Nanocrystals produced by ARTcrystal®-technology

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1 INTRODUCTION

1.1 The role of solubility in drug development

In the development of new pharmaceutical actives, poor solubility is a serious issue, as it leads to manyfold problems. Poor solubility can be responsible for a low oral bioavailability, but can also be problematic for paranteral applications by requiring volumes of solvent too large for injection or infusion [1]. Up to 70% of the new developed drug actives are considered as poorly soluble [2], therefore classified as class II or IV substances in the Biopharmaceutics Classification System (BCS) [3, 4]. The BCS was established by Amidon et al. in 1995 and divides drug actives into four classes, depending on their membrane permeability and solubility (Figure 1) [4].

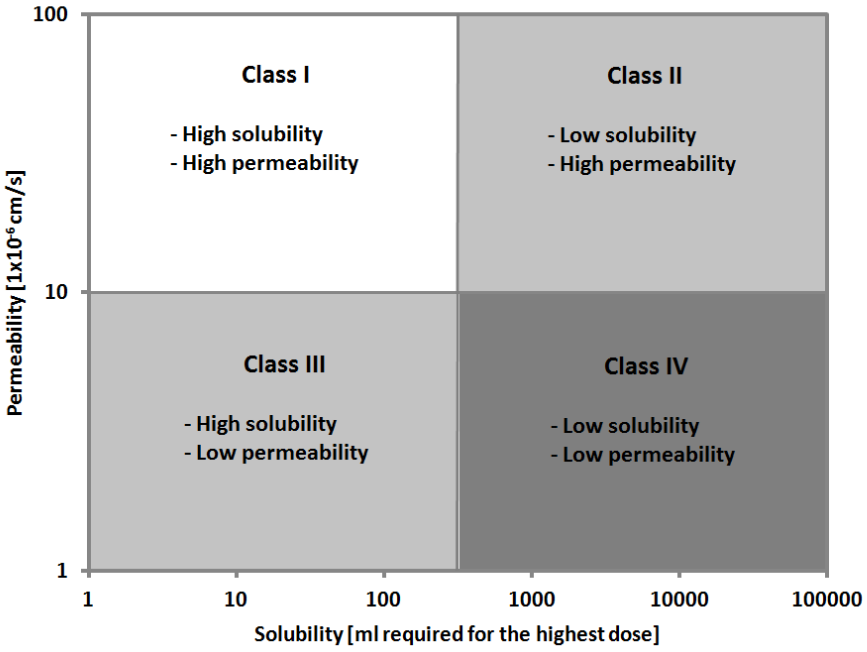

Figure 1 **The Biopharmaceutics Classification System (modified after [4])**

As the number of practically insoluble drug actives rises, the need for technologies improving the solubility steadily increases. Several approaches were made to improve the solubility of actives, e.g. salt formation of ionizable drug actives, the use of cosolvents or cyclodextrins as well as amorphization, micronization or incorporation of the active into a

solid matrix ("solid solution"). An overview over existing technologies is shown in Table 1. Issues of vital importance are potential toxicological effects of pharmaceutical additives, e.g. nephrotoxicity of parenteral applied ß-cyclodextrins [5]. One easy and elegant way is the use of nanonization technologies. Nanocrystals consist of 100% pure drug active and possess only a small amount of surface-bound stabilizing agents for prevention of agglomeration. By nanonization, the dissolution velocity as well as the saturation solubility of drug actives is increased by physical means. Additionally, the high surface-to-mass ratio allows mucoadhesion of the crystals, thus besser resorption in the upper gastrointestinal tract. [6]. Nanocrystal technology is very versatile, as nanocrystals can be used as suspension or after a drying step further processed into a solid dosage form [7-12]. Therefore, this technology is suitable for all administration routes, e.g. oral [8], parenteral [13], pulmonal [14] and dermal [15] applications. This is a main advantage over many other technologies, e.g. matrix based systems are less likely suitable for parenteral application due to potential toxicological issues caused by the excipients. Furthermore, nanocrystal technology is less limited by the drug active's physical properties, e.g. the solubility in nonaqueous liquids or the molecule size in order to fit into a cyclodextrin ring [6].

Table 1 Technologies for increasing the bioavailability of poorly soluble substances

Technology	**References**
Salt formation	[16]
Solubilization	[17]
Cosolvents	[18]
Cyclodextrins	[19]
Solid dispersions/melt extrusion	[20]
Amorphization	[21, 22]
Micronization	[23-25]
Nanocrystal technology	[1, 6, 13]
Matrix nanoparticles	[26, 27]

1.2 Increase of bioavailability through nanonization

In the past, the dissolution velocity of poorly soluble drug actives could be enhanced through increasing the crystal surfacy by micronization [23-25]. As some of the developed actives possess a solubility so too low that micronization alone would not lead to a sufficient increase in solubility, the next step was the transformation of the drug crystals into the nanoscale. As the crystals reach the nanoscale, several changes in the physical properties of the crystals take place, e.g. an increase in the dissolution velocity as well as an increase of the (temporary) saturation solubility. The relevant physical backgrounds are summarized in Table 2.

Table 2 Physical equations explaining the effects taking place in the nanoscale

Noyes-Whitney	Ostwald-Freundlich	Prandtl
$\frac{dm}{dt} = \frac{DA(c_S - c)}{h}$	$c_r = c_\infty \, exp\left(\frac{2\gamma V}{r\rho RT}\right)$	$h_H = k\left(\frac{L^{1/2}}{V^{1/3}}\right)$
$\frac{dm}{dt}$= dissolution velocity D = diffusion coefficient A = particle surface area c_s = saturation solubility c = concentration in the surrounding liquid h = thickness of the diffusional layer	C_r = solubility for a particle with the radius r c_∞= solubility for an infinite big particle (r=∞) γ = interfacial surface tension V = particle volume r= particle radius ρ = density R = gas constant T = temperature	h_H = hydrodynamic boundary layer thickness k = constant L = length of the surface in the direction of flow V = relative velocity of the liquid Flow
Reference: [28]	Reference: [29, 30]	Reference: [31]

The Noyes-Whitney equation describes the dissolution velocity of crystals in dependency on the concentration difference between the saturation solubility and the concentration in the surrounding liquid (c_s-c), the crystal surface (A) and the diffusion distance (h; the thickness of the layer around the crystal with an increased drug active concentration).

The Ostwald-Freundlich equation explains the dependence of the vapor pressure on the interfacial energy (γ) and radius (r). It must be noted that differences in the vapor pressure and thus an increase in solubility do only apply for crystals with sizes <1 µm [30].

The Prandtl equation further explains the increase in saturation solubility in the nanoscale. As the particle size decreases, the curvature increases and the length of surface in the direction of flow (L) decreases. With decreasing length, the diffusional distance (h_h) decreases and the dissolution velocity increases [31]. For an in-detail physical explanation of the effects, the reader is referred to [28-33].

In conclusion, the following benefits from transferring crystals into the nanoscale can be summarized:

- Increase in saturation solubility [31]
- Increase in dissolution velocity [28]
- Increased mucoadhesion (oral application) [1]
- More constant bioavailability, less variability induced by food intake [34-36]

Decreasing the crystal size by the factor 10 leads to an theoretical increase of the surface area by the factor 10. This means, in theory, reducing the size of a crystal from 50 µm to 500 nm results in an increase in surface by the factor 100. With increased surface, the dissolution velocity increases. Furthermore, a reduction in size leads to an increase of the surface-to-mass ratio. Smaller crystals can adhere to the mucosa of the proximal gastrointestinal tract (stomach, duodenum, proximal jejunum). This allows poorly soluble substances to dissolve where they can be absorbed, instead of passing the gastrointestinal tract in a crystalline form and leaving the body undissolved [1].

1.3 Nanosuspensions: Physical stability and further processing

While nanosuspensions feature many beneficial characteristics, the stability of nanosuspensions is an important topic for assurance of the product's shelf life. Furthermore, nanosuspensions are typically not stored as suspensions, but further processed into dosage forms. Therefore, the next chapter gives a brief overview over the stability of nanosuspensions and the options for further processing into a final dosage form.

1.3.1 Physical stability

To retain their original properties and crystal size, nanosuspensions have to be stabilized by using the right stabilizing agents. Stabilizers are typically ionic or nonionic tensid molecules, which adhere to the crystal surface and prevent agglomeration and aggregation as well as caking of the solid particles. Depending on the stabilizer, the main stabilizing mechanism can be electrostatic repulsion (ionic stabilizers) or steric hindrance [37, 38]. The physical stability of a nanosuspension depends on several factors, such as storage conditions and in process temperature, crystal size distribution and agglomeration/aggregation tendencies due to the crystal morphology. Stabilizer molecules adsorbed onto the nanocrystal surface can prevent crystal growth and agglomeration. An insufficient surface coverage lowers the physical stability. Additionally, the nanocrystal surface covered by stabilizer molecules provides protection from degradation by oxygen and light. Only uncovered areas of the crystal are accessible to water and light [39].

An indicator for the long term stability of nanosuspensions is the analysis of the zeta potential. The zeta potential is the potential measured at the hydrodynamic shear plane. It can be determined from the particle mobility by applying an electric field. For ionic stabilizers, a zeta potential of at least -30 mV is frequently considered as indicator for physical stability [38, 40]. Steric stabilizers carry no electric charge. They adsorb onto the surface of the particles and form a thick layer around the particles due to their high molecular weight. Therefore, the plane of shear is shifted and lower zeta potentials are measured with increasing thickness of the stabilizing layer. As the value of -30 mV only applies for ionic stabilizers, suspensions containing steric stabilizers can still be physically stable with zeta potentials close to zero. The most efficient mechanism of stabilization is, however, a combination of steric and electrostatic stabilization, where electrostatic repulsion and steric hindrance complement each other [41].

Another important issue for long term physical stability is the particle size distribution. Broad size distributions promote Ostwald ripening, an effect where larger crystals grow on the expense of smaller ones, narrowing the size distribution while increasing the mean crystal size in the process [29]. Furthermore, most stabilizers used for nanosuspension stabilization can increase the solubility of the drug active, therefore enhancing Ostwald ripening [42]. In order to prevent Ostwald ripening, stabilizers with low solubility of the active enhancing potential should be selected. However, the most important parameter is to define production parameters, which efficiently destroy larger crystals as well as agglomerates, thus leading to a narrow size distribution and increased physical stability therefore.

1.3.2 Solidification and further processing

For increasing storage stability and application convenience, solidification of nanosuspensions is an important step. By solidification, chemical and physical stability issues, e.g. hydrolysis, agglomeration, caking and Ostwald ripening can be circumvented, thus increasing the shelf life of the product. As tablets or capsules are easier to handle and allow better dosage of highly potent substances, solidification is commonly applied when nanocrystals are aimed to be applied orally. Several techniques for solidification of nanosuspensions exist. The most common techniques for solidification are spray drying [43] and freeze drying [44] of a nanosuspension, as well as implementation of the nanosuspension into pellets by pelletization or spray coating [45, 46]. The dried nanopowder can be further processed into tablets by direct compression or after a granulation step [8]. A detailed review on this topic was recently published as part of this thesis [47].

1.4 Recent marketed products containing nanocrystals

Almost 25 years have passed from the final idea of nanocrystals until now [48]. In this time, several production techniques were established. In the year 2000, the first pharmaceutical product containing nanocrystals was marketed by Wyeth (Rapamune®/Sirolimus). Nanonization was performed by wet bead milling. Several more products followed and different nanonization techniques were applied (Table 3). Increasing numbers of nanocrystal containing products are to be expected in the next years due to increasing solubility issues as well as the economic benefits of the nanocrystal technology.

Table 3 Marketed pharmaceutical products containing nanocrystals, modified after [49].

Product	Application route	Drug active	Company	Nanonization method
Rapamune®	oral	Sirolimus	Wyeth	BM
Emend®	oral	Aprepitant	Merck	BM
Tricor®	oral	Fenofibrate	Abbott	BM
Triglide™	oral	Fenofibrate	First Horizon Pharma	HPH
Cesamet®	oral	Nabilone	Lilly	Co-precipitation
Megace®	oral	Megesterol acetate	Par Pharma	HPH
Invega Sustenna®	parenteral	Paliperidone palmitate	Johnson&Johnson	HPH
Gris-PEG®	oral	Griseofulvin	Novartis	Co-precipitation

1.5 Methods for the production of nanocrystals

There are many methods available for the production of nanocrystals. In this section, an overview over existing methods as well as their benefits and disadvantages is presented.

1.5.1 Currently available methods

The production processes for nanocrystals can be divided into two subgroups, the bottom-up approach and the top-down approach. In the bottom-up approach, nanocrystals are formed by in-situ crystallization during mixing a saturated drug solution with a non-solvent [50]. Top-down techniques break larger microscale or macroscale crystals down into nanocrystals using mechanic forces.

In order to achieve even smaller crystal sizes, several methods can be combined with each other or with a pretreatment step. An overview over existing techniques is given in Table 4.

Table 4 Established nanonization techniques

Method	Patent Name	Type	References
Precipitation	Hydrosols®	Bottom-up	[51]
Precipitation	Nanomorph®	Bottom-up	[22]
High pressure homogenization (HPH)	DissoCubes®	Top down	[6]
Wet bead milling (BM)	NanoCrystal®	Top down	[52]
Precipitation/HPH	Nanoedge®	Bottom-up/top down	[53]
Spray drying/HPH	H42	Bottom-up/top down	[43]
Precipitation/HPH	H69	Bottom-up/top down	[54]
BM/HPH	CT	Top-down	[55]
Freeze drying/HPH	H96	Bottom-up/top down	[56]

1.5.1.1 *Bottom-up technologies*

The drug active is dissolved in a solvent and by adding an antisolvent to the mixture, precipitation of nanocrystals takes place. The crystal growth has to be controlled in order to prevent the growth of microcrystals. Depending on the process parameters, the resulting particles can have a crystalline or amorphous form [50].

Commonly known precipitation techniques are the Hydrosols®-technology and the Nanomorph®-technology. The Hydrosols®-technology is the basic precipitation process, consisting of mixing a saturated drug solution with a non-solvent and produces drug nanocrystals [51]. The Nanomorph®-technology is a further development of the Hydrosols®-technology and uses an aqueous polymer solution for the precipitation step. The main advantage is the formation of nanocrystals as well as amorphous nanoparticles, depending on the process parameters [22]. An alternative bottom-up approach is freeze-drying or spray-drying of saturated drug solutions [57].

1.5.1.2 *Top-down technologies*

Two main top-down processes are known and commonly used: High pressure homogenization (HPH) and wet bead milling (BM) [6], [13].

1.5.1.2.1 High pressure homogenization

HPH uses cavitational forces in order to break larger crystals into smaller nanocrystals. These cavitational forces occur when the suspension is forced to pass a small gap under the application of high pressures. The homogenization gap possesses a size of about 25 µm, varying with applied pressure [6]. According to Bernoulli's law, the flow volume of a liquid substance inside a closed system per cross-section is constant. By reducing the diameter during the transfer from the feeding tube to the gap, an increase in dynamic pressure takes place, and the static pressure decreases. When the vapor pressure reaches the static pressure of the environment, the liquid begins to boil, resulting in the formation of gas bubbles. After passing the gap, the diameter increases, resulting in an increase of the static pressure and implosion of the gas bubbles. These cavitations are the main driving force for particle size reduction in the HPH process. Shear forces during the gap passage do occur, but contribute only slightly to the process of crystal breakage. Standard conditions for nanocrystal production are 20 passages at 1,500 bar pressure [6]. This technology is well-investigated and has been applied for the nanonization of many substances [8, 14, 58-61].

1.5.1.2.2 Wet bead milling

BM achieves nanonization by subjecting macrocrystals to shearing and grinding between milling beads inside a milling container. A drawback of this technique is the high abrasion of the milling beads, resulting in possible contamination of the final product. By using resistant materials, e.g. yttrium stabilized zirconium oxide, abrasion and thus contamination can be minimalized [62]. For sufficient milling results, a large amount of milling beads, up to 50% of the slurry volume, is needed. Suspensions with drug concentrations of 2-30% (w/w) can be milled by wet bead milling. Milling beads possess typically a size of 0.5 – 1 mm, although larger or smaller beads with a size of 0.1 mm are commercially available [52]. Depending on the applied milling speed and material properties, milling can take from 30 minutes [63] to up to several days [64]. When choosing a milling speed, energy intake and thus overheating of the product must be taken into account. Besides HPH, BM is one of the most commonly used nanonization techniques [13, 52]. BM produces smaller nanocrystals with a more narrow size distribution in comparison to HPH, as proven for several substances [65, 66].

1.5.1.2.2.1 Ultra cryo-milling

The ultra cryo-milling process is an optimization approach to classical wet bead milling. The main difference is suspending the drug active in liquid nitrogen and replacing the milling beads with dry ice beads. This new process holds the advantage of generating no wear from the beads, thus no product contamination occurs. Furthermore, the dry ice beads sublimate after the milling step, eliminating the filtration step in order to remove the milling beads from the slurry. One disadvantage of this method is the increase in process time, as the processing time may increase by the factor 20, thus the afore mentioned economical benefits are cancelled out [67].

1.5.1.3 ***Combination technologies***

In order to achieve even smaller crystal sizes in comparison to the standard processes, several combination processes have been developed. The drawback of these processes is an increase in production time and costs.

1.5.1.3.1 Nanoedge®

The Nanoedge®-technology combines a microprecipitation step (solvent-antisolvent precipitation) with subsequent HPH as annealing step [68]. This combination process is a decoupled process, meaning HPH and precipitation are two separate processing steps. The additional HPH annealing step breaks needle-shaped crystals, formed during the precipitation step and removes amorphous structures inside the particle [69, 70]. The drawback of this method is the use of organic solvents, which have to be removed from the final product and a larger crystal size distribution compared to standard technologies and combination processes. Crystal growth might take place between the precipitation and the HPH step.

1.5.1.3.2 H42-technology

The H42-process consists of a spray drying part and subsequent HPH. Spray drying serves here as precipitation step. After dissolution in an organic solvent, the drug active is spray dried. To improve spray drying results, sugars are often added to the drug solution. Spray dried particles are more brittle and thus better breakable in a subsequent top-down step [71]. Subsequent HPH is applied under standard conditions (20 cycles at 1500 bar). The resulting particles tend to have a spherical morphology as well as partly amorphous regions

[43]. Due to the thermic stress during spray drying, this method is not suitable for thermolabile substances.

1.5.1.3.3 H69-technology

The H69-technology is an improved Nanoedge®-process. The main difference is the immediate application of HPH while the precipitation takes place, unlike the Nanoedge®-process, which is decoupled [72]. The dissolved drug active is pumped to the homogenization gap, while shortly before reaching the gap, the antisolvent is added. Therefore, precipitation takes place, while the solvent passes the homogenization gap, where immediate breakage of larger crystals and annealing is applied. This step prevents further crystal growth and reduces the amount of possible forming amorphous regions. Through the flow speed and solvent-antisolvent ratio, control over the crystallization process is possible [72].

1.5.1.3.4 H96-technology

Another combination process consisting of a drying step as pretreatment and subsequent HPH is the H96-process. The main difference to the H42-process is the use of freeze drying instead of spray drying, making the H96-technology more suitable for thermosensitive drugs [73]. The drawback of this technique is the time and cost consuming nature of the freeze-drying step [74]. As proven by Salazar et al., the subsequent HPH increases the variable degree of crystallinity after freeze drying and allows production of finer nanocrystals in comparison to classical HPH [56]. The brittle, porous and less crystalline intermediate after freeze drying is more prone to diminution by cavitation forces in comparison to micro- and nanocrystals from coarse powder.

1.5.1.3.5 Combination technology (CT)

Combining a short-time BM with subsequent HPH, the BM part of this process mills a suspension down to about 600 nm - 1.5 µm, followed by 1-3 cycles of HPH at reduced pressures [75]. While this technology is able to produce fine nanocrystals in a shorter time than standard HPH, partitioning of the milling beads from the nanocrystals as well as contamination due to abrasion can be problematic.

1.5.2 Comparison of common nanonization technologies

All nanonization technologies have their advantages and disadvantages. The most relevant facts are summarized in Table 5.

Table 5 Overview of advantages and disadvantages of available nanonization technologies

Technology	Advantages	Disadvantages
Precipitation	Applicable for very resistant and ductile materials, possibility to form amorphous nanoparticles	Use of organic solvents, larger crystal size distribution in comparison to top-down methods
HPH	Faster processing in comparison to BM	Larger crystal sizes in comparison to BM
BM	Smaller final crystal size in comparison to HPH	Possible contamination through abrasion, long processing times, limited scalability, higher energy intake
Nanoedge®	More homogeneous size of crystals and better stability in comparison to traditional precipitation	Use of organic solvents, larger crystal size distribution in comparison to top-down methods
H42	Smaller crystal sizes in comparison to HPH or BM	Long processing times
H69	Smaller crystal sizes in comparison to HPH or BM	Long processing times
H96	Smaller crystal sizes in comparison to HPH or BM	Long processing times
CT	Smaller crystal sizes in comparison to HPH or BM	Long processing times

1.5.3 ARTcrystal-technology®: A new combination process

The ARTcrystal®-technology is a novel top-down combination process, combining continuous high speed stirring (HSS) and HPH, aiming at a reduction of HPH cycle number and pressure, thus economizing nanocrystal production. This process was first developed and patented by Keck in 2011, allowing the nanonization of rutin crystals within 5 cycles at 300 bar pressure after 5 minutes of HSS pretreatment [76].

The MICCRA D27, designed by ART Prozess- und Labortechnik, is an in-line rotor-stator system, which is capable of achieving maximum tip speeds up to 70 m/s (36,000 rpm). This means, the D27 is more powerful than conventional rotor-stator systems, which reach typically tip speeds in the range of 10-50 m/s [77]. The system comes with a 5 liter product container, but also larger vessels can be mounted. In-process cooling is provided by an external cooling thermostat, cooling the double-walled product container. Cooling during the process is of vital importance, as nanocrystals tend to agglomeration at temperatures <30°C [78].

As continuous rotor stator system, the milling chamber contains a rotor and a stator and is fed from the product container. High shear stress is applied during passage of the rotor and stator slits as well as inside the shear gap, which is the main driving force for desagglomeration, disaggregation, milling and emulsification purposes [77]. After passing rotor and stator, the product then is returned from the milling chamber to the product container through the product circulation tube.

In comparison to traditional HPH, the main benefit of this process is the elimination of a premixing and premilling step, usually performed by a batch mixer and 2-4 cycles of subsequent HPH with increasing pressures in the range of 250-1000 bar [6, 8, 14, 15, 78, 79]. This pretreatment is required to prevent blockage of the homogenization gap by large particles [6]. The intermediate product is even finer and more homogeneous after the HSS step in comparison to traditional premixing and premilling..

From a technical point of view, the benefits of this process are first initial homogenization of the raw suspension by a short, but effective application of hydrodynamic shear stress onto the particles. The power of the hydrodynamic shear stress is limited, but powerful enough to destroy aggregates as well as agglomerates and large monolithic crystals in a mass fraction step [80]. As the attrition step is not economically reasonable, the HSS process should be stopped after complete mass fraction. Due to the increased homogeneity of the suspension, less cycles of HPH are required, as breakage/mass fraction of microscale crystals into nanocrystals occurs stepwise. One cycle of HPH even at extremely high pressures, e.g. 4000 bar, could never be enough to transform all crystals into the nanoscale, as mass fracture does not completely take place in one single step. The HSS pretreatment has the advantage of a high suspension throughput per time, while homogenization of the same amount of raw

suspension takes distinctly longer (when using lab scale homogenizers). Also, even large microscale crystals with sizes >100 µm can be processed by HSS without the possibility of blockages.

In the patent [76], the ARTcrystal®-technology was described as a process, which allows faster production as well as finer crystal sizes in comparison to HPH or BM with rutin as testing material. The production takes only a time of about 20 minutes, while production by HPH or BM takes several hours (in the laboratory scale). Also, achievement of even smaller sizes in comparison to traditional approaches is possible. The main feature of the ARTcrystal®-technology is the possibility of producing large amounts of nanosuspensions within a short amount of time. This economic efficiency and the scaling possibilities render this technology interesting for industrial applications.

1.5.4 ARTcrystal®-technology: Outlook and need for further research

Unlike the other combination technologies, which aim at maximum size reduction, the ARTcrystal®-method aims at an industrial application by economizing the production. While the patent is based on promising first data, the process itself was not further investigated in detail. Rutin could be nanonized by the ARTcrystal®-method, but it is not known if the results are transferable to other materials or if the results could be optimized in any way. The impact of the starting material's physicochemical properties (particle size, morphology, ductility) on the final results as well as on the method's efficacy compared to other approaches needs to be investigated. The rotor-stator pretreatment step holds possibilities for optimization, as the process itself was applied once, but no further in-detail investigation of this step was performed. Therefore, further research is required to prove the full potential of the ARTcrystal®-technology.

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2 AIM OF THE THESIS

This work aims at investigating the milling potential of the ARTcrystal®-technology in order to better understand and optimize this process. As described in the patent [76], the process holds a great potential for nanonization at a industrial scale. It has to be noted that this combination process did not undergo intense research, therefore influencing and critical parameters as well as possibilities for the optimization of the process were unknown. Furthermore, the D27 rotor-stator-system prototype used in the patent, had only a maximum stirring rate of 24,000 rpm (tip speed ~45 m/s), while the system was further developed to allow a rate of 36,000 rpm, increasing the potential of this process. Critical process parameters were not investigated up to this point. Additionally, the process was only tested for rutin, so transferability for substances with different physical properties (crystal size, hardness, morphology) was not yet known. In order to finally evaluate the potential of this technology, in-depth research had to be performed.

In summary, the aim of this thesis can be subdivided into the following steps:

- **Investigation of relevant process parameters during the rotor- stator pretreatment**
- **Optimization of the rotor-stator pretreatment step**
- **Evaluation of the ARTcrystal®-method's efficacy by comparison to traditional nanonization approaches**
- **Further optimization of the ARTCrystal®-method**
- **Investigation of further application potential of rotor stator technology**

3 RESULTS AND DISCUSSION

Due to the rising numbers of new developed drug actives with poor solubility, the importance of nanocrystal technology for formulation development will increase. As several single techniques and combination processes for the production of nanocrystals already exist, it is vital to focus on time-saving and economic solutions for the production of nanocrystals. While combination techniques allow achieving smaller crystal sizes in comparison to standalone processes, it has to be kept in mind that these processes come with an increase in both processing time and costs. Also scalability is an issue, as not all processes are equally suited for large scale production [50]. This gap can be filled by the ARTcrystal®-technology, as it combines scaling possibilities with a reduction in processing time.

3.1 Investigation of relevant process parameters

In order to understand and optimize the rotor-stator pretreatment step, relevant parameters had to be determined and investigated. During the first experiments, the influence of the following parameters was investigated:

- In process temperature
- Stirring rate/tip speed
- Processing time
- Foaming during processing
- Particle size and morphology of the starting material
- Continuous versus discontinuous pretreatment

3.1.1 In process temperature

The in process temperature of a milling process is of vital importance, as post-processing agglomeration tendencies after HPH increase when temperatures during processing exceed 30°C [78]. Processing of rutin suspensions without cooling or without sufficient cooling

resulted in accordance to the literature in heavy agglomeration of the crystals after processing. Without cooling, temperatures of >50°C were measured during processing after only a few minutes of stirring at 24,000 rpm. Simple water cooling of the double-walled product container was proven to be not sufficient, as temperatures of 30°C were reached after 3-4 minutes of processing at 24,000 rpm [81]. This observation complied with the data stated in the patent [76]. Therefore, cooling was supplied by a cooling thermostat at a temperature of -10°C. Under this circumstances, processing of up to 15 minutes was possible. As the application of stirring rates >24,000 rpm further increases the energy intake, higher stirring rates can only be applied when using a cooling thermostat. Even with application of a cooling thermostat, the energy intake was so high that in process temperatures at 36,000 rpm reached 30°C within 3 minutes of processing. Because of this, work was mainly carried out at 24,000 rpm, as cooling temperatures below -10°C were not applicable. The main problem is the device design, as a double-walled product return pipe could serve as flow-through cooler. However, as complex redesigns of the device were not achievable within time, this approach might be relevant for further designs of the D27 device.

3.1.2 Stirring rate/tip speed

The tip speed (V_T; m/s) is the most relevant process parameter due to its influence on both processing time and final particle/droplet size. It is defined as the circumferential speed of the outer rotor surface:

$$\boldsymbol{V_T = \pi * D * N}$$

with

V_T tip speed (m/s)

D rotor diameter (m)

N stirring rate (rpm)

While the flow rate ($\vec{\vartheta}$) through the mixing head is proportional to the stirring rate (N) and thus the tip speed (V_T), the energy dissipation and consequently the potential for disruption of particles and droplets rises by N^3 [82]. As a result of the energy dissipation increase, the

energy intake into a liquid dispersion system increases exponential with the tip speed, leading to an increase in process temperatures. Depending on the material being processed, this has to be taken into account. While emulsions benefit from increased process temperatures due to the reduction of viscosity of the lipid phase [83], dispersion and wet milling of microscale and nanoscale crystals at increased temperatures (>30°C) promotes the formation of agglomerates after processing [78]. For scaling purposes, the influence of the rotor diameter on the tip speed and thus the energy dissipation is of vital importance.

The D27 system had initially a maximum stirring rate of 24,000 rpm. After further development, the system can reach stirring rates of up to 36,000 rpm (tim speed of ~70 m/s), which is considered very high by comparison to other commercially available systems.

As the energy intake rises in the third dimension of the stirring rate, intense cooling of the product is essential for processing at the maximum stirring rate of 36,000 rpm. After implementation of a cooling thermostat, processing at 36,000 rpm was possible, however, cooling was only sufficient for processing times below 3 minutes, which is too short for adequate pretreatment.

One critical weakness of the system is the sealing between the milling chamber and the motor. Stirring at 24,000 rpm worked fine, but at 36,000 rpm, the sealing rings wore off, contaminating the product with gummi particles as well as leading to a leaking milling chamber, with the leaking occurring well before the product was contaminated with gummi particles due to abrasion. Leakage began after 20-30 minutes of stirring (seperated into several experiments, as stirring times of 5 minutes were typically applied). This issue could not be fixed within this study, as there was no alternative sealing available for this high stirring rate. The only option was frequent change of the sealing rings everytime initial slight leakage was detected.

3.1.3 Processing time

As described in the literature [80], focusing on the mass fraction step is most important regarding economic aspects. In the case of the D27 system, short processing times are most economic, as further processing only has a small influence on further crystal size reduction. Depending on the material properties, milling of rutin at 24,000 rpm was proven to be most

efficient for a short time (1-3 minutes), while further processing only had a small impact on the overall crystal size [66, 84].

The aim of the ARTcrystal® technology must lie on a short HSS pretreatment step, as longer processing only leads to economically inefficient attrition of the crystals. The reason or this is the two-step mechanism of the crystal breakage (Figure 2), as in the first step (mass fraction), the crystal breaks into smaller crystals, which are then rounded in the second step during further processing (attrition [80]). This mechanism was proven true during this study.

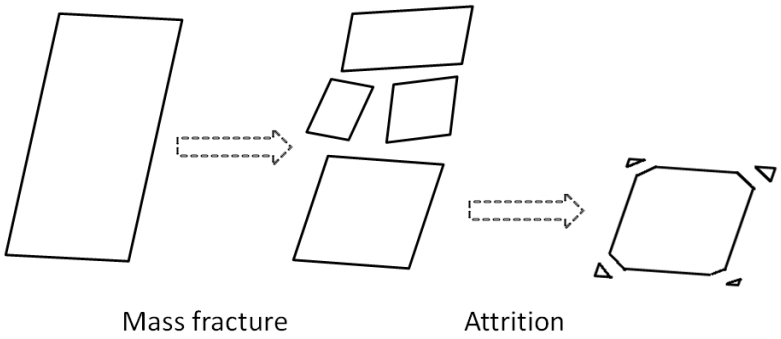

Figure 2 Schematic display of crystal breakage during processing (modified after [66, 80])

3.1.4 Foaming during processing

During the processing of rutin suspensions or stabilizer solutions, intense foaming during initial processing was observed. The foaming was so intense, that the 5 liter container (containing 1 liter of product) was completely filled with foam after only 2 minutes of stirring at 24,000 rpm. This foaming was especially strong when the stabilizer Plantacare® 2000 was used. Other stabilizers (e.g. Tween® 80) tend to foam less, but still high amounts of foam were observed during processing. However, as Plantacare® 2000 was proven to be the best stabilizer for rutin suspensions in earlier studies [85], this stabilizer was chosen for further work. Also, the use of a "problematic" surfactant is especially suited to investigate relevant process parameters, as the process should be adjusted to the formulation and not vice-versa.

3.1.5 Particle size and morphology of the starting material

By processing two qualities of rutin (coarse and jet-milled), first data regarding the influence of the crystal morphology on the final crystal size was produced. Both qualities contained many large particles (d(v)0.99 of 90-120 µm, d(v)0.95 of 60-80 µm). The jet-milled rutin possessed a larger d(v)0.50 of 10-12 µm, while the coarse rutin had a d(v)0.50 of 5-6 µm. Light microscopy revealed that the jet-milled material consisted of small needle-shaped particles in the lower µm-range with many large aggregates, while the coarse material consisted of monolithic cuboid crystals with varying sizes.

Results could show that a deaggregration process is performed within 1-2 minutes of stirring at 24,000 rpm, with nearly no changes in size after further processing. Milling monolithic crystals takes longer, with best results regarding the time-size ratio being obtained after 5 minutes (Figure 3). Further processing leads only to a small decrease in size, mainly by destruction of single remaining larger crystals [81]. In general, it was shown that monolithic crystals below a size of 20-30 µm could not be further broken down, also particles below this range seem to be mainly unaffected during the whole pretreatment process.

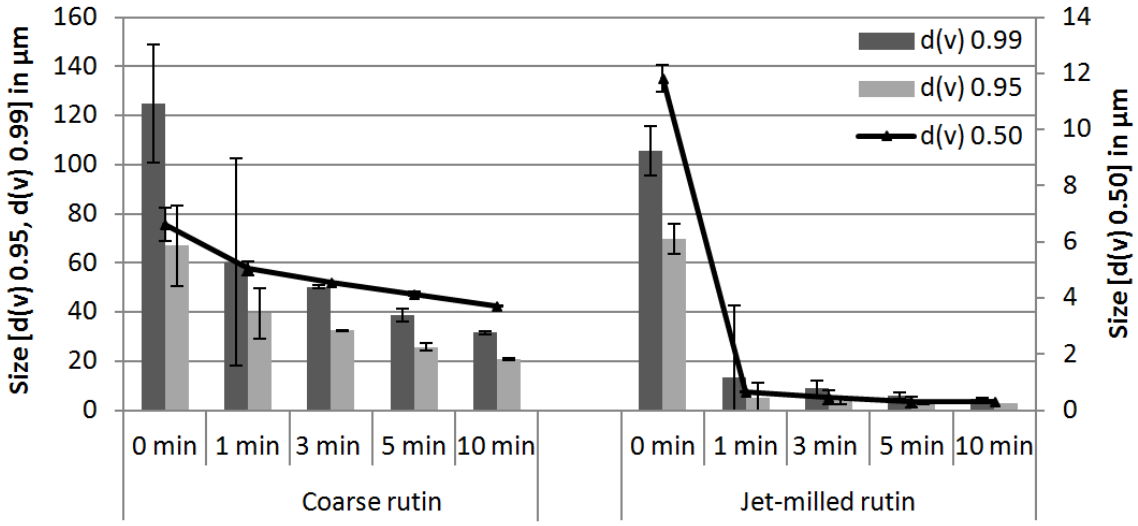

Figure 3 **High speed stirring pretreatment at 24,000 rpm of rutin suspensions (coarse material and jet-milled material; modified after [81])**

3.2 Optimization of the rotor-stator pretreatment step

3.2.1 Reduction of foaming

Foaming can be reduced by several means, e.g. vacuum or a modification of the product circulation tube geometry to prevent air intake. However, an extension of the tube is not beneficial, as it leads to reduced product circulation inside the container, thus feeding the returning material directly again to the milling chamber. As complex redesigns of the system were not achievable in time, reduction of the foam volume should be achieved by reduction of the product flow rate, as high flows tend to increase the air intake when the product stream hits the product surface inside the product container.

Foaming could be reduced by varying the valve position of the product circulation tube. Choosing a valve position of 45° resulted in a reduction of the foam volume by 87% [81]. However, as the flow rate of the product was modified, the impact of the reduced flow rate needed to be investigated.

However, analysis of the foam showed only minor retention of larger crystals inside the foam, thus the foam is supposed to have no direct impact on the milling time by retaining larger particles. Furthermore, the foam tends to stay above the surface of the product in the product container, as reduced foam volume did not seem to impact the milling results [81].

3.2.2 Influence of the flow rate of the product

Reduction of foaming was achieved by changing the valve position of the product circulation tube. However, while the stirring rate was not modified, the product flow was reduced by 61% (from 26.5 liter/min to 10.3 liter/min). This lead conclusively to a slight prolongation of the process time, but did not impact the final crystal sizes and size distributions. For jet-milled and coarse rutin, differences, mainly in the d(v)0.95 and d(v)0.99 values, could be seen after 1 minute of processing time, whereas after 3 minutes, only minor differences in the crystal sizes did occur. After 5 minutes, differences were nivellated [81]. Therefore, working with a reduced product flow is acceptable as long as no complex redesign of the system is undertaken.

3.3 Evaluation of the method's efficacy by comparison to traditional approaches

As the HSS step was proven to be more potent than the classical premixing and premilling HPH step , the number of cycles needed to reach the nanoscale could be reduced from 20 cycles at 1500 bar (classical HPH) to 5 cycles at 500-750 bar (ARTcrystal®) with 5 minutes of pretreatment at 24,000 rpm. Additionally, processing times were distinct shorter in comparison to traditional top-down methods. For example, an experienced user can nanonize rutin with HPH in the laboratory scale within 2 hours, while bead milling takes 4-8 hours. Other combination technologies might even take longer times. Using the ARTcrystal®-technology, nanonization can be achieved within a production time of one hour.

Thus, the ARTcrystal®-technology is superior to classical HPH regarding processing time as well as final crystal size and particle size distribution. This fact could be proven for the flavonoids rutin, hesperidin and apigenin. The results are summarized in Figure 4. As the milling was performed at 24,000 rpm, the results may even be optimized when applying a higher stirring rate. The nanosuspensions were physically stable for at least 90 days at room temperature [86].

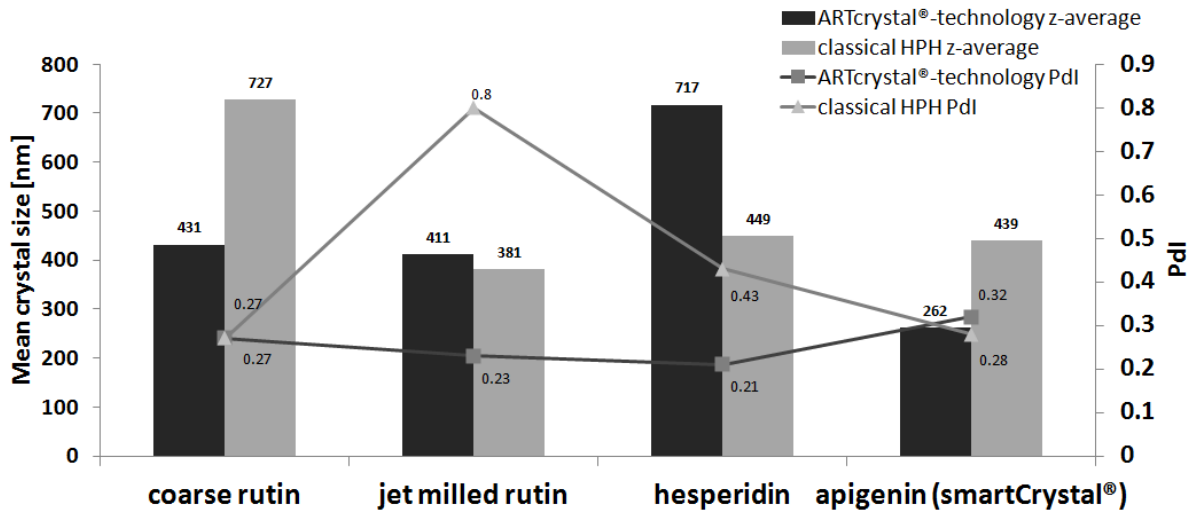

Figure 4 **Comparison of nanonization results (ARTcrystal vs. HPH) for several flavonoids (modified after [86])**

3.4 Further optimization of the ARTCrystal®-method

While these first results were promising, the method still leaves room for improvement. The milling efficacy could be improved by:

- Comparison of continuous versus discontinuous pretreatment
- Increase in stirring rate
- Optimization of the rotor and stator geometry

3.4.1 Continuous versus discontinuous pretreatment

During processing, turbulences inside the product container were observed. As these turbulences can lead to retention of particles in the product container, while other particles, returning from the milling chamber, may be directly fed to the milling chamber again, without circulating inside the container. This may reduce the milling efficacy by leading to an unhomogeneous number of passages of each particle through the milling chamber in a given time. To investigate this influence, continuous processing was compared to discontinuous processing, using coarse rutin and a stirring rate of 24,000 rpm. In the discontinuous part, the product return pipe was turned and the complete suspension was caught in a seperate vessel before it was returned to the product container manually for another cycle.

The results showed an influence of the turbulences inside the product container on the milling efficacy. The raw stirring time could be reduced by the factor 4-5 when discontinuous processing was applied [84]. However, as the process had to be stopped after every cycle and the suspension had to be refilled into the product container manually, the actual processing time can be considered longer in comparison to continuous stirring. Nevertheless, these results are valuable for potential redesign, e.g. coupling of two devices where complete passage of the whole suspension through one milling chamber can be assured before a new passage, thus reducing energy intake into the product and reducing the requirements for cooling.

3.4.2 Increase in stirring rate

In the patent as well as during the first tests, the working stirring rate was 24,000 rpm. However, as the system is able to work at a maximum stirring rate of 36,000 rpm, the process was optimized in order to support this high stirring rate.

As redesign of the system to support better cooling was not possible, cycles of high and low intensity were applied. The high intensity part was carried out at 36,000 rpm, while the low intensity part was carried out at 5,000 rpm. This way, the energy intake was reduced during the low intensity part to allow cooling, while eliminating the problem of particle sedimentation and possible subsequent blockage of the milling chamber.

Tests have shown that cycles of 1 minute at 36,000 rpm, followed by 5 minutes at 5,000 rpm, are best suited for processing at temperatures below 30°C. in this constellation, several cycles can be applied, thus enabling working at 36,000 rpm. Reduction of the low intensity part resulted in an insufficient time for the suspension to cool down in the product container [84]. However, as mentioned in chapter 3.1.2, the sealing issue is still not fixed.

3.4.3 Optimization of the rotor and stator geometry

The rotor and stator used for this work were a standard rotor and stator with a gap size of 0.1 mm and a slit size of 1.0 and 0.5 mm, respectively. As hydrodynamic shear stress is the main driving force for particle size reduction in rotor-stator processes, the shear stress can be increased by varying and optimizing the geometry of the rotor and stator. Six combinations of rotors and stators were tested to estimate influences of the rotor stator geometry. Rotors and stators differed in slit size, gap size and surface properties. In order to assess the milling potential, ibuprofen was chosen as testing material, as it is very ductile and thus hard to nanonize by top-down methods [87].

Processing at 24,000 rpm with these six setups revealed that the surface geometry and orientation of rotor and stator teeth had the strongest impact on the milling results. The combination of a sharp saw-toothed rotor with slant slits and a standard stator with narrow slits achieved the smallest crystal sizes within 5 minutes of stirring (d(v)0.50 of 6.2 µm, d(v)0.99 of 35,0 µm). The values between the setups differed mainly in the range of d(v)0.90-0.99, indicating that only the largest crystals are affected when the shear stress is varied by changes in the rotor and stator geometry. In general, small slit and gap sizes are desirable and achieve better milling results, but slant rotor slits in combination with straight stator slits lead to the strongest increase in shear stress [66].

3.4.4 Application of optimized parameters

Applying these optimized parameters (optimized cooling, stirring rate of 36,000 rpm, optimized rotor and stator geometry), ibuprofen was milled by ARTcrystal®-technology and the results were compared to HPH and BM. It was shown that even in case of resistant materials like ibuprofen, the HSS pretreatment allows reduction of the required HPH cycle number. The HSS pretreatment replaced the traditional pretreatment, allowed the reduction of HPH cycles from 40 to 15 and enabled nanonization of monolithic crystals with sizes >100 µm. Equal crystal sizes could be achieved (933 vs. 929 nm). While reduction of the pressure is only for materials with limited crystal hardness and ductility applicable, the HSS pretreatment optimizes the HPH process regarding economical efficacy. Wet bead milling could achieve crystal sizes below 400 nm, although the process took a large amount of time (60 hours) and was performed in a very small scale (2 mL) under cooling over the whole duration. Therefore, while being superior regarding the final crystal size, wet bead milling is limited by its process duration as well as scalability [66].

3.5 Further application possibilities for the D27 system

With an increased stirring rate of 36,000 rpm, the D27 system could be useful for the production of nanoemulsions, as it can process large quantities in a short time. According to the literature, rotor-stator systems are not capable of producting nanoemulsions, they are only acle to produce fine emulsions with mean droplet sizes in the lower µm-range [88, 89]. In this work, additional to the ARTcrystal®-technology, the potential of the D27 system as standalone system for nanoemulsion production was tested and assessed.

While the stirring rate of 24,000 rpm could produce an emulsion in the submicron range (255 nm), the resulting emulsion still processed droplets in the micrometer scale (d(v)0.99 1.95 µm), thus a broad droplet size distribution. Therefore, 24,000 rpm as stirring rate was not powerful enough for nanoemulsion production. Increasing the stirring rate to the maximum of 36,000 rpm allowed droplet size reduction into the nanoscale (136 nm), with a more narrow size distribution and no microscale droplets present (d(v)0.99 0.28 µm). The nanoemulsions showed no sign of coalescence or Ostwald ripening during 90 days of storage at room temperature [83].

Regarding the final droplet size, the process is inferior to HPH (136 vs. 93 nm), with nearly similar maximum droplet sizes (d(v)0.99 of 0.24 vs. 0.28 µm). Nevertheless, 1 liter of nanoemulsion could be produced within 3-5 minutes, pointing out the potential for economically efficient large scale nanoemulsion production [83].

In addition to the nanoemulsification experiments, the potential of the D27 system to destroy bacteria was tested. High pressure homogenization is able to destroy bacteria by cavitational forces. It was revealed that the D27 system is not capable of sterilizing a solution containing E.coli bacteria at 24,000 rpm as well as at 36,000 rpm. With a starting concentration of 6,1 * 10^8 and 4,0 * 10^6 bacteria/ml, the concentration was reduced to 7,0*10^6 and 2,6*10^6 bacteria/ml after 5 minutes at 24,000 rpm or 10 minutes after 36,000 rpm, respectively. The temperature was kept below 30 °C at all times. By comparison, high pressure homogenization using an Emulsiflex C50 resulted in a reduction from 4,1*10^9 bacteria/ml from the start to 2,4*10^4 after three passages [90]. Therefore, it must be concluded that the D27 system is not capable of producing bacteria-free products.

4 PUBLICATIONS

4.1 ARTcrystal®-process for industrial nanocrystal production - Optimization of the ART MICCRA pre-milling step

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Scholz, P.; Arntjen, A.; Müller, R.H.; Keck, C.M.; ARTcrystal®-process for industrial nanocrystal production - Optimization of the ART MICCRA pre-milling step. International Journal of Pharmaceutics, 465 (2014): p. 388-395

http://dx.doi.org/10.1016/j.ijpharm.2014.02.026

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

4.2 Microcrystals and Nanocrystals produced by Rotor-Stator-High-Speed-Stirring

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Scholz, P.; Arntjen, A.; Keck, C.M.; Microcrystals and Nanocrystals produced by Rotor-Stator-High-Speed-Stirring. PharmInd 08/16, 2016, p. 1196-1207.

http://www.ecv.de/suse_item.php?suseId=Z|pi|8540&susePattern=

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

4.3 Flavonoid nanocrystals produced by ARTcrystal®-technology

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Scholz, P.; Keck, C.M.; Flavonoid nanocrystals produced by ARTcrystal®-technology. International Journal of Pharmaceutics, 482 (2015): p. 27-37.

http://dx.doi.org/10.1016/j.ijpharm.2014.11.008

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

4.4 Ibuprofen nanocrystals produced by ARTcrystal-technology

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Scholz, P.; Keck, C.M.; Ibuprofen nanocrystals produced by ARTcrystal-technology. PharmInd 9/16, 2016, p. 1340-1349.

http://www.ecv.de/suse_item.php?suseId=Z|pi|8570&susePattern=

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

4.5 Nanoemulsions produced by rotor-stator high speed stirring

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Scholz, P.; Keck, C.M.; Nanoemulsions produced by rotor-stator high speed stirring. International Journal of Pharmaceutics, 482 (2015): p. 110-117.

http://dx.doi.org/10.1016/j.ijpharm.2014.12.040

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

5 CONCLUSION

This work could prove that the ARTcrystal®-method is able to reduce processing times in comparison to classical top-down methods while holding the potential for easy upscaling. After an initial investigation of relevant process parameters and optimization of the pretreatment step, the process allowed the efficient production of nanosuspensions. As shown for the flavonoids rutin, hesperidin and apigenin, a short HSS pretreatment was sufficient to reduce both the required cycle number as well as the required pressure in the HPH step for nanonization.

It was shown that the rotor geometry impacts the HSS pretreatment results. Especially the surface and orientation of the teeth were proven to be important factors for increasing shear forces and thus enhance the breakdown of the crystals. The slit size and gap size played a minor role, as slit and gap sizes of 1mm/0.1mm were proven to be efficient. Further reduction of the slit size did not improve the diminution efficacy, while an increase in the gap size did slightly reduce the milling efficacy. As proven by laser diffraction, the changes in rotor geometry mainly affected the final size of the remaining larger crystals (d(v)0.99), while the main population of smaller crystals (d(v)0.50) was not further diminuted. As the HSS pretreatment aims at the production of a homogeneous intermediate suspension with a narrow size distribution, the elimination of larger crystals is vital. The stirring rate was proven to be the most important factor, as it is mainly responsible for the energy intake and thus the generation of shear stress for the crystal breakage.

After further optimization, even mechanical resistant materials (in this study ibuprofen) could be nanonized. A reduction of the required pressure could not be achieved, nevertheless, the HSS pretreatment reduced the required cycle number from 40 to 15, thus economically optimizing the process by reducting the production time.

The development of the MICCRA D27 allowed an increase of the stirring rate from initial 24,000 rpm to 36,000 rpm. While stirring at 24,000 rpm was not sufficient for the production of fine nanoemulsions with a narrow droplet size distribution, processing at 36,000 rpm allowed the formation of nanoemulsions. Earlier rotor-stator systems were only able to achieve droplet sizes in the range of 1-2 µm, but at a maximum stirring rate of 36,000 rpm, the D27 was able to break the nanoscale border. The formation of nanoemulsions by rotor-

stator technology has become accessible due to this technological breakthrough. Furthermore, as the production of nanoemulsions is possible, the system should be able to produce lipid nanoparticles as well, as the process temperature can be controlled by using an external thermostat.

In conclusion, due to the recent development of in-line rotor-stator technology, the ARTcrystal®-method is able to produce nanosuspensions faster and more economically in comparison to classical top-down methods. Even when used as standalone process, rotor-stator technology can produce lipid based nanoparticulate systems. This makes rotor-stator technology versatile as well as economically interesting for industrial applications in the field of pharmaceutical nanotechnology.

6 SUMMARY

Poor solubility of newly developed active pharmaceutical ingredients is an issue of increasing importance, as many new substances are poorly soluble in water. Several approaches to circumvent solubility issues have been made, e.g. solubilization, amorphization or solid solutions. One elegant approach is the nanozation of actives in order to increase the dissolution velocity as well as the temporary saturation solubility. Another advantage of nanocrystals is the reduction in the variation of the oral bioavailability between fasted and fed state.

In the recent years, several techniques have been developed and optimized to achieve nanocrystals with sizes as small as possible. While the focus of these techniques was on the final crystal size, scalability and economic aspects of these techniques were neglected. These factors are now addressed by a new combination technology called ARTcrystal®-method. The ARTcrystal®-method consists of a rotor-stator pretreatment step and subsequent high pressure homogenization at reduced pressure and cycle numbers. Prior to this work, this concept was initially tested and it was revealed that this method holds the potential to be used for industrial applications due to faster and cheaper processing of suspensions. However, the ARTcrystal®-method itself was not further investigated in detail, so that critical process parameters as well as optimization possibilities and limits of this method were unknown. The aim of this thesis was to investigate these aspects in order to fully reveal the potential of the ARTcrystal®-method for nanocrystal production as well as to investigate further uses and potentials for the high speed stirring pretreatment step.

Combining a HSS pretreatment with subsequent HPH was proven to shorten the production time of nanosuspensions. Initial investigation and optimization of the pretreatment step showed that a short pretreatment of 5 minutes is the most effective way for milling crystals. The HSS pretreatment mainly affected aggregates, agglomerates and large microscale crystals, thus producing a more homogeneous intermediate suspension. As discussed in the literature, it was shown that cooling is important for preventing post-processing agglomeration of micro- and nanocrystals. Foaming during the pretreatment could be reduced, however, its impact on the milling results is neglectable.

The nanonization of several flavonoids resulted in nearly similar final sizes compared to traditional HPH, but in a shorter process time. 1 liter of suspension can be processed at 24,000 - 36,000 rpm within 5 minutes into an intermediate suspension with particle sizes in the lower microscale. This intermediate suspension could be transformed into a nanosuspension within 5 cycles of reduced pressure (300-750 bar for flavonoids, depending on the material properties).

During further process optimization, it was shown that the high-speed stirring process requires intensive cooling. As the energy intake rises with the stirring rate, the product is heated to over 50 °C during processing at 36,000 rpm within minutes when no cooling is applied. Since in-process temperature is of vital importance for the stability of the final suspension, powerful cooling in combination with an alternating process intensity had to be applied to keep the temperature below 30 °C at a stirring rate of 36,000 rpm. When the system is redesigned to allow discontinuous processing, the process can be even more efficient, as shorter stirring times and thus less energy intake into the product is necessary to achieve similar results in comparison to continuous processing.

The stirring rate was proven to be the most influencing parameter on the final crystal size, followed by the rotor surface and teeth orientation. Reduction of the rotor slit size below 1.0 mm did not improve the milling results, also an increase of the gap size did only slightly affect the milling efficacy.

For ibuprofen, a stirring rate of 36,000 rpm had to be applied in order to achieve the smallest possible crystal sizes. Nanonization of ibuprofen was possible within 15 cycles of HPH at 1500 bar after a 5 minute pretreatment. Given the fact that classical HPH required 40 cycles to achieve the nanoscale, the short pretreatment reduced the production time massively. While bead milling can achieve even smaller final crystal sizes in comparison to high pressure homogenization or ARTcrystal®-technology, the process is very time consuming and has limited scalability possibilities. Processing of such resistant materials as ibuprofen can take several days in order to achieve minimum possible crystal sizes.

At a maximum stirring rate of 36,000 rpm, the MICCRA D27 was able to produce large quantities of nanoemulsions within minutes. Reduction of the slit size did slightly reduce the processing time. The final droplet size was 135 nm, distinctly larger in comparison to high pressure homogenization (< 100 nm). Nevertheless, a volume of 1 liter could be processed

within 3-5 minutes, depending on the rotor-stator setup. This means high speed stirring is able to produce large amounts of nanoemulsions faster than high pressure homogenization (lab scale equipment). The D27 system was able to reduce the bacteria count in a solution but is not able to sterilize the product during processing. Therefore, when a sterile product must be obtained, additional sterilization methods must be applied.

The ARTcrystal®-technology was proven to be able to produce nanocrystals in a distinctly shorter time compared to bead milling and high pressure homogenization. Therefore, this technology is interesting for industrial applications aiming at a fast and economic production of nanocrystals.

7 ZUSAMMENFASSUNG

Die schlechte Löslichkeit neuer Arzneistoffe in Wasser ist ein Problem mit zunehmender Bedeutung für die Entwicklung neuer Arzneimittel. Es gibt verschiedene Ansätze, dieses Problem zu lösen, wobei die Nanonisierung des Arzneistoffes einen besonders einfachen und eleganten Weg darstellt. Durch die Überführung der Kristalle in den Nanomaßstab wird die Lösungsgeschwindigkeit sowie die temporäre Sättigungslöslichkeit erhöht. Ein weiterer Vorteil ist die Verringerung von Schwankungen in der Bioverfügbarkeit bei oraler Applikation bedingt durch Einflüsse des Mageninhaltes zum Zeitpunkt der Applikation.

Über die Jahre wurden verschiedene Technologien zur Nanonisierung von Wirkstoffen entwickelt und optimiert, um möglichst kleine Nanokristalle zu produzieren. Hierbei wurde typischerweise eine Verringerung der Kristallgröße auf Kosten der Möglichkeit des Upscalings sowie unter Vernachlässigung ökonomischer Aspekte erreicht. Ein neuer Prozess, die ARTcrystal®-Technologie fokussiert nun genau diese Aspekte. Hierbei handelt es sich um ein Kombinationsverfahren bestehend aus einer Rotor-Stator-Vorbehandlung, gefolgt von einem Hochdruckhomogenisationsprozess bei reduziertem Homogenisationsdruck und verringerter Zyklenanzahl. Im Vorfeld wurde ein erster Versuch durchgeführt, der zeigte, diese Technologie aufgrund stark verkürzter Produktionszeiten ein großes Potenzial für die industrielle Anwendung besitzt. Da die ARTcrystal®-Technologie allerdings nicht genauer untersucht wurde, waren kritische Prozessparameter, Optimierungsmöglichkeiten sowie Grenzen in der Anwendbarkeit nicht bekannt. Somit war es das Ziel dieser Arbeit, diese Aspekte näher zu untersuchen, um das volle Potenzial sowie Limitierungen dieser Technologie aufzuzeigen.

Die Kombination einer kontinuierlichen Rotor-Stator-Vorbehandlung einer Suspension mit anschließender Hochdruckhomogenisation erlaubt eine enorme Verkürzung der Prozessdauer. Durch die Rotor-Stator-Vorbehandlung werden Agglomerate, Aggregate und größere monolithische Kristalle zerstört, wodurch die resultierende Suspension eine verbesserte Homogenität aufweist. Zur Vorbehandlung reicht typischerweise eine fünfminütige Prozessierung bei 24.000 rpm aus. Kühlung des Produktes wurde als kritischer Prozessparameter identifiziert, da eine unzureichende Kühlleistung zu Agglomeration von Kristallen nach der Produktion führt. Die intensive, durch den Stabilisator bedingte

Schaumbildung konnte reduziert werden, hat sich aber bezüglich der Mahlleistung als vernachlässigbar erwiesen.

Es konnte gezeigt werden, dass die ARTcrystal®-Technologie Flavonoid-Suspensionen nach fünfminütiger Vorbehandlung innerhalb von 5 Zyklen bei 300-750 bar Homogenisationsdruck nanonisieren kann. Somit erwies sich diese Technologie als überlegen gegenüber klassischer Hochdruckhomogenisation.

Aufgrund des sehr hohen Energieeintrags bei hohen Umdrehungsgeschwindigkeiten, insbesondere bei 36.000 rpm, wird für den Prozess eine intensive Kühlung benötigt. Ohne Kühlung konnten während des Prozesses Temperaturen von über 50 °C gemessen werden. Da die Inprozesstemperatur aber einen entscheidenden Einfluss auf die Stabilität von (Nano)Suspensionen hat, musste eine leistungsstarke Kühlvorrichtung in Kombination mit wechselnder Prozessintensität verwendet werden, um eine Inprozesstemperatur von unter 30 °C sicherzustellen. Ein Redesign des Systems, welches eine diskontinuierliche Prozessierung erlaubt, könnte die Effizienz noch steigern, da sich die effektive Mahldauer und somit der Kühlungsbedarf reduzieren lässt.

Die Umdrehungsgeschwindigkeit zeigte den größten Einfluss auf das Mahlergebnis, gefolgt von der Beschaffenheit der Rotoroberfläche und Orientierung der Rotor-/Statorzähne. Eine weitere Reduktion der Rotorschlitzbreite unter 1,0 mm konnte die Mahlgüte nicht verbessern, ebenso eine Vergrößerung der Spaltgröße erwies sich als untergeordnete Einflussgröße.

Für die Nanonisierung von Ibuprofen musste eine Umdrehungsgeschwindigkeit von 36.000 rpm gewählt werden, um eine ausreichende Zerkleinerungsleistung zu erzielen. Für Ibuprofen konnte eine Reduktion des Druckes nach fünfminütiger Vorbehandlung nicht erreicht werden, aber die Zyklenanzahl konnte von 40 auf 15 reduziert werden. Hierdurch lässt sich die Prozessdauer stark verkürzen. Im Vergleich zur Hochdruckhomogenisation kann eine Perlmahlung noch kleinere Kristallgrößen erreichen, der Prozess ist aber nur begrenzt skalierbar und sehr zeitintensiv.

Als Prozessparameter mit dem größten Einfluss wurde die Rotationsrate ermittelt, gefolgt von der Oberflächengeometrie und Schlitzorientierung des Rotors. Die weitere Reduktion

der Rotorschlitzgröße führte zu keinem verbesserten Mahlergebnis, während die Vergrößerung des Spaltes zwischen Rotor und Stator nur einen kleinen Einfluss ausübte.

Bei 36.000 rpm konnte der MICCRA D27 einen Liter Nanoemulsion innerhalb von 3-5 Minuten herstellen. Eine Reduktion der Rotorschlitzbreite führte zwar nicht zu kleineren Tröpfchengrößen, konnte aber die Prozessdauer leicht verkürzen. Die finale Tröpfchengröße lag bei 135 nm, somit oberhalb mittels Hochdruckhomogenisation erzielten Tröpfchengrößen (< 100 nm). Da sich aber Nanoemulsionen in großen Mengen innerhalb kurzer Zeit herstellen lassen, ist der Prozess dennoch auch für die Nanoemulsifikation interessant. Sterilisation mittels Rotor-Stator-Technologie hat sich als nicht durchführbar erwiesen, wobei aber zumindest eine Reduktion der Keimzahl erreicht werden konnte. Um ein steriles Produkt zu erhalten, müssen demnach zusätzliche Maßnahmen getroffen werden.

Schlussfolgernd kann festgestellt werden, dass die ARTcrystal®-Technologie in der Lage ist, schneller und ökonomischer Nanosuspensionen herzustellen als klassische Methoden. Daher ist die ARTcrystal®-Technologie insbesondere für industrielle Applikationen interessant.

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9 LIST OF PUBLICATIONS

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Nano modified antioxidants for the prevention and treatment of neurogenerative diseases

DPhG-Jahrestagung, Düsseldorf, 23.-25. September 2015

10 CURRICULUM VITAE

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