug nanocrystals make them a pharmacologically effective drug delivery system [21]. The size of the drug nanocrystals is below 1000 nm, typically 100-300 nm or lower. The term "nanosuspension" refers to the drug nanocrystals being dispersed in a liquid [20, 22]. Depending on the drug properties and the production parameters, the drug nanocrystals can be obtained in the crystalline or amorphous state. The solid state behavior of the drug particles has a great influence on their solubility [25]. The following explains the distinctive physicochemical properties of the drug nanocrystals (Fig. 1).

Drug nanocrystals: physicochemical properties

Noyes-Whitney	Ostwald-Freundlich	Prandtl

$$\frac{dC}{dt} = \frac{AD(C_s - C)}{h}$$

$$\ln\frac{S}{S_0} = \frac{2M\gamma}{\rho r RT}$$

$$h_H = k(L^{1/2}/V^{1/2})$$

$\frac{dC}{dt}$ = dissolution velocity

A = surface area of the particle

D = diffusion coefficient

C_s = saturation solubility of the drug

C = drug concentration in the surrounding liquid

h = thickness of the diffusional layer

S = drug solubility at temperature T

S_0 = solubility for an infinite big particle ($r=\infty$)

M = molecular weight of the compound

γ = interfacial surface tension

ρ = density of the compound

r = radius of the particle

R = gas constant

T = temperature

h_H = hydrodynamic boundary layer thickness

K = constant

L = length of the surface in flow direction

V = relative velocity of the flowing liquid

Figure 1: Distinctive physicochemical properties of the drug nanocrystals.

The dissolution velocity of a compound is directly proportional to its diffusion coefficient (D), the difference between saturation solubility (Cs) and drug concentration in the bulk medium (C) and the surface area (A) presented by the particles (the Noyes-Whitney equation). Due to the extremely small particle size of the drug nanocrystals, their surface area is increased compared to the micronized material. This increase in surface area leads to an enhanced dissolution rate according to the Noyes-Whitney equation [26]. The enhanced dissolution rate can lead to a higher oral bioavailability of poorly soluble compounds after oral administration [27].

The small particle radius of the drug nanocrystals increases the saturation solubility of the nanosized compounds according to the Ostwald-Freundlich equation (Fig. 1), which describes the vapor pressure as a function of the curvature of solid particles in a liquid phase [28, 29].

S is the drug solubility at the given temperature T, S_0 is the solubility for an infinite big particle (radius r=∞), M is the molecular weight of the compound, γ is the interfacial surface

tension, ρ is the density of the compound and R is the gas constant. The dissolution pressure of a particle in a liquid improves by increasing the curvature (i.e., by reducing the particle size) of the particle. However, the influence of the particle size on the saturation solubility begins with particle sizes below 1 μm [18]. Another factor influencing the solubility is the crystallinity. Drugs having a high solid density (with a high melting point) are less soluble than amorphous compounds with a lower density [30].

Finally, the Prandtl equation demonstrates how the increased curvature of the drug nanocrystals reduces the diffussional distance h, which improves the dissolution velocity (Fig. 1).

Besides the enhanced dissolution rate and saturation solubility, there are some other benefits of employing drug nanocrystals as formulation tools. These benefits include reduced fed/fasted variability [31], reduced absorption variability between patients, faster action onset, an enhanced absorption rate and better compliance due to reduced oral unit intake [6, 7].

Compounds belonging to Class II of the Biopharmaceutics Classification System (BCS) are poorly soluble (aqueous solubility < 0.1 mg/ml) and highly permeable [2, 32]. Class II drugs, in general, show a bioavailability that depends on the dissolution rate as a limiting factor [33]. Consequently, their formulation as drug nanocrystals for dissolution rate enhancement could eventually solve their oral bioavailability issues [9].

An important aspect is the proper analysis of the drug nanocrystals or nanosuspensions. The particle size of the drug nanosuspensions are characterized usually by photon correlation spectroscopy (PCS) and laser diffractometry (LD). The first analytical technique determines the mean particle diameter (z-average) as a light intensity-weighted size of the bulk population and the polydispersity index (PDI) as a measure for the width of the particle size distribution of the drug nanocrystals in the liquid nanosuspension. The second technique determines the volume-based particle size distribution of the nanosuspensions, which is sensitive to large particles irrespective of their quantity. For example, the diameter of 90% (d90%) value indicates that 90% of the particle volume is below the given particle size in micrometers [34]. The first technique has a measuring range of 3 nm-3 μm, which means that larger particles remain undetected. The second technique has a lower measuring range of 0.05-80 μm (depending on the equipment) and an upper measurement level of up to 2000 μm. By working with the two combined analytical techniques, it is possible to properly characterize the particle size and size distribution of the nanosuspensions [18]. Another important parameter is the charge of the particles, which determines the stability of the

nanosuspensions by electrostatic repulsion. The particle charge is usually calculated by electrophoresis and expressed as electrophoretic mobility, which is converted to zeta potential (in milivolts) employing the Helmholtz-Smoluchowski equation [35].

Scanning electron microscopy (SEM), powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC) are standard analytical tools to analyze pharmaceutical powders in terms of morphology and solid state behavior [36, 37].

By employing the SEM technique, it is possible to characterize the particle structure of the drug powders and to assess the structure modification due to pre-treating processes such as freeze-drying (FD) and spray-drying (SD). Porous and brittle drug structures are suitable for a particle size reduction process, as they are easier to nanosize [38].

PXRD and DSC are both techniques employed to analyze the solid state of pharmaceutical powders. The degree of crystallinity (DC) of modified drug powders compared to the DC of the micronized starting material is important to determine both the crystallinity modification induced by a pre-treatment process and to what extent the modification could facilitate the production of drug nanocrystals [39].

1.2 Production techniques for drug nanocrystals

There are basically two approaches to produce drug nanocrystals, with a third one (the combinative approach) being a combination of the first two techniques. The first process type produces drug nanocrystals by precipitating dissolved molecules. This approach is called bottom-up, as the size of the particles is increased. This group involves processes such as microprecipitation and chemical synthesis. The second process type involves particle size reduction or comminution. This approach is called top-down, as the size of already existing particles is decreased [40]. The third approach involves combinations of bottom-up and/or top-down steps to improve the particle size reduction effectiveness of the single-unit processes. The first step is usually a bottom-up process employed as a drug pre-treatment to obtain a brittle, friable starting material for a subsequent comminution step. Thereby, the drug material is easier to nanosize. Combinations of two different top-down steps (such as bead milling followed by high pressure homogenization) have also been developed [6, 41].

1.2.1 Precipitation processes (bottom-up)

The drug dissolved in a solvent is precipitated by adding an antisolvent. This is a classical precipitation process, also known as *via humida paratum* (Latin for "produced in a wet process"). The challenges of this technique are to minimize the crystal growth into the nanometer range (controlled crystallization) and to control the solid state of the crystals, i.e., to produce them in crystalline or amorphous form [6].

The "hydrosols" technology is the first process involving a bottom-up step to produce drug nanoparticles; this technology was developed by Sucker and nowadays belongs to Novartis [42, 43]. The process has the advantage of producing crystalline drug nanoparticles. However, this technology has a set of drawbacks. The drug has to be soluble in at least one solvent and the process involves organic solvents that need to be removed. There are apparently no products on the market that use this technology, perhaps due to the difficulty of avoiding the crystal growth.

Another precipitation technology is the Nanomorph® process developed by Auweter [44, 45]. This technology yields amorphous drug nanoparticles, which have the advantage of higher saturation solubility and a faster dissolution rate compared to the crystalline form. However, drawbacks include undesired compound re-crystallization to the crystalline state with a subsequent decrease in bioavailability [25].

Another bottom-up technology is the controlled crystallization during FD [46]. This technology involves a precipitation process (lyophilization) to produce nanocrystalline particles. Subsequently, the freeze-dried powders can be directly employed to manufacture tablets. This makes it a simple single-unit precipitation process. Additionally, drug releases of up to 80% after 10 min of dissolution testing were reported. The special features of this bottom-up technology are the production of crystalline drug nanoparticles and the ability for large-scale production [46, 47].

FD as well as SD are precipitation processes widely employed in the pharmaceutical industry to obtain dry intermediates or final drug powders. Both bottom-up technologies can also be employed to modify drug materials to make them more suitable (i.e., friable and brittle) for a subsequent comminution process [24].

1.2.2 Comminution processes (top-down)

1.2.2.1 Wet bead milling (WBM, NanoCrystal® technology)

The pearl or bead milling technique was developed by Liversidge [48] and is owned by Alkermes plc. It is referred to as the first-generation production method for drug nanocrystals. This technology comprises a milling chamber with an agitator, which is loaded with the milling material, a dispersium medium (usually water), surfactants as a stabilization system and the drug to be nanosized. The drug particles are reduced in size by mechanical attrition. The milling material is usually small beads of stainless steel, glass, ceramic (e.g., yttrium stabilized zirconium dioxide) or highly cross-linked polystyrene resin, the last two being preferred due to reduced contamination to the product. The milling pearls have different sizes (e.g., 0.1, 0.2 or 0.5 mm). The collision frequency during the comminution process is increased with the reduction in size of the milling beads. Thus, the particle size reduction effectiveness can be enhanced [49]. Other factors affecting the comminution effectiveness are the hardness of the drug, the surfactant and its concentration, temperature, the viscosity of the dispersion medium, etc. The forces producing the particle size reduction include shear forces and particle collision produced by the movement of the milling material inside the chamber. The NanoCrystal® technology is regarded as a successful technology: the first product containing drug nanocrystals (Rapamune® by Wyeth Pharmaceuticals in 2000) came to the market only 10 years after the development of the technology [7, 50].

1.2.2.2 High pressure homogenization (HPH)

The HPH technique is a high-energy disintegration process that employs high pressure to reduce the particle size of drug particles in liquid media with surfactants for stabilization purposes. The HPH involves the principles of piston-gap homogenization and jet-stream homogenization (microfluidization) [21, 22].

1.2.2.2.1 Piston-gap homogenization

When the piston-gap HPH technique is employed, the particle size reduction is achieved by cavitation, shear forces and particle collision. The suspension is forced through a small gap,

which reduces the diameter from 3 cm to approximately 25 µm [34]. Because of the tremendous diameter change, according to Bernoulli's law, the dynamic pressure raises and the static pressure falls. As a result of the latter, the liquid starts boiling in the homogenization gap, as the static pressure is lower than the vapor pressure of the liquid. This phenomenon results in the formation of gas bubbles that implode after leaving the gap (cavitation). The shear forces and the particle collisions are developed during the process due to the high pressures involved (usually up to 1500 bar). The equipment employed for piston-gap homogenization is produced, for example, by APV, Gaulin and Avestin [9, 21].

Employing piston-gap homogenizers, Müller and co-workers developed the Dissocubes® technology (now belonging to Skyepharma plc) and the Nanopure® technology (now belonging to Abbott GmH & Co. KG) [21, 51]. These technologies produce drug nanocrystals by employing high pressures of up to 1500 bar. However, they employ different process media for the homogenization process. The Dissocubes® technology produces drug nanoparticles in an aqueous dispersion at room temperature. On the contrary, the Nanopure® process employs non-aqueous media (e.g., oils or liquid polyethylene glycols) or water-reduced media (e.g., employing glycerol/water mixtures) [23]. One interesting feature of the Nanopure® technology is that the oil dispersions can be employed to subsequently fill capsules as the final dosage form [6].

1.2.2.2.2 Jet-stream homogenization (microfluidization)

The microfluidization technology (Microfluidizer®, Microfluidics Inc., USA) is based on the jet-stream principle. The drug is suspended in aqueous media with surfactants for stabilization. Then, the suspension is put into the homogenizer device for processing. During the homogenization process, two jet streams are forced to circulate at high pressure (up to 1700 bar) through two different interaction chambers (Y and Z). This produces particle collision and shear forces as well as cavitation, which results in the disintegration of the drug particles [52]. SkyePharma Canada Inc. employs a Microfluidizer® homogenizer for its IDD-P™ (insoluble drug delivery particles) technology, which produces submicronic nanosuspensions [53].

1.2.3 Combinative technologies

In summary, the known limitations of the standard processes (WBM, HPH) for the production of drug nanocrystals are the necessity of a micronized drug as the starting material and the long runtimes for the top-down equipment [23]. The combinative particle size reduction techniques have been developed to overcome these drawbacks and to improve the particle size reduction effectiveness of the standard processes. Nowadays, five combinative methods are known: NANOEDGE™ (microprecipitation followed by a high-energy step such as HPH), H 69 (microprecipitation immediately followed by HPH, also called "cavi-precipitation"), H 42 (SD followed by HPH), H 96 (FD followed by HPH), and the CT combinative technology (media milling followed by HPH) [6]. The applications of the combinative technologies for a variety of drugs are shown in Table 1.

1.2.3.1 NANOEDGE™ technology

The NANOEDGE™ technology from Baxter is the first combinative particle size reduction method developed for the production of drug nanosuspensions. This production technique combines a microprecipitation step (a solvent-antisolvent technique) followed by a high-energy process. The drug is first dissolved in a suitable solvent, usually a water-miscible organic solvent. The drug solution is then mixed with a second aqueous liquid in which the drug is less soluble. The aqueous liquid can contain surfactants for stabilization, and it is added to the drug solution in a controlled manner using, for example, an infuser device. Subsequently, the precipitation occurs due to the change in solubility. The microprecipitation is a pre-treatment and the drug particles can be obtained in amorphous or semi-crystalline form. Then, the drug particles are reduced in size and transformed to the more stable crystalline state after employing a high-energy annealing step, such as HPH [54, 55]. The objective of the annealing step is to improve the thermodynamic stability of the nanosuspensions by preventing the crystal growth of the precipitated particles to the micrometer range. This is achieved by single or repeated energy applications, followed by thermal relaxation. The change to the more stable form is produced by the high-energy input of the top-down step, which promotes the formation of low-energy, more stable structures, either by enhancing the crystallinity of the particles (reordering of the lattice structure) or by rearrangement of the stabilizing system at the surface of the drug nanocrystals. The fast

9

microprecipitation improves the particle size reduction effectiveness of the top-down step due to induced friable material, drug crystal defects and dendritic morphology. The top-down process is usually piston-gap HPH but other techniques such as sonication or microfluidization can also be employed [55, 56].

The residues of organic solvents in the nanosuspension are a major problem associated with this combinative technology, which becomes more complicated in the case of large-scale production (i.e., larger amounts of solvent to be removed from the final drug product). This is difficult when a nanosuspension is needed. Another drawback is that this technology achieves particle sizes markedly bigger than with standard technologies. As the Baxter development is mainly focused on injectables, the solvent-removal processes as well as the production lines need to be performed under sterilized conditions. This situation makes the process more complicated and expensive. This technology has no marketed products to date, as I.V.-injectable products are more complicated to develop as oral products [6].

1.2.3.2 NANOEDGE™ applications

The NANOEDGE™ platform has been employed to formulate poorly soluble anti-cancer drugs such as paclitaxel to improve the plasma concentration and thereby the pharmacologic efficacy. Nanosuspensions formulated with 1-5% w/v drug presented particle sizes of around 1000 nm. The plasma levels achieved by the antineoplastic drug nanocrystals were tested in animal models. The drug nanosuspensions produced by this technology could achieve higher drug loads and a more flexible administration, such as oral and injectable routes [57].

The therapy efficacy for an anti-cancer compound described as "A" was established in rat tumor models. The nanosuspension formulation showed better tolerability in rats than the drug formulated with standard techniques, which enable the implementation of higher doses. However, the efficacy of the tumor suppression was comparable after I.V. injection and after oral administration. With another poorly soluble, poorly bioavailable anti-cancer drug described as "B", a linear relationship was found between the decreasing in nanosuspension particle size (particle sizes of 400-1000 nm, administered at 300 mg/kg) and the increase of oral bioavailability measured from the plasma of rats. In this case, the formulation as a nanosuspension employing the NANOEDGE™ process resulted in an up to 30-fold bioavailability increase in the rat models compared to the control formulation [57].

The NANOEDGE™ combinative technology was also employed to reformulate a paclitaxel product (Taxol®, Bristol Myers Squibb Company). The objective was to eliminate Chremophor® EL (polyethoxilated castor oil) as an excipient to avoid its incompatibilities and toxicity. Functionalized polyethylene glycols were employed as surfactants to minimize the opsonization of the drug nanocrystals, which had a mean particle size of 200 nm [30, 57].

The poorly soluble drug itraconazol was processed employing the NANOEDGE™ technology. It was dissolved in N-methyl-2-pyrrolidone (NMP) and then precipitated by adding an aqueous diluent with surfactants. Sonication for one minute at 10000 Hertz (Hz) and 400 watts (W) employed as the annealing step resulted in drug nanoparticles with a mean particle size of 177 nm [55].

In another study, an itraconazol nanosuspension for I.V. administration was developed employing the NANOEDGE™ process. Its resulting bioavailability was compared to the results of a marketed itraconazol solution (Sporanox® IV, Janssen Pharmaceutica), which is formulated with cyclodextrin technology and presents some degree of toxicity due to the high cyclodextrin load. In this case, HPH was used as the annealing step, achieving a final mean particle size of 581 nm. Subsequently, in vivo studies were performed in rat models. The nanosuspension formulation led to better bioavailability and tolerability, enabling the use of higher drug doses. The subject survival was superior with the nanoparticulated itraconazol due to higher drug concentrations in the target organs compared to the standard solution formulation [30, 58].

Carbamazepin, prednisolone and nabumetone were also processed with this technology. The drugs were separately dissolved in NMP and then precipitated by adding distilled water. Carbamazepin and prednisolone presented a needle-shaped form and a mean particle size of approximately 2 µm after precipitation. An Avestin C50 homogenizer (Avestin Inc., Canada) was employed for the high-energy step to process the three drug macrosuspensions, which resulted in final mean particle sizes of 400 nm for carbamazepin, 640 nm for prednisolone and 930 nm for nabumetone [55].

There are also a variety of drugs processed by "Nanoedge-like" processes involving a microprecipitation step followed by a high-energy process (HPH or sonication), although they are a not addressed as "Nanoedge". The anti-tumor alkaloid 10-hydroxycamptothecin (10-HCPT) was processed by employing a microprecipitation-homogenization process. The drug was dissolved in dimethyl sulfoxide (DMSO), and then it was precipitated by adding an aqueous surfactant solution. The drug suspension was then homogenized employing an ATS

AH110D piston-gap homogenizer (ATS Engineer Inc., China). The drug particles were obtained in the amorphous state and the best final mean particle size of 131 nm was obtained by homogenizing the drug suspension for 20 cycles at 1000 bar [59].

Isradipine was also processed employing a microprecipitation-HPH technique. The drug was dissolved in 2-propanol and then precipitated by adding an aqueous solution containing surfactants for stabilization purposes. This macrosuspension was then processed by HPH employing a piston-gap homogenizer (GEA Niro Soavi Inc., USA) for 30 cycles at 1200 bar. The resulting nanosuspension had a mean particle size of 469 nm [60].

The non-steroidal anti-inflammatory drug meloxicam was also processed employing a combinative approach. This drug was dissolved in dimethylformamide and then precipitated by adding the drug solution to an aqueous solution containing surfactants. The drug particles were then further processed either by ultrasonication (20 min 300 W with a FS-5 sonicator, Frontline Ltd., India) or by HPH (15 cycles at 500 bar with an ATS AH110D homogenizer). The results showed a final mean particle size of 259 nm with the sonication method and 212 nm with the HPH technique. However, the amount of larger crystals was considerably smaller when HPH was used as the reduction step. Additionally, low pressure could be maintained (500 bar), as higher pressures (or a higher number of homogenization cycles) did not improve the particle size reduction [61].

In the case of nitrendipine, the drug was processed by employing a microprecipitation-ultrasonication process. The drug was first dissolved in a 1:1 PEG 200:acetone mixture, and then it was precipitated by adding a polyvinyl alcohol aqueous solution. The drug particles were subsequently processed by employing ultrasonication (Ningbo Scientz Biotechnology Co. Ltd., China). The best mean particle size result of 209 nm was obtained by employing 20000 Hz and 400 W as sonication conditions for 15 min. Nitrendipine suffered no substantial crystallinity change after the process [62].

All-trans retinoic acid is a poorly soluble, heat-sensitive anti-cancer drug. A microprecipitation-sonication process under controlled temperature was chosen to produce nanoparticles of the drug to eventually improve its dissolution rate dependent bioavailability. The drug was first dissolved in acetone, and then it was mixed with demineralized water to produce the precipitation. The drug particles were immediately sonicated employing an EQ-250E medical ultrasonator (Kunshan Ultrasonic Instrument Corporation, China) for 30 min. A final mean particle size of 155 nm was obtained. However, this result was only slightly improved compared to the precipitation process without sonication (176 nm mean particle

size). Additionally, the precipitation process produced, in general, amorphous drug particles. [63].

Hydrocortisone was processed performing experiments with a microprecipitation-sonication technique. This drug was dissolved in ethanol and then precipitated by adding an aqueous surfactant solution. The drug particles were immediately sonicated after precipitation for 5 min. The process factors that were investigated included solvent:antisolvent flow rate and drug concentration. A mean particle size of 80 nm could be achieved under optimized conditions. The precipitation process modified the high crystallinity of the starting material leading to amorphous drug nanoparticles [64, 65].

Finally, a microprecipitation-sonication process was performed with ibuprofen. The drug was dissolved in acetone and then added to an aqueous solution containing surfactants. The precipitated drug particles were further sonicated for 60 min employing a Sonic Dismembrator model 550 (Fisher Scientific International Inc., USA). Different surfactants were screened for the precipitation step and the best stabilization results were achieved with HPMC K3, which led to a mean particle size of 702 nm after sonication [40].

1.2.3.3 H 69 technology

The H 69 process was developed by Müller and Möschwitzer, and it belongs to the smartCrystal® technology family. This combinative process is similar to the NANOEDGE™ approach. It combines a microprecipitation step involving organic solvents, followed by HPH for particle size reduction. The difference is that with the H 69 technology, the cavitation takes place at the same time as the particle formation ("cavi-precipitation") or at most two seconds thereafter. To employ this combinative technique, the drug is dissolved in a suitable solvent (liquid 1), which is then mixed with an aqueous non-solvent (liquid 2). The non-solvent is added to the solvent in a controlled manner using, for example, an infuser device such as the Perfusor® from B. Braun Melsungen, Germany. To do this, different pump rates can be adjusted. The liquid flows come in contact, which results in the precipitation of the drug. The particle formation takes place in the high-energy zone of a homogenizer, where the just-formed drug particles are immediately treated with cavitation, particle collision and shear forces. The Microfluidizer® or the EmulsiFlex C5 from Avestin are suitable homogenizers to process the liquid flows directly in the high-energy zone of the device [66].

As with all precipitation methods, the challenge is to control the particle crystallization by avoiding crystal growth. Nucleation can be stopped by employing this "cavi-precipitation" technique, where the drug particles formed are immediately treated with a high-energy annealing process. The top-down step not only reduces the particle size but also stabilizes the drug nanocrystals with the energy application. Another advantage of the annealing step is that it promotes the more stable crystalline form [66]. See chapter 1.2.3.1 for more information about the annealing step. A drawback of this combinative process is that the resulting nanosuspensions contain organic solvent residues that need to be removed before further processing, just as with the NANOEDGE™ technology.

1.2.3.4 H 69 applications

Prednisolone was processed employing this combinative technology. The drug was dissolved in ethanol, mixed with demineralized water as a non-solvent for precipitation and then directly homogenized at high pressure. A mean particle size of 113 nm could be achieved after one minute of homogenization. These results improved to 27 nm after 5 min and to 22 nm after 6 min. Afterwards, the drug nanocrystals dissolved due to the increased dissolution pressure at these small particle sizes [66].

The drugs hydrocortisone acetate (HCA) and omeprazol were processed employing the H 69 process, achieving mean particle sizes of 787 nm and 921 nm, respectively after 20 cycles of homogenization at 1500 bar [66].

Ibuprofen and resveratrol are other examples of drugs processed with the H 69 technology. In the case of ibuprofen, best results were achieved when the drug was dissolved in tetrahydrofuran and then precipitated by adding demineralized water with surfactants. These drug particles then showed a mean particle size of about 10 μm. The drug crystals were immediately homogenized employing a Micron LAB 40 device (APV Gaulin, Germany) for 10 cycles at 1500 bar or the EmulsiFlex C5 homogenizer for 10 cycles at 1200 bar. The latter equipment produced the smallest ibuprofen nanocrystals, which presented a mean particle size of 170 nm. The EmulsiFlex C5 has the advantage that the precipitated drug particles can be directly homogenized at the high-energy zone of the device. Thus, it is possible to immediately stabilize the drug nanocrystals to ensure small particle sizes. In the case of resveratrol, best results were achieved by dissolving the drug in a DMSO/acetone mixture. The drug was then processed with the Avestin C5 as described for ibuprofen. A final mean

particle size of 150 nm could be achieved in the case of resveratrol. The reduction of the time between precipitation and the top-down step and the proper selection of the organic solvent to dissolve the drug prior to the precipitation step were identified as critical factors in obtaining the smallest drug nanocrystals [67].

1.2.3.5 H 42 technology

The H 42 process was developed by Möschwitzer, and it also belongs to the smartCrystal® technology platform. This combinative technology combines SD as a precipitation and pre-treatment step, followed by HPH for particle size reduction. The organic solvent is eliminated during the bottom-up step, which differentiates this technology from the NANOEDGE™ and H 69 processes. In the first unit operation (SD) the poorly soluble compound is dissolved in organic solvents. Surfactants such as poloxamer or sugars such as mannitol can be added to the drug solution to improve the results of the drying step. The solvent selection is critical to improve the performance of the process. The ideal organic solvent should possess good dissolving properties as well as suitable both boiling point and vapor pressure to ensure an efficient process and spray-dried powders free of solvent residues. Additionally, the selected solvent should ideally possess a low toxicity [24].

The objective of the drug modification by means of SD is to produce suitable, more breakable drug powders for the subsequent comminution process. The obtained spray-dried drug powders are then dispersed in aqueous media containing surfactants for stabilization purposes. The suspensions are further processed to nanosuspensions by employing the HPH technique, using homogenization equipment such as the Micron LAB 40 [68].

The H 42 combinative technology has advantages such as relatively short processing times during SD, solvent-free dry intermediates and small drug nanocrystals after a reduced number of HPH cycles. Its drawback is the employment of high temperatures during SD, which could make this technology unsuitable to process thermolabile compounds.

1.2.3.6 H 42 applications

In the first experiments, ibuprofen was processed employing this combinative technology. The drug was dissolved in ethanol and then spray-dried. The modified powders were then homogenized for 20 cycles at 1500 bar, reaching a mean particle size of 636 nm (original

value without modification: 1172 nm). The spray-dried ibuprofen powders showed almost no crystallinity change compared to the unprocessed material, which was confirmed by employing the DSC technique. The melting points and the normalized melting enthalpies of unmodified and spray-dried modified ibuprofen were compared and showed almost no difference. In this case, the improved reduction effectiveness was not linked to a change in the solid state behavior of the drug, but to the enhanced friability of the starting material [68].

Amphotericin B was also processed employing the H 42 technology. This model compound was dissolved in a 1:19 DMSO/methanol mixture and then spray-dried. The drug powders were homogenized at 1500 bar for 20 cycles using PEG 300 as a dispersion medium with the purpose of employing the nanosuspension to directly fill capsules. The process yielded a final mean particle size of 172 nm [68].

The model compound HCA was also processed employing the H 42 technology. The drug was dissolved in ethanol, with different amounts of poloxamer 188 being added to the drug solution. The spray-dried drug processed with a 9:1 drug/surfactant ratio brought the best particle size results, which were 281 nm after 20 homogenization cycles at 1500 bar. Additionally, this finely dispersed nanosuspension presented high storage stability. The micronized, unmodified HCA led to a final mean particle size of 551 nm under the same process conditions [23].

The improved drug structure of the best spray-dried powder was analyzed employing the SEM technique, which showed spherical drug particles. Further, the solid state behavior of the spray-dried powders was analyzed by using the PXRD technique. These results showed that the spray-dried powders stayed as crystalline as the unmodified drug material. The SD process did not modify the crystallinity of HCA. Small amounts of the surfactant positively impacted the characteristics of the spray-dried powders, such as flowability and millability. On the contrary, high surfactant amounts (i.e., 1:1 drug/surfactant ratio) negatively impacted the powders' characteristics and the subsequent particle size reduction effectiveness. The processing times could also be drastically reduced. When the best modified material was employed, only one cycle at 1500 bar was necessary to achieve smaller particle sizes than by homogenizing micronized drug material for 20 cycles [23].

Glibenclamide was further processed employing the H 42 technology. The influence of both surfactant and drug concentration during the bottom-up step was tested in this study. The effect of these parameters on the solid state behavior and morphology of the drug, as well as on the particle size reduction effectiveness of the top-down step, was analyzed. The DC of the

drug powders was established employing the DSC technique. It was discovered that the spray-dried glibenclamide powders showed, in general, a reduced crystallinity (DCs of between 20% and 30%) compared to the unmodified drug (100% DC). However, the drug solutions processed with medium and high drug concentrations (both sprayed with a 0.2% docusate sodium salt ethanolic solution) produced spray-dried powders with very low DCs: 8.1% and 8.3%. Both powders led after the homogenization step to nanosuspensions presenting mean particle sizes of about 236 nm, which were the best of all the results. Additionally, the SEM analysis of these glibenclamide samples revealed the formation of spherical drug particles. Both solid state modification leading to an amorphous drug and the morphology change due to the precipitation process positively impacted the particle size reduction effectiveness of the top down-step [24].

With the antioxidant compound resveratrol, experiments were also performed with the H 42 process employing a design of experiments. Resveratrol was dissolved in ethanol containing different amounts of the surfactant sodium cholate, and then the drug solutions were spray-dried for further homogenization. The best mean particle size obtained was 200 nm, which was improved compared to the 428 nm mean particle size obtained with unmodified resveratrol. Additionally, the amount of larger crystals was drastically reduced by employing the spray-dried modified drug instead of micronized material: from 2.2 μm (d90%) to 0.736 μm (d90%). Finally, the number of HPH cycles at 1500 bar necessary to achieve a proper nanosuspension could be reduced from 20 cycles with the standard method to only one cycle with the modified drug. This is one of the most important features of the H 42 technology. However, it was difficult to establish a link between the DC and the smallest drug particle sizes with resveratrol as the model drug [69].

1.2.3.7 H 96 technology

The H 96 combinative technology was developed by Möschwitzer and Lemke and belongs to the smartCrystal® technology family (Abbott/Soliqs, Germany). This process involves FD as a bottom-up and pre-treatment step, followed by HPH for particle size reduction. The bottom-up step eliminates the organic solvent content, just as with the H 42 technology. The FD step involves the dissolution of poorly soluble drugs employing organic solvents. The drug solution is then frozen (e.g., with instant freezing or snap-freezing) with liquid nitrogen and

further freeze-dried. The aim of the drug pre-treatment is to modify the starting material to improve the particle size reduction effectiveness of the HPH [6, 70].

The solvents need to be carefully selected to optimize the process and the characteristics of the freeze-dried powders. The critical solvent characteristics that determine the process performance are, among others, the freezing point, vapor pressure and toxicity. For FD purposes, it is important to employ organic solvents presenting relatively high freezing points. In this way, it is ensured that the solvent crystallizes completely during the lyophilization process. The selected solvent should also possess a high vapor pressure to ensure a complete elimination during the primary drying step. The complete removal of residues of organic solvents is necessary to ensure patient safety and product quality [39, 71].

Mixtures of organic solvents can also be implemented to improve the performance of the lyophilization process. For example, experiments using glibenclamide as a model compound had employed mixtures of DMSO and tert-butyl alcohol (TBA) for FD. DMSO dissolves the model compound but has a low vapor pressure, which resulted in low-quality freeze-dried powders (i.e., wet and sticky due to incomplete elimination of the solvent). TBA has both a high freezing point and vapor pressure, which makes it an ideal solvent for lyophilization purposes. DMSO contributed to the process with the necessary dissolving force for glibenclamide and TBA was added to the solution to improve the characteristics of the freeze-dried cakes [38, 71].

The H 96 technology is especially suitable to process thermolabile or expensive drugs due to the low temperatures and the high yields of the FD. Additionally, as the lyophilization step eliminates the organic solvent content, the subsequently produced nanosuspensions are ready to be further processed or used. Its drawback is the extension of the lyophilization step.

1.2.3.8 H 96 applications

During first experiments employing the H 96 technology, amphotericin B was dissolved in DMSO, snap-frozen with liquid nitrogen and then lyophilized. The freeze-dried drug powder was processed to a nanosuspension employing a Micron LAB 40 homogenizer for five cycles at 1500 bar producing drug nanocrystals of a 62 nm mean particle size. The snap-freezing or instant freezing with liquid nitrogen was necessary to achieve this very low particle size, as slowly freezing the drug solution resulted in bigger particle sizes after the top-down step (186 nm). In addition to the ultra-small particle size, the process became extremely cost-effective

by reducing the number of homogenization cycles from 20 with the standard technique to only one cycle at 1500 bar with the combinative technology [70]. In another study, human erythrocytes were loaded with an amphotericin B nanosuspension produced with the H 96 technology. The antifungal treatment could be improved due to the enhanced pharmacological profile of the amphotericin B nanocrystals [72, 73].

HCA was also processed as described for amphotericin B. After dissolving the drug in DMSO, the drug solution was snap-frozen with liquid nitrogen. The drug powder was processed to a nanosuspension employing the HPH technique for 10 cycles at 1500 bar. The final mean particle size was 414 nm for this drug nanosuspension.

Another drug processed with the H 96 process was cyclosporin A. This compound was dissolved in a 1:1 ethanol:DMSO mixture, freeze-dried employing the snap-freezing technique and further homogenized for 15 cycles at 1500 bar. A mean particle size of 440 nm was reported in this case [70].

Further experiments employing glibenclamide revealed a relationship between the crystallization conditions and the particle size reduction effectiveness of the top-down step. The different ratios of a DMSO-TBA mixture (90:10 to 10:90 v/v) and the drug concentration during the bottom-up process modified the solid state behavior of the drug as well as its morphology. The micronized and freeze-dried glibenclamide powders were analyzed with the DSC technique to determine their DC. The micronized glibenclamide possess a DC of 100% while most of the lyophilized powders showed DCs between 50% and 60%. However, when a design of experiments for the assessment of the critical crystallization factors was employed, it was found that solvent mixtures containing a high TBA proportion (i.e., DMSO:TBA:10:90 v/v) and a low drug concentration favored the formation of highly amorphous glibenclamide. This modified drug powder showed a DC of 1%. It was found that the H 96 technology is able to produce drug powders in either a crystalline or an amorphous state, depending on the process conditions and additives. Additionally, the process conditions modified the morphology of glibenclamide from a hard, rough structure to a fine, subtle and brittle structure, determined by the SEM technique. The drug modification by means of FD was advantageous for the HPH step. A mean particle size of 164 nm could be obtained under optimized conditions, which was markedly improved compared to the unmodified glibenclamide (772 nm). The homogenization length could also be reduced from 20 cycles to only one cycle, which was sufficient to produce a nanosuspension with a smaller particle size than after 20 cycles with the standard method [38].

In another study also employing glibenclamide as a model drug, the comminution effectiveness of the WBM and HPH processes when employing lyophilized drug as a starting material was compared. The FD solvent were DMSO:TBA mixtures prepared with solvent ratios of 90:10 to 10:90 (v/v). The drug concentration was kept constant at 5% for both top-down steps. Both methods were an improvement over the standard process using unmodified material. In the case of the WBM, the process time was reduced from 24 hours to only one hour to achieve a proper nanosuspension. Smaller particle sizes can be achieved much faster by modifying the drug structure. In the case of the HPH, the number of homogenization cycles was reduced from 20 to only five cycles to achieve a sufficiently small particle size. A mean particle size of 160 nm was reported employing WBM on modified material after 24 hours of processing. In addition, a mean particle size of 335 nm was obtained using HPH on a freeze-dried modified drug. Both methods benefited from the freeze-dried drug modification. However, the processes benefited from different drug characteristics. For the WBM process the higher friability and volume of the drug powders, which remained crystalline, was beneficial. For the homogenization process, the change in the drug crystal behavior from crystalline to amorphous to achieve smaller particle sizes was beneficial. This feature was confirmed by assessing the DC of the drug powders employing the DSC technique [39].

1.2.3.9 Combination technology (CT)

The CT technology is the only combinative process that does not employ organic solvents. The CT process combines a low-energy pearl milling step, followed by HPH for particle size reduction. The shear forces and particle collision are combined with the cavitation for an innovative particle size reduction process [41]. The pre-treatment of the drug involves the milling of its macrosuspension. This step achieves, in general, drug particle sizes between 600 nm and 1500 nm. The subsequent homogenization process improves the homogeneity of the nanosuspension by reducing the particle size and the amount of larger crystals. The latter feature also enhances the physical stability by avoiding crystal growth (Ostwald ripening), which improves the long-term stability of the drug nanosuspensions during storage [74]. Interestingly, it was reported that lower homogenization pressures (100-500 bar) resulted in smaller drug nanocrystals and more homogeneous nanosuspensions than higher homogenization pressures (1500 bar) after the pearl milling step [41]. The advantages of this technology are the reduction of the homogenization pressure and process length, as well as

the improved physical stability of the nanosuspensions. However, the CT process leads to particle sizes that are relatively bigger compared to the other combinative technologies.

1.2.3.10 CT applications

The flavonoid hesperidin is an example of a poorly soluble drug processed with the CT technology. A mean particle size of 599 nm was reported for hesperidin nanosuspensions, which also showed improved long-term stability [75]. Special features of the production of nanoparticulated hesperidin employing the CT process were the reduction of the homogenization cycles (from 20 to five) and of the necessary pressure (from 1500 bar to 1000 bar) to achieve a nanosuspension. In this manner, it is possible to reduce the energy input and the wearing of the machines [41].

Rutin and apigenin are also poorly soluble drugs processed with the CT technology. These drugs are flavonoids showing antioxidant properties with potential applications in pharmaceutical and cosmetic products. The first cosmetic product formulated employing nanotechnology contains rutin nanocrystals and was launched by Juvena, Switzerland [76]. Hesperidin nanocrystals can be found in the Platinum Rare cosmetic product (La Prairie, Switzerland) [21]. The topical route has been reported as full of potential for nanoparticulate applications, as the drug nanocrystals enhance the compound penetration to the skin. Furthermore, the CT technology produces drug nanosuspensions with increased stability again electrolytes. The electrolytes could lead to aggregation by reducing the zeta potential (i.e., the electrostatic repulsion) of the drug nanocrystals, thus producing the loss of their fast dissolution properties [41].

Employing apigenin, the CT technology led to a final mean particle size of 275 nm after only one homogenization cycle at 300 bar using an Avestin C50 homogenizer. The pearl-milled product presented a mean particle size of 412 nm, which was further reduced by the homogenization step. Interestingly, in this case lower pressures had an advantage in achieving smaller drug nanocrystals.

In the case of rutin, a suspension of the drug was pearl-milled with zirconium oxide beads (0.3 mm) to a mean particle size of about 1000 nm. This pre-milled suspension was then homogenized employing the Avestin C50 for one cycle at different pressures. The best mean particle size of 604 nm was achieved employing low pressure (100 bar).

An up-scaling with apigenin was also performed using this technology. The nanosuspension production could be scaled from a 20 g batch to a 3 kg batch. The milling process was performed using an agitating pearl mill Bühler PML 2 (Bühler AG, Switzerland) with zirconium oxide beads (bead size: 0.4-0.6 mm). The homogenization part of the CT process was performed employing the Avestin C50 for one cycle at 300 bar. The pre-milling step resulted in drug particles with a mean particle size of 413 nm, which remained constant after the homogenization process. However, the homogenization produced a narrowing of the particle size distribution, manifested through a decreasing PDI. This feature is critical to enhance the physical stability of the nanosuspensions. The particle size, crystallinity and physical stability of the nanosuspension was maintained when up-scaling the process, which is necessary for industrial production. Further, the CT technology could drastically reduce the number of homogenization cycles to just one, which is more cost-effective [77].

Table 1: Different drugs processed with the combinative particle size reduction technologies.

combinative technology	pre-treatment	particle size reduction technique	drug	smallest reported mean particle size	length of the nanosuspension production	administration focus	reference
NANOEDGE™	microprecipitation	HPH	paclitaxel	200 nm	20000 psi for 30 min (Avestin C50)	I.V. (re-formulation as nanosuspension to eliminate chremophor EL as excipient)	[57]
NANOEDGE™	microprecipitation	HPH	nabumetone	930 nm	20000 psi for 30 min (Avestin C50)	I.V.	[55]
NANOEDGE™	microprecipitation	HPH	prednisolone	640 nm	10000 psi for 15 min (Avestin C50)	I.V.	[55]
NANOEDGE™	microprecipitation	HPH	carbamazepin	400 nm	20000 psi for 15 min (Avestin C50)	I.V.	[55]
NANOEDGE™	microprecipitation	HPH	itraconazol	581 nm	20000 psi for 30 min (Avestin C50)	I.V.	[58]
NANOEDGE™	microprecipitation	sonication	itraconazol	177 nm	1 min 10000 Hz (400 W)	I.V.	[55]
"Nanoedge-like"	microprecipitation	HPH	meloxicam	212 nm	15 cycles 500 bar (ATS AH110D)	oral	[61]
"Nanoedge-like"	microprecipitation	HPH	isradipine	469 nm	30 cycles 1200 bar (GEA Niro Soavi)	oral	[60]
"Nanoedge-like"	microprecipitation	HPH	10-hydroxycamptothecin (10-HCPT)	131 nm	20 cycles 1000 bar (ATS AH110D)	oral	[59]
"Nanoedge-like"	microprecipitation	sonication	hydrocortisone	80 nm	5 min	oral	[64]
"Nanoedge-like"	microprecipitation	sonication	ibuprofen	702 nm	60 min	oral	[40]
"Nanoedge-like"	microprecipitation	sonication	nitrendipine	209 nm	15 min 20000 Hz (400 W)	oral	[62]
"Nanoedge-like"	microprecipitation	sonication	all-trans retinoic acid	155 nm	30 min	oral	[63]
"Nanoedge-like"	microprecipitation	sonication	meloxicam	259 nm	20 min (300 W)	oral	[61]
H 69	cavi-precipitation	HPH	ibuprofen	170 nm	10 cycles 1200 bar (Avestin C5)	oral	[67]
H 69	cavi-precipitation	HPH	hydrocortisone acetate (HCA)	787 nm	20 cycles 1500 bar (Micron LAB 40)	oral	[66]
H 69	cavi-precipitation	HPH	resveratrol	150 nm	10 cycles 1200 bar (Avestin C5)	oral	[67]
H 69	cavi-precipitation	HPH	omeprazol	921 nm	20 cycles 1500 bar (Micron LAB 40)	oral	[66]
H 69	cavi-precipitation	HPH	prednisolone	22 nm	1500 bar for 6 min (Micron LAB 40)	oral	[66]
H 42	spray-drying	HPH	amphotericin B	172 nm	20 cycles 1500 bar (Micron LAB 40)	oral	[68]
H 42	spray-drying	HPH	glibenclamide	236 nm	20 cycles 1500 bar (Micron LAB 40)	oral	[24]
H 42	spray-drying	HPH	hydrocortisone acetate (HCA)	281 nm	20 cycles 1500 bar (Micron LAB 40)	oral	[23]
H 42	spray-drying	HPH	ibuprofen	636 nm	20 cycles 1500 bar (Micron LAB 40)	oral	[68]
H 42	spray-drying	HPH	resveratrol	200 nm	1 cycle 1500 bar (Micron LAB 40)	oral	[69]
H 96	freeze-drying	HPH	amphotericin B	62 nm	5 cycles 1500 bar (Micron LAB 40)	oral	[70]
H 96	freeze-drying	HPH	glibenclamide	164 nm	20 cycles 1500 bar (Micron LAB 40)	oral	[38]
H 96	freeze-drying	HPH	cyclosporin A	440 nm	15 cycles 1500 bar (Micron LAB 40)	oral	[70]
H 96	freeze-drying	HPH	hydrocortisone acetate (HCA)	414 nm	10 cycles 1500 bar (Micron LAB 40)	oral	[70]
CT	pearl milling	HPH	rutin	604 nm	1 cycle 100 bar (Avestin C50)	topical/oral	[41]
CT	pearl milling	HPH	hesperidin	599 nm	5 cycles 1000 bar (Micron LAB 40)	topical/oral	[41]
CT	pearl milling	HPH	apigenin	275 nm	1 cycle 300 bar (Avestin C50)	topical/oral	[41]

2. DISCUSSION

New formulation technologies are the key to overcoming the increasing problem of poor aqueous solubility among emerging compounds [1]. The combinative particle size reduction processes have been presented as a part of the new enabling technologies. A schematic description of the standard particle size reduction processes (left side) and the combinative technologies (right side) is shown in Figure 2. Employing the combinative methods, the micronization step is replaced by a pre-treatment [23].
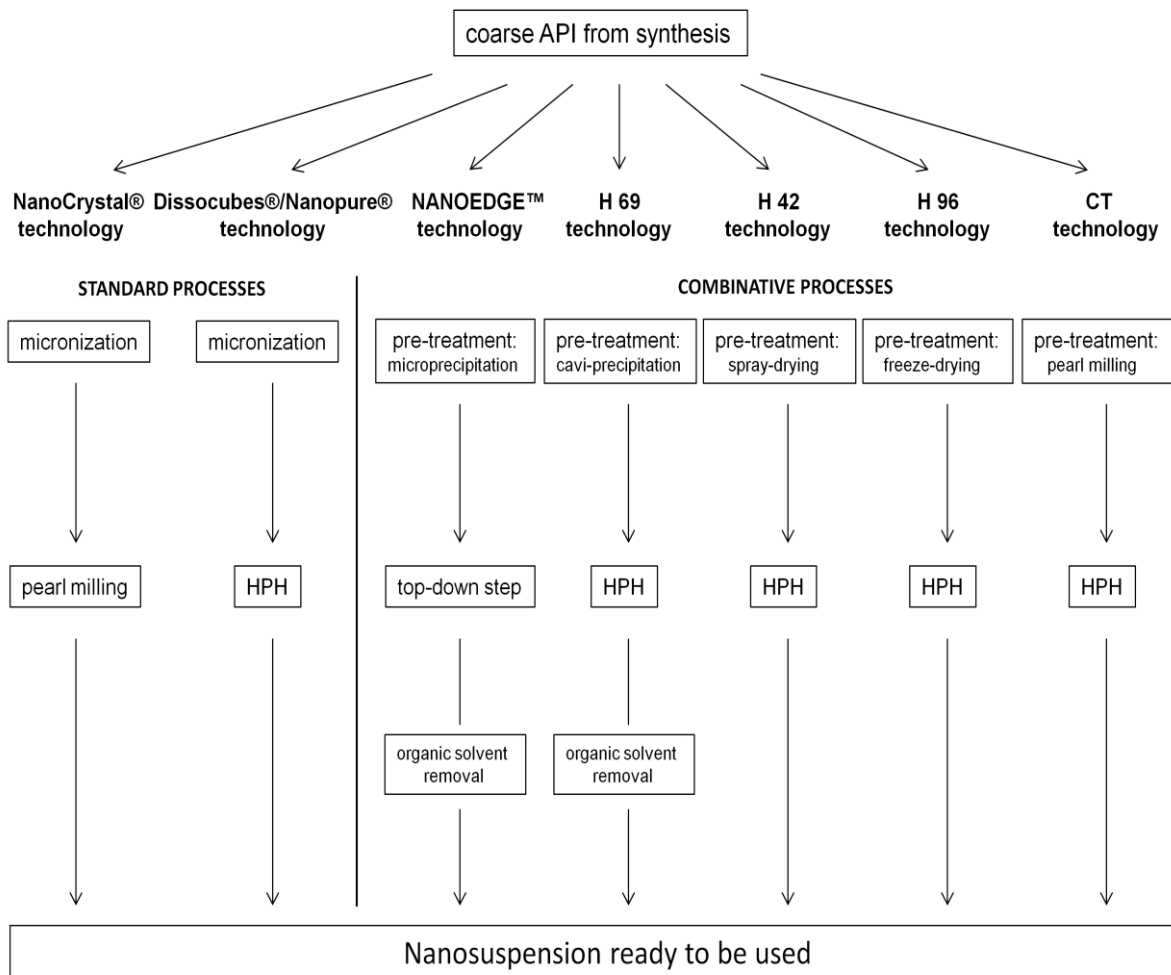
Figure 2: Schematic description of standard and combinative particle size reduction technologies.

2.1 Evaluation and comparison of the combinative particle size reduction technologies

Combinative processes such as the NANOEDGE™, H 69, H 42 and the H 96 technologies enable the direct processing of a drug solution after synthesis without previously performing a crystallization step. However, as the H 69 and NANOEDGE™ technologies involve the precipitation of particles in liquid media that usually contain organic solvents, these nanosuspensions are not ready to be used further (see Fig. 2). Extra drying steps need to be performed to eliminate the organic solvent content, which makes the process longer, more expensive and more complicated regarding regulatory aspects [6, 71]. On the contrary, when employing the H 42 and H 96 technologies, the organic solvent necessary to dissolve the poorly soluble drugs is eliminated during the bottom-up step. In this manner, the nanosuspensions produced with the dried intermediates can be directly used or downstreamed for the production of solid dosage forms [6, 21, 78].

A wide variety of drugs processed with the combinative technologies are shown in Table 1. These results were also described in the respective chapter of the combinative technologies. The NANOEDGE™ technology is the only process with a main focus on injectables (I.V. administration). The other technologies are focused on nanosizing for dissolution rate improvement for oral administration or formulation for topical administration. The formulation for cosmetic and nutraceutical applications, such as those discussed by Petersen in the CT technology patent, has also been successful [41].

When comparing the production length of the processes, the NANOEDGE™ and the H 69 techniques are relatively fast due to the rapid precipitation step. However, the organic solvent content of the nanosuspensions needs to be removed when employing these technologies, and so they lose the advantage of producing nanosuspensions in a fast process. The H 96 technology is more time-consuming due to the lengthy FD process. However, the lyophilization technique results in yields near to 100%, which is important in the case of expensive compounds. Additionally, the H 96 nanosuspensions do not contain amounts of organic solvents, which enable their direct usage after production. Finally, the H 42 technology produces nanosuspensions in a fast process. The SD is a rapid production step that can be performed in continuous mode. The H 42 nanosuspensions can also be subsequently directly processed or used, as they do not contain organic solvents.

Regarding the particle size reduction effectiveness, the H 96, H 69 and H 42 technologies are the processes achieving the smallest particle sizes for a variety of drugs (Table 1). Also, some

microprecipitation-high-energy approaches (NANOEDGE™ and "Nanoedge-like") led to small mean particle sizes.

By processing the same drug with different combinative techniques, it is possible to compare the reduction effectiveness and performance of the technologies. For example, amphotericin B and glibenclamide were both more effectively processed with the H 96 technology than with the H 42 process. In the case of amphotericin B, final mean particle sizes of 62 nm and 172 nm were achieved employing the H 96 and H 42 technologies, respectively [68, 70].

In the case of glibenclamide, final mean particle sizes of 164 nm and 236 nm were achieved employing the H 96 and H 42 technologies, respectively. The factors influencing the particle size reduction effectiveness were the porosity and the crystallinity of the drug powders. Both technologies produced drug powders with porous and brittle drug structures as well as with modified crystallinity. The H 96 technology produced, under optimized conditions, glibenclamide powders with 1% DC, which subsequently led to the low mean particle size of 164 nm. In comparison, the H 42 process led to glibenclamide powders with relatively higher DC (8.1%), which also resulted in relatively bigger particle sizes after the homogenization step (236 nm). However, both technologies achieved homogeneous dispersed nanosuspensions with a low particle size. Additionally, the H 42 technology had the advantage of being a much faster process [24, 38].

When comparing the H 42 and H 69 performances, the results are diverse. In the case of ibuprofen, better particle size results were achieved employing the H 69 process. These results were 170 nm with the H 69 process and 636 nm with the H 42 technology. However, the homogenizing equipment was different: the Avestin C5 for 10 cycles at 1200 bar in the case of the H 69 process and the Micron LAB 40 for 20 cycles at 1500 bar in the case of the H 42 process [67, 68]. Ibuprofen was also processed employing a microprecipitation-sonication technique. However, this approach led to bigger mean particle sizes than with the other combinative approaches (702 nm) [40].

With HCA as a model drug, the best results were achieved with the H 42 process with a final mean particle size of 281 nm after 20 cycles at 1500 bar. However, the H 96 process achieved a final mean particle size of 414 nm after 10 cycles at 1500 bar. Finally, the H 69 process produced with HCA a nanosuspension with a mean particle size of 787 nm after 20 cycles at 1500 bar. The latter result was considerably bigger than with the first two techniques [23, 66].

In the case of resveratrol as a model compound, the H 69 combinative processes led to a final mean particle size of 150 nm and the H 42 process achieved a final mean particle size of

200 nm. However, the homogenization conditions were different: the H 69 process was performed with an Avestin C5 for 10 cycles at 1200 bar and the H 42 technology was performed with a Micron LAB 40 for 1 cycle at 1500 bar [67, 69].

With meloxicam as model drug, a "Nanoedge-like" approach employing either sonication or HPH as the annealing step led to similar mean particle sizes (259 nm and 212 nm, respectively) [61].

In general, the particle size reduction effectiveness depends on several factors: the technology and equipment employed, as well as the physicochemical characteristics of the drug, such as solid state behavior, hardness, porosity and morphology. A technique that produces amorphous drugs and/or highly brittle, porous and friable structures can also lead to smaller particle sizes after the comminution step [38].

2.2 Comparison of the particle size reduction effectiveness and performance of combinative technologies with standard techniques

The particle size reduction performances of standard and selected combinative processes with glibenclamide as a model compound are compared in Figure 3. The graphic description shows the superior particle size reduction effectiveness of the combinative technologies regarding the process length to achieve a nanosuspension and the smallest final mean particle size. The HPH and WBM standard techniques achieved a final mean particle size of 772 nm and 191 nm at the end of their respective processes (after 20 cycles of HPH and 24 hours of WBM). However, these processes presented a slower particle size reduction progress than the combinative methods.

When the H 96 technology (black columns) was employed, the nanosuspension had a mean particle size of about 200 nm after one cycle of HPH. At this point, the standard HPH presented a mean particle size of 1417 nm. Additionally, the standard WBM presented a mean particle size of 840 nm after one hour of milling. This mean particle size result of 200 nm after only one cycle of HPH was markedly improved than with standard HPH (772 nm) and almost the same as standard WBM (191 nm) till the end of these processes. Finally, the H 96 process achieved a final mean particle size of 164 nm after 20 HPH cycles [38].

When the H 96 technology was employed with WBM as the top-down step (white columns), the nanosuspension had a mean particle size of 269 nm after only one hour of milling and

160 nm after 24 hours of processing. Both results were also markedly improved compared to the standard approaches [39].

In the case of processing glibenclamide with the H 42 technology (grey columns), the nanosuspension had a mean particle size of 384 nm after one cycle of HPH and a final mean particle size of 236 nm after 20 cycles of homogenization [78]. Again, these particle size results were improved compared to the standard techniques employing untreated drug material.

In general, the combinative particle size reduction processes perform faster than the standard methods to produce nanosuspensions and achieve smaller final mean particle sizes.
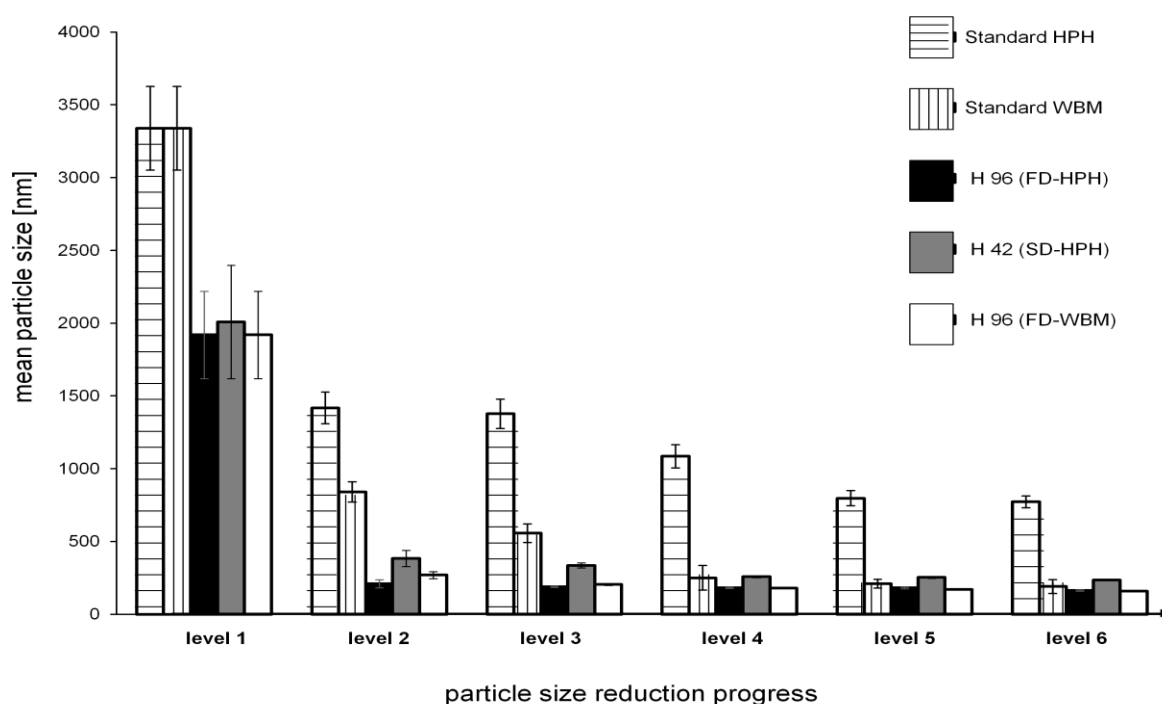
Figure 3: Particle size reduction performance of standard and combinative technologies. Six levels: pre-milling (1), 1 HPH cycle at 1500 bar/1 hour of WBM (2), 5 cycles/2 hours (3), 10 cycles/4 hours (4), 15 cycles/8 hours (5) and 20 cycles/24 hours (6).

Substantial parts of the introduction and discussion were submitted for publication as:

Salazar, Jaime; Müller, Rainer H.; Möschwitzer, Jan P., (2013), "Combinative particle size reduction technologies for the production of drug nanocrystals", Journal of Pharmaceutics.

3. AIM OF THE THESIS

The research objective of the present thesis was the systematical investigation of the combinative particle size reduction technologies H 96 and H 42 for the production of drug nanocrystals. The research was focused on the identification of the critical process parameters influencing the particle size reduction effectiveness of the combinative methods, and on the comparison of the effectiveness of different top-down techniques, including the standard comminution technologies. The optimization of the processes conditions was done by using design of experiment principles. Finally, the transfer of the drug nanocrystals from the liquid nanosuspension to tablets was investigated. This was based on nanosuspensions prepared with the H 96 and H 42 technologies, the results of which were compared with the products of standard high pressure homogenization.

The objective of **Publication 1**, which involved the H 96 process, was to identify critical process parameters during non-aqueous freeze-drying. The influence of the solvent composition during the bottom-up step on the particle size reduction effectiveness of the top-down step was investigated. The reduction effectiveness of employing untreated/treated material was analyzed. Both high pressure homogenization and wet bead milling were employed as the top-down step of the H 96 process to compare their reduction performances.

The experiments of **Publication 2** were performed in order to systematically investigate and identify optimal process parameters for the H 96 process. A two-factorial, five-level design of experiment was employed to determine the influence of the variables of drug concentration and organic solvent composition during freeze-drying on the particle size reduction effectiveness of the high pressure homogenization. Additionally, the reproducibility of the H 96 process was tested by employing freeze-dried drug powders produced under different bottom-up conditions.

Publication 3 was focused on the H 42 technology for nanosuspension production and its transfer to tablets. The influence of drug and surfactant concentrations during spray-drying on the particle size reduction effectiveness of the high pressure homogenization was analyzed. The use of different downstream methods and process conditions to obtain the drug nanocrystals in powder form was studied. The drug powders were compressed to immediate release tablets, which were further tested in a dissolution test to determine their drug release.

Finally, in **Publication 4**, the performances of the H 96 and H 42 technologies were compared for a complete assessment of the processes. The technological features that were compared included bottom-up conditions, additives, and yield, morphology and solid state modification due to the precipitation processes. The effect of the latter on particle size reduction, smallest achievable particle sizes, reproducibility and process length was also analyzed. In addition, tablets were prepared with H 96 and H 42 nanosuspensions to test their dissolution rate improvement.

4. PUBLICATIONS

4.1 "Nanocrystals: Comparison of the size reduction effectiveness of a novel combinative method with conventional top-down approaches"

Jaime Salazar [1]; Antoine Ghanem [2]; Rainer H. Müller [1]; Jan P. Möschwitzer [1,3]

[1] Institute of Pharmacy, Dept. of Pharmaceutics, Biopharmaceutics and NutriCosmetics, Freie Universität Berlin, Kelchstr. 31, 12169 Berlin, Germany

[2] Materials Development and Analysis Competence Centre, Solvay Research and Technology, Rue de Ransbeek 310, B-1120 Brussels, Belgium

[3] Pharmaceutical Development, Abbott GmbH & Co. KG, Knollstr. 50, 67061 Ludwigshafen am Rhein, Germany

The author of this work conceived and realized the experiments and the written reports independently. The SEM characterization was performed by the co-authors.

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

Jaime Salazar [1]; Oliver Heinzerling [2]; Rainer H. Müller [1]; Jan P. Möschwitzer [1, 2]

[1] Institute of Pharmacy, Dept. of Pharmaceutics, Biopharmaceutics and NutriCosmetics, Freie Universität Berlin, Kelchstr. 31, 12169 Berlin, Germany

[2] Pharmaceutical Development, Abbott GmbH & Co. KG, Knollstr. 50, 67061 Ludwigshafen am Rhein, Germany

The author of this work conceived and realized the experiments and the written reports independently. The SEM characterization and the DoE modeling were performed by the co-authors.

4.3 "Application of the combinative particle size reduction technology H 42 to produce fast dissolving glibenclamide tablets"

Jaime Salazar [1]; Rainer H. Müller [1]; Jan P. Möschwitzer [1,2]

[1] Institute of Pharmacy, Dept. of Pharmaceutics, Biopharmaceutics and NutriCosmetics, Freie Universität Berlin, Kelchstr. 31, 12169 Berlin, Germany

[2] Pharmaceutical Development, AbbVie Deutschland GmbH & Co. KG, Knollstr. 50, 67061 Ludwigshafen am Rhein, Germany

The author of this work conceived and realized the experiments and the written reports independently.

4.4 "Performance comparison of two novel particle size reduction technologies"

Jaime Salazar [1]; Rainer H. Müller [1]; Jan P. Möschwitzer [1,2]

[1] Institute of Pharmacy, Dept. of Pharmaceutics, Biopharmaceutics and NutriCosmetics, Freie Universität Berlin, Kelchstr. 31, 12169 Berlin, Germany

[2] Pharmaceutical Development, AbbVie Deutschland GmbH & Co. KG, Knollstr. 50, 67061 Ludwigshafen am Rhein, Germany

The author of this work conceived and realized the experiments and the written reports independently.

5. CONCLUSIONS

This thesis was able to demonstrate that combinative particle size reduction technologies can be used for the very efficient production of nanosuspensions. These novel techniques have been developed to circumvent the drawbacks of the standard techniques, and eventually lead to faster production and smaller particle sizes. The latter aspect has a direct impact on the dissolution rate and bioavailability of poorly soluble drugs after oral, topic and I.V. administration. More research needs to be performed to solve the technical challenges of the different technologies in order to achieve improved particle size reduction effectiveness and better formulations. In the future, it is expected that more screenings will be performed employing design of experiment principles to analyze systematically the critical factors influencing the production of drug nanosuspensions.

Publication 1 has shown that the composition of the organic solvent during freeze-drying has an influence on the porosity and on the solid state of the modified drug powders. Both factors affected particle size reduction. The use of porous drug powders that remained crystalline was beneficial for WBM. For HPH, it was beneficial to use drug powders that showed an amorphous behavior as well as high porosity. The H 96 process employing HPH or WBM as a top-down step performed markedly faster and more efficiently than the standard methods.

The design of experiment (DoE) realized in **Publication 2** revealed that both drug concentration and solvent composition during freeze-drying influenced particle size reduction effectiveness. Low drug concentrations and a high TBA proportion in the DMSO:TBA mixture resulted in highly amorphous, porous drug, and led to the smallest particle sizes after HPH. The usefulness of DoE for the optimization of the production of drug nanocrystals was confirmed. In addition, the H 96 technology led to reproducible results.

From the H 42 study realized in **Publication 3**, it can be concluded that both the surfactant and the drug concentration during spray-drying influence the particle size reduction effectiveness of the subsequent top-down step. Medium surfactant concentrations and high drug concentrations during spray-drying favored the formation of amorphous, brittle drug powders. This feature led to the smallest particle size results. A nanosuspension transfer to tablets that maintained the fast dissolution properties of the drug nanocrystals was successfully achieved by optimizing the downstream methods.

Finally, the performance comparison of the H 96 and H 42 technologies in **Publication 4** revealed that both processes impact the morphology and the solid state of the modified drugs in a manner dependent on the bottom-up conditions. Based on the example of glibenclamide, the H 96 process led to relatively smaller particle sizes and higher yields that are suitable for the processing of expensive and thermolabile drugs. However, the H 42 technology also led to sufficiently small particle sizes in a much faster process. It can therefore be concluded that both combinative technologies can be employed for the very efficient production of nanosuspensions.

6. SUMMARY

Poor aqueous solubility among new drugs emerging from synthesis and present in the development pipelines is an increasing problem for the pharmaceutical industry. Poorly soluble compounds show drawbacks such as poor oral bioavailability, food effects (fed/fasted variation), erratic absorption, non-linear pharmacokinetic profiles and high patient variability. Standard formulation approaches are, for example, salt formation, pH adjustment, solubilization with co-solvents, emulsions and molecular complexation. Although these approaches can be successfully applied for a variety of drugs, they are not sufficient to formulate new, challenging compounds.

Particle size reduction is a viable, non-specific approach for formulating poorly soluble drugs. The decrease in particle size results in an increase in surface area. The outcome is an increased rate of dissolution, which can lead to a better oral absorption for drugs showing a dissolution rate dependent oral bioavailability (Class II of the Biopharmaceutics Classification System). There are different production approaches for producing drug nanocrystals. The first process type produces drug nanocrystals by precipitating dissolved molecules. It is described as "bottom-up", as the size of the particles is increased. The second process type involves particle size reduction or comminution. It is referred to as being "top-down", as the size of the already existent particles is decreased. Standard techniques employed for the production of drug nanocrystals are the top-down processes of high pressure homogenization (HPH) and wet bead milling (WBM). These techniques have been already employed to formulate poorly soluble drugs successfully. However, these technologies still present drawbacks, including long processing times and the necessity to employ micronized drug as starting material. Therefore, the third approach, i.e., the combinative particle size reduction technique, has been developed in order to overcome these drawbacks and to improve the particle size reduction effectiveness of the standard processes. It involves combinations of bottom-up and/or top-down steps to improve the particle size reduction effectiveness of the single-unit processes. The first step is usually a bottom-up process employed as a drug pre-treatment in order to obtain a brittle, friable starting material for a subsequent comminution step. Nowadays, five combinative technologies are known: NANOEDGE™ (microprecipitation followed by a high-energy step such as HPH), H 69 (microprecipitation immediately followed by HPH, also called "cavi-precipitation"), H 96

(freeze-drying followed by HPH), H 42 (spray-drying followed by HPH) and the CT combinative technology (media milling followed by HPH). This thesis is focused on the H 96 and H 42 processes for the production of drug nanocrystals. These are promising technologies that show some very important features for the formulation of poorly soluble drugs, such as solvent-free products and very small particle sizes.

During screening experiments with the H 96 process, glibenclamide was employed to analyze the influence of the organic solvent composition during the freeze-drying step on the particle size reduction effectiveness of the subsequent top-down step. The modified drug powders were then processed employing WBM or HPH. The crystal habit of glibenclamide was modified by freeze-drying from dimethyl sulfoxide (DMSO)/tert-butanol (TBA) solvent mixtures using different ratios. The resulting drug powders were characterized by scanning electron microscopy, powder X-ray diffraction and differential scanning calorimetry. It was shown that the combinative approach can significantly improve the particle size reduction effectiveness of both top-down methods over conventional approaches. Drug lyophilization using DMSO:TBA in 25:75 and 10:90 (v/v) ratios resulted in a highly porous and breakable material. In addition, the 25:75 ratio produced amorphous drug, which was especially suitable for the HPH step. The milling time to achieve nanosuspensions was reduced from 24 hours with the jet-milled micronized glibenclamide to only one hour with the modified starting material. The number of homogenization cycles was decreased from 20 with unmodified glibenclamide to only 5 with the modified drug. The smallest mean particle size, achieved on modified samples, was 160 nm (d50% of 0.117 µm and d90% of 0.212 µm) by WBM after 24 hours and 355 nm (d50% of 0.503 µm and d90% of 1.908 µm) by HPH after 20 homogenization cycles at 1500 bar. The use of porous drug powders that remained crystalline was beneficial for WBM. For HPH, it was beneficial to use drug powders that showed an amorphous behavior alongside their high porosity.

The screening experiments with glibenclamide showed a relationship between the lyophilization conditions and the final particle size. Subsequently, a systematic investigation using a design of experiments (DoE) was conducted to identify optimal process parameters. The influence of the independent variables of drug concentration and organic solvent composition during freeze-drying was tested by conducting a two-factorial, five-level DoE. The model drug was dissolved in DMSO:TBA mixtures in different concentrations. It was then freeze-dried and subsequently homogenized. Low drug concentrations and a DMSO:TBA proportion of 10:90 (v/v) favored the formation of highly amorphous and brittle

drug, which was beneficial for the comminution effectiveness of HPH. Using these optimized process conditions, the particle size after 20 cycles was very small: 164 nm (mean particle size), 0.114 µm (d50%) and 0.209 µm (d90%). 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. On the contrary, for untreated, micronized glibenclamide, the particle size analysis revealed a mean particle size of 772 nm and volume-based size distribution values of 2.686 µm (d50%) and 14.423 µm (d90%). It was shown that the structure modification of the drug by means of freeze-drying can significantly improve the particle size reduction effectiveness of HPH. The study also confirmed the usefulness of DoE for the production of drug nanocrystals.

Next, the H 42 combinative technology was systematically analyzed. This process consists of a drug pre-treatment by means of spray-drying followed by standard HPH. During this research, spray-drying process parameters influencing the diminution effectiveness, such as drug and surfactant concentration, were systematically analyzed. The untreated and pre-treated drug powders were subsequently homogenized for 20 cycles at 1500 bar. The use of pre-treated material (10:1 glibenclamide/docusate sodium salt ratio spray-dried as ethanolic solution) was beneficial for the comminution process. These process conditions turned the crystalline drug into an amorphous compound, which resulted in a mean particle size of 236 nm and volume-based size distribution values of 0.131 µm (d50%) and 0.285 µm (d90%) after HPH. These results were markedly improved compared to the standard HPH process (results as mentioned above). The conditions during spray-drying affected the solid state behavior and the morphology of the drug, as well as the particle size reduction effectiveness of the HPH step. The H 42 combinative technology could be successfully applied in the production of small drug nanocrystals.

In the following step, solid dosage forms were prepared with the nanosuspensions. This is a critical part of the formulation of poorly soluble drugs, which is very important for, e.g., stability, compliance and marketing aspects. The nanosuspensions were transferred into immediate release tablet formulations. Wet granulation, freeze-drying and spray-drying were investigated as downstream methods to produce dry intermediates. In the case of the last two downstream techniques, mannitol was added to the nanosuspensions in different concentrations prior to the drying step to investigate its effect on the drug release. Regarding the dissolution rate, the rank order of the downstream processes was as follows: spray-drying > freeze-drying > wet granulation. The best drug release (90% within 10 minutes) was

obtained for tablets produced with spray-dried nanosuspension containing 2% mannitol as matrix former. In comparison, the tablets processed with micronized glibenclamide showed a drug release of only 26% after 10 min. A nanosuspension transfer to tablets that maintained the fast dissolution properties of the drug nanocrystals was successfully achieved. The key factors were the downstream technique employed and the mannitol concentration selected. It is critical to avoid agglomeration during the process to maintain the improved dissolution properties of the drug nanocrystals. Therefore, it is important to carefully screen the optimal downstream conditions to achieve the fast dissolution of the solid dosage forms.

Finally, for a complete evaluation of these combinative techniques, the characteristics and performances of the H 96 and H 42 technologies were compared. Technology features that were analyzed included bottom-up conditions and yield, morphology and solid state modification due to the precipitation processes. The latter's effect on particle size reduction, smallest achievable particle sizes, reproducibility and process length were also analyzed. Both combinative technologies employ a solvent elimination step as pre-treatment that modifies the morphology and the solid state behavior of the drug, in a manner dependent on the bottom-up conditions. In this way, is possible to obtain porous, brittle drug powders in either a crystalline or an amorphous state that are both highly suitable for a comminution step. The nanosuspensions produced with freeze-dried (low drug concentration, high TBA solvent composition) and spray-dried powders (high drug concentration, middle surfactant concentration) showed a very small particle size, narrow size distribution and reproducible results. The H 96 process led to relatively smaller mean particle sizes (164 nm) and higher yields (near 100%) that are suitable for the processing of expensive and also thermolabile drugs due to the low temperatures involved. On the other hand, the lyophilization step makes it a rather slow process. The H 42 technology leads to sufficiently small particle sizes (236 nm) in a much faster process. However, spray-drying is not suitable for thermolabile drugs and the lab-scale equipment gives low yields of about 50%. In addition, tablets prepared by wet granulation with H 96 and H 42 nanosuspensions led to drug releases of 80% after 10-15 minutes. On the contrary, tablets prepared with the standard nanosuspension achieved the same drug release after 70 min. The H 96 and H 42 tablets performed markedly better than the tablets prepared with the standard nanosuspension.

Both H 96 and H 42 techniques can be successfully employed for the formulation of poorly soluble drugs. These combinative particle size reduction technologies have improved the drawbacks of the standard techniques, leading to faster production and smaller particle sizes.

This study has shown that the combinative techniques enable the selective modification of the drug characteristics in terms of crystallinity and porosity to enhance the particle size reduction effectiveness of top-down steps. Thereby, it is possible to reach very small particle sizes.

The optimized transfer of the nanosuspensions to tablets could maintain the fast dissolution properties of the drug nanocrystals. The formulation of poorly soluble drugs can be successfully achieved by employing both H 96 and H 42 combinative technologies.

7. ZUSAMMENFASSUNG

Eine schlechte Wasserlöslichkeit von neuen Arzneistoffen stellt ein zunehmendes Problem für die Pharmaindustrie dar. Schwerlösliche Arzneistoffe zeigen häufig Nachteile wie z.B. schlechte orale Bioverfügbarkeit, Nahrungsmitteleffekte, erratische Absorption, ein nichtlineares pharmakokinetisches Profil, sowie große Schwankungen zwischen den Patienten. Standardverfahren zur Erhöhung der Bioverfügbarkeit sind z.B. Salzbildung, pH-Einstellung, Kosolventien, Emulsionen und molekulare Komplexbildung. Obwohl diese Verfahren bei einer Vielzahl von Arzneistoffen erfolgreich angewandt werden können, reichen sie nicht aus, um neue schwierige Verbindungen zu formulieren.

Partikelgrößenreduktion, speziell die Nanonisierung von Wirkstoffen, ist ein praktikabler und universeller Formulierungsansatz, um schwerlösliche Arzneistoffe zu verarbeiten. Die Abnahme der Partikelgröße hat eine Zunahme der Oberfläche zur Folge. Das Ergebnis ist eine höhere Auflösungeschwindigkeit. Dies kann zu einer besseren oralen Absorption führen, insbesondere bei Arzneistoffen, die eine Bioverfügbarkeit abhängig von der Auflösungsgeschwindigkeit zeigen (siehe Klasse II des biopharmazeutischen Klassifikationssystems).

Es gibt verschiedene Produktionsverfahren, um Arzneistoffnanokristalle herzustellen. Der erste Verfahrenstyp erzeugt Arzneistoffnanokristalle mittels Ausfällung gelöster Moleküle. Dieses aufbauende Verfahren wird aufgrund der Vergrößerung der Partikelgröße als „Bottom-up" bezeichnet. Im Gegensatz dazu gibt es auch abbauende Verfahren, bei denen eine Partikelgrößenreduktion erfolgt. Diese werden daher auch als „Top-down"-Verfahren bezeichnet. Hochdruckhomogenisation (*high pressure homogenization*, HPH) und Naßmahlung (*wet bead milling*, WBM) sind Standardtechniken, die für die Herstellung von Arzneistoffnanokristallen angewandt werden. Diese Techniken wurden bereits mehrfach erfolgreich angewandt, um schwerlösliche Arzneistoffe zu formulieren. Nichtsdestotrotz besitzen auch diese Technologien immer noch Nachteile, wie z.B. relative lange Prozeßzeiten sowie die Notwendigkeit der Verwendung von mikronisierten Arzneistoffen als Ausgangsmaterial. Um diese Nachteile zu adressieren, wurde ein dritter Verfahrenstyp entwickelt, der als kombiniertes Partikelgrößenreduktionsverfahren bezeichnet wird. Diese neuen Methoden kombinieren Bottom-up und/oder Top-down-Prozesse. Der erste Schritt ist normalerweise ein Bottom-up-Prozess, der als Arzneistoffvorbehandlung angewandt wird, um

ein sprödes, bröckeliges Ausgangsmaterial für den anschließenden Schritt der Zerkleinerung zu erhalten. Heute sind fünf kombinatorische Technologien bekannt: NANOEDGE™ (Mikropräzipitation gefolgt von einem hochenergetischen Schritt wie HPH), H 69 (Mikropräzipitation unmittelbar gefolgt von HPH, auch *cavi-precipitation* genannt), H 96 (Gefriertrocknung gefolgt von HPH), H 42 (Sprühtrocknung gefolgt von HPH) und die CT kombinatorische Technologie (Nassmahlung gefolgt von HPH).

Diese Dissertation beschäftigt sich insbesondere mit der systematischen Untersuchung der H 96 und H 42 Technologien zur Herstellung von Glibenclamid-Nanokristallen. Beides sind vielversprechende Herstellungsmethoden für die Formulierung schwerlöslicher Arzneistoffe, die z.B. die Möglichkeit bieten, hoch effizient sehr kleine Partikel herstellen zu können.

Zunächst wurden Screening-Experimente mit dem H 96-Prozesses durchgeführt. Dabei wurde der Einfluss der Zusammensetzung des organischen Lösemittels während der Gefriertrocknung auf die Effektivität der Partikelgrößenreduktion des anschließenden Top-down-Vorgangs hin analysiert. Die modifizierten, pulverförmigen Arzneistoffe wurden dann per WBM oder HPH weiter prozessiert. Der Kristallhabitus des Glibenclamid wurde durch Gefriertrocknung aus Dimethylsulfoxid (DMSO)/tert-Butanol (TBA) Lösemittel-Mischungen unter Verwendung verschiedener Mischungsverhältnisse modifiziert. Die daraus resultierenden, pulverförmigen Arzneistoffe wurden durch Rasterelektronenmikroskopie sowie mit Röntgendiffraktometrie und Dynamischer Differenzkalorimetrie charakterisiert. Im Vergleich zu den Standardverfahren zeigte sich, dass durch die Anwendung von dem kombinatorischen Verfahren die Effektivität der Partikelgrößenreduktion beider Top-down-Methoden signifikant verbessert wurde. Die Arzneistoff-Gefriertrocknung unter Verwendung von DMSO:TBA im Verhältnis 25:75 und 10:90 (v/v) resultierte in einem hoch-porösen und -brechbaren Material. Außerdem wurde mit dem 25:75-Verhältnis ein amorpher Arzneistoff produziert, der speziell für den HPH-Schritt geeignet war. Die Mahldauer zur Erreichung von Nanosuspensionen wurde von 24 Stunden mit mikronisierten Glibenclamid auf eine Stunde mit dem modifizierten Ausgangsmaterial reduziert. Die Zahl der Homogenisationszyklen wurde von 20 mit dem nicht-modifizierten Glibenclamid auf 5 mit dem modifizierten Arzneistoff reduziert. Die kleinste durchschnittliche Partikelgröße, erzielt mit dem modifizierten Arzneistoff, war 160 nm (d50%-Wert von 0.117 µm und d90%-Wert von 0.212 µm) durch WBM nach 24 Stunden und 355 nm (d50%-Wert von 0.503 µm und d90%-Wert von 1.908 µm) durch HPH nach 20 Homogenisationszyklen bei 1500 bar. Für WBM war es vorteilhaft poröse, pulverförmige und kristalline Arzneistoffe zu verwenden. Für HPH war die

Verwendung von pulverförmigen Arzneistoffen, die neben einer hohen Porosität auch eine reduzierte Kristallinität aufwiesen, von Vorteil.

Die Screening-Experimente mit Glibenclamid zeigten einen Zusammenhang zwischen den Bedingungen der Gefriertrocknung und der final erzielbaren Partikelgröße. Deshalb wurde anschließend eine systematische Untersuchung unter Verwendung einer statistischen Versuchsplanung (*design of experiment*, DoE) durchgeführt, um die optimalen Prozess-Parameter zu identifizieren. Der Einfluss der unabhängigen Variablen, Arzneistoff-Konzentration und Zusammensetzung des organischen Lösemittels während der Gefriertrocknung, wurde anhand einer zweifaktoriellen Versuchsplanung mit fünf Stufen getestet. Glibenclamid wurde in DMSO:TBA-Mischungen verschiedener Konzentrationen aufgelöst, gefriergetrocknet und anschließend homogenisiert. Niedrige Arzneistoff-Konzentrationen und ein DMSO:TBA-Verhältnis von 10:90 (v/v) begünstigten die Bildung eines hoch-amorphen und -brüchigen Mittels, das vorteilhaft für die Zerkleinerungseffektivität des HPH war. Die Verwendung dieser optimierten Prozessbedingungen führte zu einer sehr kleinen Partikelgröße nach 20 Zyklen: 164 nm (mittlere Partikelgröße), 0,114 µm (d50%-Wert) und 0.209 µm (d90%-Wert). Allerdings konnten auch überraschend gute Partikelgrößenergebnisse erzielt werden, wenn der Modifikationsschritt hoch poröses und kristallines Glibenclamid hervorbrachte. Daher ist Porosität offenbar ein wichtiger Parameter.

Im Gegensatz dazu kam es bei unbehandeltem mikronisierten Glibenclamid zu einer durchschnittlichen Partikelgröße von 772 nm und eine volumenbasierte Größenverteilung von 2,686 µm (d50%) und 14,423 µm (d90%). Damit wurde bewiesen, dass die Strukturveränderung des Arzneistoffes durch Gefriertrocknung die Effektivität der Partikelgrößenreduktion des HPH deutlich verbessern kann. Die Studie konnte außerdem die Nützlichkeit des DoE für die Produktion von Arzneistoffnanokristallen bestätigen.

In darauf folgenden Versuchen wurde die H 42 Technologie systematisch untersucht. Dieser Prozess besteht aus einer Arzneistoff-Vorbehandlung mittels Sprühtrocknung, gefolgt von HPH. Im Rahmen dieser Untersuchung wurden die Parameter des Sprühtrocknungsprozesses, die die Effektivität der Partikelgrößenreduktion beeinflussen, so wie Arzneistoff- und Tensidkonzentration, systematisch analysiert. Die unbehandelten und vorbehandelten Arzneistoffpulver wurden anschließend homogenisiert mit 20 Zyklen bei 1500 bar. Die Verwendung vorbehandelten Materials (10:1 Glibenclamid/Tensid Verhältnis, sprühgetrocknet als ethanolische Lösung) erwies sich als förderlich für den

Zerkleinerungsprozess. Unter diesen Prozessbedingungen kam es zur Bildung von amorphem Glibenclamid, welcher in einer durchschnittlichen Partikelgröße von 236 nm und einer volumenbasierten Größenverteilung von 0,131 µm (d50%) und 0,285 µm (d90%) nach HPH resultierte. Diese Ergebnisse bedeuten eine entscheidende Verbesserung gegenüber dem Standard-HPH-Prozess (siehe oben). Die Bedingungen während der Sprühtrocknung beeinflussten die Kristallinität und die Morphologie des Arzneistoffes sowie die Effektivität der Partikelgrößenreduktion des HPH-Prozesses. Die H 42-Technologie konnte somit erfolgreich zur Herstellung von Glibenclamid-Nanokristallen angewendet werden.

Einen weiteren Untersuchungsschwerpunkt stellte die Herstellung von Tabletten dar, die mit Glibenclamid-Nanokristallen beladen waren. Dies ist ein kritischer Punkt bei der Formulierung schwerlöslicher Arzneistoffe, welcher sehr wichtig zum Beispiel für Stabilität und Compliance ist. Feuchtgranulierung, Gefriertrocknung und Sprühtrocknung wurden als Trocknungsmethoden untersucht, um pulverförmige Zwischenprodukte zu erhalten. Im Falle der letzten beiden Trocknungstechniken wurde der Nanosuspension vor dem Trocknungsprozess Mannitol in verschiedenen Konzentrationen hinzugefügt, um den Einfluss des Hilfsstoffes auf die Arzneistofffreisetzung zu untersuchen. In Bezug auf die Freisetzung konnte die folgende Reihenfolge identifiziert werden: Sprühtrocknung > Gefriertrocknung > Feuchtgranulierung. Die beste Arzneistofffreisetzung (90% innerhalb von 10 Minuten) wurde mit Tabletten aus sprühgetrockneter Nanosuspension mit 2% Mannitol als Matrixformer erzielt. Im Vergleich dazu zeigten die Tabletten, die mit mikronisiertem Glibenclamid prozessiert worden waren, nur 26% Arzneistofffreisetzung nach 10 Minuten. Der Transfer von den Nanosuspensionen zu Tabletten, die die schnelle Auflösung der Nanokristalle behielten, war demzufolge ebenfalls erfolgreich. Die entscheidenden Faktoren waren sowohl die angewendeten Downstream-Techniken, als auch die gewählte Mannitol-Konzentration. Es ist entscheidend, eine Agglomeration während des Prozesses zu verhindern, um die verbesserten Auflösungseigenschaften der Arzneistoffnanokristalle beizubehalten. Deshalb ist es wichtig, die optimalen Downstream-Bedingungen vorsichtig zu überprüfen, um eine schnelle Freisetzung zu erreichen.

Abschließend wurden die Kombinations-Technologien und die damit erzielbaren Ergebnisse noch einmal direkt miteinander verglichen. Technologische Eigenschaften, die analysiert wurden, beinhalteten Bottom-up-Konditionen und -Erträge, die Morphologie und Festkörpermodifikation aufgrund der Ausfällungsprozesse und den Effekt auf die Partikelgrößenreduktion, der kleinsten zu erreichenden Partikelgröße, Reproduzierbarkeit und

Prozesslänge. Beide kombinatorischen Techniken verwenden einen Trocknungsschritt als Vorbehandlung, der die Morphologie und das Verhalten des Arzneistoffes im festen Zustand in Abhängigkeit von den Bottom-up-Bedingungen modifiziert. Auf diese Weise ist es möglich, poröse, brüchige pulverförmige Arzneistoffe entweder in kristallinem oder in amorphem Zustand zu gewinnen, die hochgeeignet für einen Zerkleinerungsschritt sind. Die Nanosuspensionen, die mit gefriergetrockneten (niedrige Arzneimittelkonzentration, hohe TBA Zusammensetzung) und sprühgetrockneten (hohe Arzneimittelkonzentration, mittlere Tensidkonzentration) Pulvern hergestellt wurden, wiesen eine sehr kleine Partikelgröße, eine enge Größenverteilung und reproduzierbare Ergebnisse auf. Der H 96-Prozess führte zu verhältnismäßig kleineren Partikelgrößen (164 nm) und höheren Erträgen (beinahe 100%), die aufgrund der niedrigen Temperaturen geeignet sind, teure und auch thermolabile Arzneimittel zu verarbeiten. Negativ ist aufgrund des Gefriertrocknungsschrittes, dass die H 96 Technologie zu einem eher langsamen und teuren Prozess wird. Die H 42-Methode führt zu ausreichend kleinen Partikelgrößen (236 nm) in einem viel schnelleren Prozess. Nachteilhaft an der Sprühtrocknung ist, dass die Laborausstattung nur niedrige Erträge von etwa 50% abwirft. Des Weiteren könnte es sein, dass sie nicht für thermolabile Arzneistoffe geeignet ist. Außerdem führten Tabletten, die durch Feuchtgranulierung von Nanosuspensionen aus dem H 96- bzw. dem H 42-Prozess hergestellt wurden, zu Arzneistofffreisetzungen von 80% nach 10-15 Minuten. Im Gegensatz dazu erreichten Tabletten, die mit der Standard-Nanosuspension hergestellt wurden, die gleiche Arzneistofffreisetzung nach 70 Minuten. Die H 96- und H 42-Tabletten setzten den Wirkstoff also wesentlich schneller frei als die, die mit der gewöhnlichen Nanosuspension erzeugt wurden.

Die Verwendung sowohl der H 96- als auch der H 42-Methode konnte erfolgreich zur Formulierung des schwerlöslichen Arzneistoffes Glibenclamid eingesetzt werden. Diese kombinatorischen Partikelgrößenreduktionstechnologien haben die Nachteile der üblichen Methoden ausgeglichen, was eine optimierte Produktion sowie kleinere Partikelgrößen zur Folge hat. Diese Arbeit hat gezeigt, dass die kombinatorischen Methoden die Modifikation der Arzneistoffeigenschaften in Hinblick auf Kristallinität und Porosität ermöglichen, um die Effektivität der Partikelgrößenreduktion von Top-down-Schritten zu steigern. Auf diese Weise ist es möglich, sehr kleine Partikelgrößen zu erhalten. Der optimierte Transfer der Nanosuspensionen zu Tabletten konnte die schnelle Auflösung der Arzneistoffnanokristalle bewahren.

Zusammenfassend kann festgestellt werden, dass beide kombinatorische Technologien erfolgreich für die Formulierung schwerlöslicher Arzneistoffe verwendet werden können.

8. REFERENCES

1. Chaubal, M.V., *Application of formulation technologies in lead candidate selection and optimization.* Drug Discov. Today, 2004. **9**: p. 603-609.
2. Wong, J., et al., *Suspensions for intravenous (IV) injection: A review of development, preclinical and clinical aspects.* Adv. Drug Deliver. Rev., 2008. **60**(8): p. 939-954.
3. Lipinski, C.A., et al., *Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings.* Adv. Drug Deliver. Rev., 1997. **23**(1-3): p. 3-25.
4. Heimbach, T., D. Fleisher, and A. Kaddoumi, *Overcoming Poor Aqueous Solubility of Drugs for Oral Delivery*, V.J. Stella, et al., Editors. 2007, Springer New York. p. 157-215.
5. Merisko-Liversidge, E.M. and G.G. Liversidge, *Drug nanoparticles: formulating poorly water-soluble compounds.* Toxicol. Pathol., 2008. **36**(1): p. 43-8.
6. Shegokar, R. and R.H. Müller, *Nanocrystals: Industrially feasible multifunctional formulation technology for poorly soluble actives.* Int. J. Pharm., 2010. **399**(1-2): p. 129-139.
7. Merisko-Liversidge, E., G.G. Liversidge, and E.R. Cooper, *Nanosizing: a formulation approach for poorly-water-soluble compounds.* Eur. J. Pharm. Sci., 2003. **18**(2): p. 113-120.
8. DiMasi, J.A., R.W. Hansen, and H.G. Grabowski, *The price of innovation: new estimates of drug development costs.* J. Health Econ., 2003. **22**(2): p. 151-185.
9. Rabinow, B.E., *Nanosuspensions in drug delivery.* Nat. Rev. Drug Discov., 2004. **3**(9): p. 785-796.
10. Li, P., L. Zhao, and S.H. Yalkowsky, *Combined effect of cosolvent and cyclodextrin on solubilization of nonpolar drugs.* J. Pharm. Sci., 1999. **88**(11): p. 1107-1111.
11. Weiner, M. and L. Bernstein, *Adverse reactions to drug formulation agents: a handbook of excipients / Murray Weiner, Leonard Bernstein*. 1989, New York: Marcel Dekker.
12. Leuner, C. and J. Dressman, *Improving drug solubility for oral delivery using solid dispersions.* Eur. J. Pharm. Biopharm., 2000. **50**(1): p. 47-60.
13. Breitenbach, J., *Melt extrusion: from process to drug delivery technology.* Eur. J. Pharm. Biopharm., 2002. **54**(2): p. 107-117.
14. Janssens, S. and G. Van den Mooter, *Review: physical chemistry of solid dispersions.* J. Pharm. Pharmacol., 2009. **61**(12): p. 1571-1586.
15. Porter, C.J.H. and W.N. Charman, *In vitro assessment of oral lipid based formulations.* Adv. Drug Deliver. Rev., 2001. **50, Supplement 1**(0): p. S127-S147.
16. Müller, R.H., K. Mäder, and S. Gohla, *Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art.* Eur. J. Pharm. Biopharm., 2000. **50**(1): p. 161-177.
17. Müller, R.H., M. Radtke, and S.A. Wissing, *Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations.* Adv. Drug Deliver. Rev., 2002. **54, Supplement**(0): p. S131-S155.
18. Müller, R.H., C. Jacobs, and O. Kayser, *Nanosuspensions as particulate drug formulations in therapy: Rationale for development and what we can expect for the future.* Adv. Drug Deliver. Rev., 2001. **47**(1): p. 3-19.

19. Müller, R.H. and K. Peters, *Nanosuspensions for the formulation of poorly soluble drugs: I. Preparation by a size-reduction technique.* Int. J. Pharm., 1998. **160**(2): p. 229-237.

20. Kesisoglou, F., S. Panmai, and Y. Wu, *Nanosizing — Oral formulation development and biopharmaceutical evaluation.* Adv. Drug Deliver. Rev., 2007. **59**(7): p. 631-644.

21. Müller, R.H., S. Gohla, and C.M. Keck, *State of the art of nanocrystals - Special features, production, nanotoxicology aspects and intracellular delivery.* Eur. J. Pharm. Biopharm., 2011. **78**(1): p. 1-9.

22. Van Eerdenbrugh, B., G. Van den Mooter, and P. Augustijns, *Top-down production of drug nanocrystals: Nanosuspension stabilization, miniaturization and transformation into solid products.* Int. J. Pharm., 2008. **364**(1): p. 64-75.

23. Möschwitzer, J. and R.H. Müller, *New method for the effective production of ultrafine drug nanocrystals.* J. Nanosci. Nanotechno., 2006. **6**(9-10): p. 3145-53.

24. Salazar, J., J. Möschwitzer, and R.H. Müller, *Performance comparison of two novel combinative particle size reduction technologies. Accepted for publication.* J. Pharm. Sci., 2013.

25. Hancock, B.C. and G. Zografi, *Characteristics and significance of the amorphous state in pharmaceutical systems.* J. Pharm. Sci., 1997. **86**(1): p. 1-12.

26. Noyes, A.A. and W.R. Whitney, *The rate of solution of solid substances in their own solutions.* J. Am. Chem. Soc. , 1897. **19 (12)**(12): p. 930-934.

27. Van Eerdenbrugh, B., et al., *Solubility Increases Associated with Crystalline Drug Nanoparticles: Methodologies and Significance.* Mol. Pharmaceut., 2010. **7**(5): p. 1858-1870.

28. Wu, W. and G.H. Nancollas, *A New Understanding of the Relationship Between Solubility and Particle Size.* J. Solution Chem., 1998. **27**(6): p. 521-531.

29. Shchekin, A.K. and A.I. Rusanov, *Generalization of the Gibbs-Kelvin-Köhler and Ostwald-Freundlich equations for a liquid film on a soluble nanoparticle.* J. Chem. Phys., 2008. **129, 154116**: p. 1-5.

30. Kipp, J.E., *The role of solid nanoparticle technology in the parenteral delivery of poorly water-soluble drugs.* Int. J. Pharm., 2004. **284**(1–2): p. 109-122.

31. Jinno, J., et al., *Effect of particle size reduction on dissolution and oral absorption of a poorly water-soluble drug, cilostazol, in beagle dogs.* J. Control. Release, 2006. **111**(1-2): p. 56-64.

32. Yu, L.X., et al., *Biopharmaceutics Classification System: The Scientific Basis for Biowaiver Extensions.* Pharm. Res., 2002. **19**(7): p. 921-925.

33. Galia, E., et al., *Evaluation of Various Dissolution Media for Predicting In Vivo Performance of Class I and II Drugs.* Pharm. Res., 1998. **15**(5): p. 698-705.

34. Keck, C.M. and R.H. Müller, *Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation.* Eur. J. Pharm. Biopharm., 2006. **62**(1): p. 3-16.

35. Müller, R.H. and C. Jacobs, *Buparvaquone mucoadhesive nanosuspension: preparation, optimisation and long-term stability.* Int. J. Pharm., 2002. **237**(1–2): p. 151-161.

36. Sebhatu, T., M. Angberg, and C. Ahlneck, *Assessment of the degree of disorder in crystalline solids by isothermal microcalorimetry.* Int. J. Pharm., 1994. **104**(2): p. 135-144.

37. Jonat, S., et al., *Mechanism of glidants: Investigation of the effect of different colloidal silicon dioxide types on powder flow by atomic force and scanning electron microscopy.* J. Pharm. Sci., 2004. **93**(10): p. 2635-2644.

38. Salazar, J., et al., *Process optimization of a novel production method for nanosuspensions using design of experiments (DoE).* Int. J. Pharm., 2011. **420**(2): p. 395-403.

39. Salazar, J., et al., *Nanocrystals: Comparison of the size reduction effectiveness of a novel combinative method with conventional top-down approaches.* Eur. J. Pharm. Biopharm., 2012. **81**(1): p. 82-90.

40. Verma, S., R. Gokhale, and D.J. Burgess, *A comparative study of top-down and bottom-up approaches for the preparation of micro/nanosuspensions.* Int. J. Pharm., 2009. **380**(1–2): p. 216-222.

41. Petersen, R., *Nanocrystals for use in topical cosmetic formulations and method of production thereof. US patent application No. 60/8866,233.* 2006.

42. Sucker, H. and P. Gassmann, *Improvements in pharmaceutical compositions, European patent 0580690.* 1992.

43. List, M. and H. Sucker, *Pharmaceutical colloidal hydrosols for injection, GB Patent 2200048.* 1988.

44. Auweter, H., et al., *Precipitated water-insoluble colorants in colloid disperse form, US patent 6494924.* 2002.

45. Auweter, H., et al., *The function of gelatin in controlled precipitation processes of nanosize particles.* J. Dispersion Sci. Technol., 1998. **19**(2-3): p. 163-184.

46. de Waard, H., W.L.J. Hinrichs, and H.W. Frijlink, *A novel bottom-up process to produce drug nanocrystals: Controlled crystallization during freeze-drying.* J. Control. Release, 2008. **128**(2): p. 179-183.

47. de Waard, H., et al., *Preparation of drug nanocrystals by controlled crystallization: Application of a 3-way nozzle to prevent premature crystallization for large scale production.* Eur. J. Pharm. Sci., 2009. **38**(3): p. 224-229.

48. Liversidge, G.G., et al., *Surface modified drug nanoparticles, US Patent 5145684.* 1992.

49. Peltonen, L. and J. Hirvonen, *Pharmaceutical nanocrystals by nanomilling: critical process parameters, particle fracturing and stabilization methods.* J. Pharm. Pharmacol., 2010. **62**(11): p. 1569-1579.

50. Merisko-Liversidge, E. and G.G. Liversidge, *Nanosizing for oral and parenteral drug delivery: A perspective on formulating poorly-water soluble compounds using wet media milling technology.* Adv. Drug Deliver. Rev., 2011. **63**(6): p. 427-440.

51. Müller, R.H., K. Mäder, and K. Krause, *Verfahren zur schonenden Herstellung von hochfeinen Micro-/Nanopartikeln, in: PCT Application PCT/EP00/06535.* 2000: Germany.

52. Khan, S. and G.W. Pace, *Composition and method of preparing microparticles of water-insoluble substances.* 2002: US Patent 6337092.

53. Majuru, S. and M.O. Oyewumi, *Nanotechnology in Drug Development and Life Cycle Management*, M.M. Villiers, P. Aramwit, and G.S. Kwon, Editors. 2009, Springer New York. p. 597-619.

54. Kipp, J.E.W., et al., *Microprecipitation method for preparing submicron suspensions. US patent 6869617.* 2001.

55. Kipp, J.E., et al., *Microprecipitation method for preparing submicron nanosuspensions. US Patent 6607784 B2.* 2003.

56. Kipp, J.E., et al., *Method for preparing submicron particle suspensions, US Patent 6884436.* 2005.

57. Rabinow, B.E., et al., *Formulation of water-insoluble antineoplastic agents as nanosuspensions using NANOEDGE formulation technology.* AACR Meeting Abstracts, 2004(1): p. 146-a-.

58. Rabinow, B., et al., *Itraconazole IV nanosuspension enhances efficacy through altered pharmacokinetics in the rat.* Int. J. Pharm., 2007. **339**(1-2): p. 251-60.

59. Pu, X., et al., *Development of a chemically stable 10-hydroxycamptothecin nanosuspensions.* Int. J. Pharm., 2009. **379**(1): p. 167-173.

60. Shelar, D., S. Pawar, and P. Vavia, *Fabrication of isradipine nanosuspension by anti-solvent microprecipitation–high-pressure homogenization method for enhancing dissolution rate and oral bioavailability.* Drug Deliv. and Transl. Res., 2012: p. 1-8.

61. Raval, A.J. and M.M. Patel, *Preparation and Characterization of Nanoparticles for Solubility and Dissolution Rate Enhancement of Meloxicam* Intl. R. J. of Pharmaceuticals, 2011. **01**(02): p. 42-49.

62. Xia, D., et al., *Preparation of stable nitrendipine nanosuspensions using the precipitation–ultrasonication method for enhancement of dissolution and oral bioavailability.* Eur. J. Pharm. Sci., 2010. **40**(4): p. 325-334.

63. Zhang, X., Q. Xia, and N. Gu, *Preparation of All-Trans Retinoic Acid Nanosuspensions Using a Modified Precipitation Method.* Drug Dev. Ind. Pharm., 2006. **32**(7): p. 857-863.

64. Ali, H.S.M., P. York, and N. Blagden, *Preparation of hydrocortisone nanosuspension through a bottom-up nanoprecipitation technique using microfluidic reactors.* Int. J. Pharm., 2009. **375**(1–2): p. 107-113.

65. Ali, H.S.M., et al., *Hydrocortisone nanosuspensions for ophthalmic delivery: A comparative study between microfluidic nanoprecipitation and wet milling.* J. Control. Release, 2011. **149**(2): p. 175-181.

66. Müller, R.H. and J. Möschwitzer, *Method and device for producing very fine particles and coating such particles.* US patent 0297565 A1, 2009.

67. Sinha, B., R.H. Müller, and J. Möschwitzer, *Precipitation followed by high presure homogenization as a combinative approach to prepare drug nanocrystals.* Tag der Pharmazie 2012 FU Berlin, Berlin, abstract V2, booklet page 6, 2012.

68. Möschwitzer, J., *Method for producing ultrafine submicronic suspensions, WO/002006094808A3.* 2006.

69. Liu, T., R.H. Müller, and J.P. Möschwitzer, *Process optimization of a novel particle size reduction technology H 42.* Tag der Pharmazie 2012 FU Berlin, Berlin, abstract P33, booklet page 25, 2012.

70. Möschwitzer, J. and A. Lemke, *Method for carefully producing ultrafine particle suspensions and ultrafine particles and use thereof, WO/2006/108637.* 2006.

71. Teagarden, D.L. and D.S. Baker, *Practical aspects of lyophilization using non-aqueous co-solvent systems.* Eur. J. Pharm. Sci., 2002. **15**(2): p. 115-133.

72. Staedtke, V., et al., *In vitro Inhibition of Fungal Activity by Macrophage-Mediated Sequestration and Release of Encapsulated Amphotericin B Nanosupension in Red Blood Cells.* Small, 2010. **6**(1): p. 96-103.

73. Möschwitzer, J.P., *Drug nanocrystals in the commercial pharmaceutical development process.* Int. J. Pharm., 2012(0).

74. Keck, C., et al., *Second generation of drug nanocrystals for delivery of poorly soluble drugs: smartCrystals technology.* Dosis, 2008. **24**(2): p. 124-128.

75. Kobierski, S. and C.M. Keck, *Nanocrystal production by BM-HPH combination technology.* Controlled Release Society, Abstract, Germany Chapter, Annual Meeting, 4-5 March, 40, 2008.

76. Keck, C.M., et al., *Second generation of drug nanocrystals for delivery of poorly soluble drugs: smartCrystals technology.* Dosis, 2008. **24**(2): p. 124-128.

77. Al Shaal, L., R.H. Müller, and R. Shegokar, *smartCrystal combination technology - scale up from lab to pilot scale and long term stability.* Pharmazie, 2010. **65**: p. 877-884.

78. Salazar, J., J. Möschwitzer, and R.H. Müller, *Application of the combinative particle size reduction technology H 42 to produce fast dissolving glibenclamide tablets. Accepted for publication.* Eur. J. Pharm. Sci., 2013.

9. ABBREVIATIONS

API	active pharmaceutical ingredient
BCS	Biopharmaceutics Classification System
CT	combination technology
DC	degree of crystallinity
DoE	design of experiments
DMSO	dimethyl sulfoxide
DSC	differential scanning calorimetry
DSS	docusate sodium salt
FD	freeze-drying
g	gram
GMP	Good Manufacturing Practice
HCA	hydrocortisone acetate
HDPE	high density polyethylene
Hz	hertz
HPH	high pressure homogenization
HPLC	high performance liquid chromatography
HTS	high throughput screening
ICH	International Conference on Harmonisation
IV	intravascular
LD	laser diffractometry
min	minute
mg	milligram
ml	mililiter
N	newton
NCE	new chemical entity
nm	nanometer
NMP	N-methyl-2-pyrrolidone
PDI	polydispersity index
PCS	photon correlation spectroscopy

PXRD	powder X-ray diffraction
RSM	response surface model
rpm	revolutions per minute
SD	spray-drying
SEM	scanning electron microscopy
SDS	sodium dodecyl sulfate
T	temperature
TBA	tert-butyl alcohol
μm	micrometer
v/v	volume/volume
W	watt
WBM	wet bead milling
WG	wet granulation
w/v	weight/volume
10-HCPT	10-hydroxycamptothecin

10. LIST OF PUBLICATIONS

Refereed journal publications

1. Salazar, Jaime; Heinzerling, Oliver; Müller, Rainer H.; Möschwitzer, Jan P., (2011), "Process optimization of a novel production method for nanosuspensions using design of experiments (DoE)", International Journal of Pharmaceutics, 420: 395-403.

2. Salazar, Jaime; Ghanem, Antoine; Müller, Rainer H.; Möschwitzer, Jan P., (2012), "Nanocrystals: Comparison of the size reduction effectiveness of a novel combinative method with conventional top-down approaches", European Journal of Pharmaceutics and Biopharmaceutics, 81: 82-90.

3. Salazar, Jaime; Müller, Rainer H.; Möschwitzer, Jan P., (2013), "Application of the combinative particle size reduction technology H 42 to produce fast dissolving glibenclamide tablets", European Journal of Pharmaceutical Sciences.

4. Salazar, Jaime; Müller, Rainer H.; Möschwitzer, Jan P., (2013), "Performance comparison of two novel combinative particle size reduction technologies", Journal of Pharmaceutical Sciences.

5. Salazar, Jaime; Müller, Rainer H.; Möschwitzer, Jan P., (2013), "Combinative particle size reduction technologies for the production of drug nanocrystals", Journal of Pharmaceutics (submitted).

Lecture presentations

1. Salazar, Jaime; Heinzerling, Oliver; Müller, Rainer H.; Möschwitzer, Jan P., (2010), "Prozessoptimierung einer neuartigen Herstellungsmethode für Nanosuspensionen unter Verwendung statistischer Versuchsplanung", Tag der Pharmazie, DPhG Berlin-Brandenburg/FU Berlin, Berlin/Germany, July 2, 2010.

2. Salazar, Jaime; Müller, Rainer H.; Möschwitzer, Jan P., (2011), "The use of a novel combinative particle size reduction technology to produce ultrasmall drug nanocrystals of fluorescein diacetate", CRS german chapter meeting, Jena/Germany, March 15-16, 2011.

Proceedings

1. Salazar, Jaime; Ghanem, Antoine; Müller, Rainer H.; Möschwitzer, Jan P., (2010), "Influence of non-aqueous freeze drying parameters on the particle size reduction effectiveness", 7th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Valetta/Malta, March 8-11, 2010. Poster presentation, abstract 135, booklet page 65.

2. Salazar, Jaime; Müller, Rainer H.; Möschwitzer, Jan P., (2012), "Novel combinative particle size reduction method combining spray drying as bottom-up step followed by high pressure homogenization as top-down process for nanosuspension production", 8th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Istanbul/Turkey, March 19-22, 2012. Poster presentation, abstract 12, booklet page 86.

Abstracts

1. Salazar, Jaime; Müller, Rainer H.; Möschwitzer, Jan P., (2009), "Systematic investigation to establish optimal process parameters for a novel particle size reduction method", Annual Meeting of the German Pharmaceutical Society (DPhG), Jena/Germany, September 28 – October 1, 2009. Poster presentation, abstract C48, booklet page 45.

2. Salazar, Jaime; Ghanem, Antoine; Müller, Rainer H.; Möschwitzer, Jan P., (2010), "Influence of freeze drying with organic solvents on the particle size reduction effectiveness of poorly soluble compounds for solubility and bioavailability enhancement", Drug Transport and Delivery, Swedish Academy of Pharmaceutical Sciences and CRS Nordic Chapter, Gothenburg/Sweden, June 28-29, 2010. Poster presentation, abstract 25, booklet page 52.

3. Möschwitzer, Jan P.; Salazar, Jaime; Heinzerling, Oliver; Müller, Rainer H.; (2010), "Optimization of a novel combinatory production method for nanosuspensions", 8th European Workshop on Particulate Systems, Paris/France, 4-5 June 2010. Lecture presentation, booklet page 15.

4. Salazar, Jaime; Ghanem, Antoine; Müller, Rainer H.; Möschwitzer, Jan P., (2010), "Gefriertrocknung organischer Lösungen zur Herstellung von porösen Arzneistoffstrukturen", Tag der Pharmazie, DPhG Berlin-Brandenburg/FU Berlin, Berlin/Germany, July 2, 2010. Poster presentation, abstract P32.

5. Salazar, Jaime; Heinzerling, Oliver; Müller, Rainer H.; Möschwitzer, Jan P., (2010), "Prozessoptimierung einer neuartigen Herstellungsmethode für Nanosuspensionen unter Verwendung statistischer Versuchsplanung", Tag der Pharmazie, DPhG Berlin-Brandenburg/FU Berlin, Berlin/Germany, July 2, 2010. Lecture presentation, abstract V4, booklet page 2.

6. Salazar, Jaime; Ghanem, Antoine; Müller, Rainer H.; Möschwitzer, Jan P., (2010), "New combinational particle size reduction method for nanosuspension production and poorly soluble API formulation for bioavailability enhancement", Encuentros 2010, Integrating Chilean Research Worldwide, Cambridge/England, August 4-6, 2010. Poster presentation, abstract 20, booklet page 11.

7. Heinzerling, Oliver; Salazar, Jaime; Müller, Rainer H.; Möschwitzer, Jan P., (2010), "The use of DoE to optimize process parameters for a novel production method for nanosuspensions", Annual Meeting of the German Pharmaceutical Society (DPhG), Braunschweig/Germany, October 4-6, 2010. Poster presentation, abstract T041, booklet page 96.

8. Salazar, Jaime; Heinzerling, Oliver; Müller, Rainer H.; Möschwitzer, Jan P., (2010), "Process optimization of a novel production method for nanosuspensions using design of experiment", PSWC+AAPS Annual Meeting, New Orleans/USA, November 13-18, 2010. Poster presentation, abstract R6156, booklet page 224.

9. Salazar, Jaime; Heinzerling, Oliver; Müller, Rainer H.; Möschwitzer, Jan P., (2010), "Production methods of ultra small nanocrystals", Metz Nanodata Event: Overview of activities in Nanotechnologies, Metz/France, November 24-25, 2010. Poster presentation P25.

10. Salazar, Jaime; Müller, Rainer H.; Möschwitzer, Jan P., (2011), "The use of a novel combinative particle size reduction technology to produce ultrasmall drug nanocrystals of fluorescein diacetate", CRS german chapter meeting, Jena/Germany, March 15-16, 2011. Lecture presentation, abstract O7, booklet page 33.

11. Salazar, Jaime; Müller, Rainer H.; Möschwitzer, Jan P., (2011), "Comparison of two novel, combinative particle size reduction methods for the production of ultrasmall drug nanocrystals", Annual Meeting of the German Pharmaceutical Society and Austrian Pharmaceutical Society (DPhG and ÖPhG), Innsbruck/Austria, September 21-23, 2011. Poster presentation, abstract PO-120, booklet page 28.

12. Salazar, Jaime; Müller, Rainer H.; Möschwitzer, Jan P., (2011), "Comparison of two combinative particle size reduction technologies which combine bottom-up steps (freeze-drying or spray-drying) followed by high pressure homogenization", AAPS Annual Meeting, Washington D.C./USA, October 23-27, 2011. Poster presentation, abstract T-3222.

13. Salazar, Jaime; Müller, Rainer H.; Möschwitzer, Jan P., (2012), "Development of fine nanosuspensions prepared by the H 42 technology and their transfer into oral solid dosage forms", AAPS Annual Meeting, Chicago/USA, October 14-18, 2012. Poster presentation, abstract T-3225.

14. Salazar, Jaime; Müller, Rainer H.; Möschwitzer, Jan P., (2012), "A novel combinative particle size reduction technology for drug nanocrystals production and further nanosuspension transfer to tablets", Annual Meeting of the German Pharmaceutical Society (DPhG), Greifswald/Germany, October 11-13, 2012. Poster presentation.

Der Lebenslauf ist in der Online-Version aus Gründen des Datenschutzes nicht enthalten.

Der Lebenslauf ist in der Online-Version aus Gründen des Datenschutzes nicht enthalten.

12. ACKNOWLEDGMENTS