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Rapid development of API nano-formulations from screening to production combining dual centrifugation and wet agitator bead milling

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ABSTRACT

Various wet ball nanomilling-screening tools for poorly soluble APIs are available which differ in their milling principle, batch size and number of samples. Here, the transferability of results from screening (small to medium-scale) to pharmaceutical production (largescale) was investigated. Wet ball milling in a dual centrifuge (DC) (10–100 mg API, 40 samples in parallel) was used to identify stable nanoformulations. In addition different sized agitator bead mills were used for scale-up to industrial scales. DC-and small-scale agitator milling (AM) resulted in small and virtually identical API-particles. Additionally, similar API-particles were obtained using two different sized agitator bead mills (batch size 1.5 and 30 kg) and applying comparable specific grinding energies (SGE). The SGE used in the trials represents the grinding limit for this API-suspension. Using lower SGEs, AM results in larger API-particles. All used milling tools had no influence on the APIs crystal structure and wear of grinding media (Zr/Y) is low. The study confirmed the importance to choose the right formulation and process parameters, which positively affect grinding efficacy, particle size distribution and wear contamination. The excellent comparability of results obtained from DC-milling and AM significantly reduces the duration for successful and predictable formulation development.

1. Introduction

Most of the newly developed APIs are poorly soluble (Loftsson and Brewster, 2010) which leads to more complex preclinical studies, clinical trials and thus pharmaceutical formulation development. To get the full pharmacological potential of an API, one has to make sure that the drug will be dissolved in an appropriate time period as a pre-requisite for its passive or active absorption in the GI tract (Liversidge and Conzentino, 1995).

To improve the solubility of poorly soluble APIs, one strategy is to reduce the API particle size to nano scale. Due to that, the surface area of the particles is strongly increased which results in much higher dissolution rates according to Noyes Whitney (Buckton and Beezer, 1992) as well as Nernst and Brunner (Brunner, 1904; Nernst, 1904) equation. Furthermore, the now strongly curved surface of the nanoparticles results in an oversaturation according to Ostwald Freundlich (Keck and Müller, 2006; Simonelli et al., 1970; Thomson, 1872).

However, not only the API solubility as such is increased due to the particle size reduction, also the dissolution rate is strongly increased, which is discussed to cause (i) an decrease of t-max and, (ii) a higher AUC (Area Under Curve) which may lead to lower therapeutic doses and reduced adverse effects as well as reduced food effects (Liversidge and Conzentino, 1995; Juenemann et al., 2011; Jinno et al., 2006; Junghanns, 2008).

The most important approach to obtain API-nanoparticles is wet bead milling of aqueous API suspensions containing polymer(s) and/or surfactant(s) for particle stabilization (e.g. avoiding of re-agglomeration) (Junghanns, 2008). As the name suggests, wet bead milling requires only water but no organic solvents as media, which is of great

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advantage. Wet ball milling is highly reproducible, the batch to batch variation of the particle sizes are low (Chin et al., 2014). To further stabilize the resulting nanosuspensions, they can subsequently be treated by lyophilisation, spray-/freeze drying or can be fixed on a carrier material as stable interim products by layering or granulation (Eerdenbrugh et al., 2008) resulting in granules or pellets. These “dry” suspensions, which cannot undergo Ostwald ripening, can finally be used to manufacture solid dosage forms like tablets and granules/pellets for capsule and stick pack filling.

There are numerous grinding machines with different basic milling principles available with the common aim of deagglomeration and true comminution of primary particles. These particles are stressed by mechanical impact forces and friction caused by grinding media (milling beads) accelerated by e.g. an agitator and/or centrifugal forces, respectively (Kesisoglou et al., 2007; Romero et al., 2016).

The agitator bead mills used during this study are equipped with horizontal stationary grinding chambers and rotating hollow slotted pin agitator shafts. With this “pin design”, higher energy densities can be achieved compared to the classic milling setup with (perforated) disc agitators (Breitung-Faes and Kwade, 2008). For the separation of the grinding media from the API-suspensions, centrifugal forces are utilized in combination with a stationary slotted screen instead of using an annular gap. Moreover, due to the high centrifugal forces present in the mill, the exposure of the separation screen cartridge to the grinding media is reduced and much smaller milling bead sizes can be used in this type of mills.

While agitator mills are perfect for medium or high batch sizes, wet bead milling of very small batches of API suspensions can successfully be performed by dual centrifugation (DC). DC differs from common centrifugation by an additional rotation of the samples during centrifugation, resulting in a very fast and powerful movement of sample material inside the vials. In combination with milling beads this leads to an effective mixing, homogenization and highly effective milling. Recently, DC has successfully been used for the very fast and effective wet bead milling of different APIs during pharmaceutical formulation development (Hagedorn et al., 2017). Effective DC-milling is possible with sample amounts down to 100 mg (corresponding to 10 mg API). 40 samples can be milled in parallel, the time to reach the grinding limit is < 90 min. Parallel DC-milling in combination with a design of experiment (DoE)-approach has already been used for the very rapid development of stable nanosuspensions of various poorly soluble APIs (Hagedorn et al., 2017).

While DC-milling is very suitable for a broad API nano-formulation screening in a very short time, its batch sizes are rather small for pharmaceutical production. Thus, to produce a distinct API nano-formulation developed by using the DC-milling approach in larger batch sizes, larger agitator bead mills have to be used. To easily transfer the formulations developed by DC-milling into the production process using agitator bead mills, it would be particularly desirable that the API-particle sizes and size-distributions of a certain formulation (API/polymer/surfactant-combination) are the same, either processed by DC- or agitator-milling (AM).

To investigate if the particle sizes and size distributions obtained from DC-milling are predictive for particle sizes and size distributions resulted from milling with agitator bead mills, in the first part of this study the production of API nanosuspensions either processed by dual centrifugation or by a small-sized agitator bead mill (DeltaVita 300, Netzsch, Selb, Germany) are compared using default milling conditions. Since small planetary ball mills have often been used for formulation development of API-nanosuspensions in the past, the milling process using a small planetary ball mill (Pulverisette 7, Fritsch GmbH; Idar-Oberstein, Germany) have been investigated as well.

In the second part of the study the comparability of API-particle sizes and distributions over all stages of pharmaceutical development as well as production were investigated based on the specific grinding energy (SGE) used for the respective milling trials. Related to the increasing grinding energy gained by typical scale-up trials using the laboratory mill DeltaVita 300 (DV300, max. 1.5 kg), pilot-scale production using the DeltaVita 600 (max. 3 kg) as well as manufacturing of the API-particles in the production scale using a DeltaVita 10,000 (max. 50 kg), the SGE could be a parameter for the prediction of API particle size distribution of the resulting nanosuspensions. However, since all tested wet milling devices have a different design which resulted in different mechanical impact forces of the grinding media, the wearing of the ceramic beads used as grinding media as well as the resulting crystal shape and structure of the APIs were investigated.

2. Materials and methods

2.1. Materials

Naproxen (Ph. Eur., 99.7% micronized) was purchased from Zhejiang Charioteer Pharmaceutical Co., Ltd. (Zhejiang, China). Fenofibrate (Ph. Eur., 99.0% micronized) was purchased from Alembic Pharmaceuticals limited (Gujarat, India). Ibuprofen (Ph.Eur., 99.8% micronized) was purchased from Shasun Chemicals and Drugs Ltd. (Puducherry, India). Hypromellose (HPMC, 3 mPa·s) was purchased from Shin-Etsu Chemical (Tokyo, Japan). Polyvinylpyrrolidon (PVP) 25 K, and Sodium Dodecyl Sulfate (SDS) were purchased from BASF SE (Ludwigshafen, Germany). Tween 80 was ordered from Merck (Darmstadt, Germany) and Sodium-Docusate (DOSS) from Solvay (New Jersey, USA). As grinding media for all trials (dual centrifugation, planetary ball milling, agitator bead milling), yttria-stabilized zirconia (YSZ) beads called TOSOH YTZ grinding media with a diameter of 0.3 mm (Nikkato Corporation, Sakai, Japan) were used. Highly stable 2 mL DC-Twist-Top-vials were purchased from Andreas Hettich GmbH & Co KG (Tuttlingen, Germany).

2.2. Milling equipment and methods

2.2.1. Preparation of API-suspensions

All amounts of the formulation components are given as percentage by mass (% w/w). In this work, three APIs were used for the preparation of the nanosuspensions. Naproxen, Fenofibrate and Ibuprofen were used in a micronized quality which is defined by a D90 value < 10 µm. For the comparison of dual centrifugation (DC) and agitator bead milling (AM) the same formulation containing 20% of Naproxen, 0.5% of DOSS and 3% of HPMC (3 mPa·s) was used.

The API-suspensions for the DC and planetary ball milling experiments were prepared as follows: Weigh milling beads and API in the DC-Twist-Top-Vial or the planetary ball milling bowls. Polymer and surfactant are pre-dissolved in purified water and added. Afterwards the suspensions are further diluted with purified water. Sample preparation for the investigation of the DC-system and the planetary ball mill differs only in batch size.

With regard to agitator-milling the given amounts of polymer and surfactant are dissolved in purified water under stirring. Subsequently, the API is added and the suspension is stirred until homogeneous distribution. The entire microsuspension is stirred until transfer to the milling chamber to avoid sedimentation of API particles. This preparation process was used for all agitator-milling trials. Fig. 1 shows that this preparation method leads to comparable initial suspensions – independent of the batch size (A) but dependent on the used API (B).

2.2.2. Dual centrifugation (DC)

DC was performed using a ZentriMix 380R (Andreas Hettich GmbH und Co KG, Tuttlingen, Germany). As milling conditions a rotation speed of 1500 rpm for a runtime of 90 min was used. For every milling trial the cooling device was set to 0 °C (measured in the rotating chamber, which results in sample temperatures of approx. 18 °C after 90 min milling at 2000 rpm/1000 mg milling beads). Since this is the maximum DC-speed and milling time used in this investigation, it can

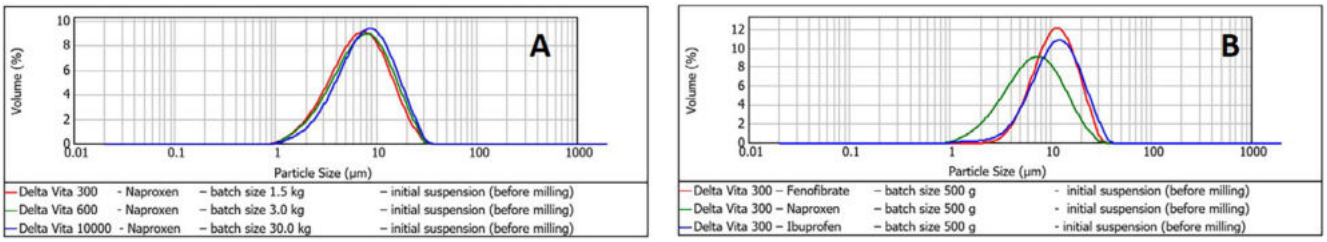

Fig. 1. A: Initially prepared Naproxen suspensions before agitator milling in different mills: Delta Vita 300 (red line), Delta Vita 600 (green line), Delta Vita 10,000 (blue line). B: Suspensions prepared with different APIs in a 500 g batch scale. Fenofibrate suspension (red line), Naproxen suspension (green line) and Ibuprofen suspension (blue line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 2. 2 DC-Twist-Top-Vial (PP), sample adapter (middle) and rotor including sample adapters (right) of dual centrifuge.

be assumed that the process temperature was always below 18 °C in all experiments. In Fig. 2 the Twist-Top-Vial, the rotor including the sample adapters and the dual centrifuge principal is shown.

2.2.3. Planetary ball mill

Planetary ball milling was performed using a Pulverisette 7 (Fritsch GmbH, Idar-Oberstein, Germany). Per milling trial two 45 mL bowls (Fritsch GmbH, Idar-Oberstein, Germany) each filled with 10 g of API-suspension. This is the minimum reasonable batch size for this equipment using the mentioned 45 mL bowls (note: smaller bowls are available as well). Milling conditions were 750 rpm over 14 cycles of 30 min interrupted by cooling breaks of 5 min (total milling time: 7 h). The defined parameters reflect a standard process often used in industry as well as academia. However, some groups work also with different milling speeds, milling bowls and milling times, but the optimization of the planetary wet ball milling process was not the topic of this work.

2.2.4. Agitator bead milling (AM)

For lab-scale purposes the following equipment (Fig. 3) was used: a DeltaVita 300 (Netzsch) with a milling chamber and agitator made of yttria-stabilized zirconia (YSZ) with a milling chamber volume of 300 mL. Regarding pilot-scale, a DeltaVita 600 (Netzsch) was operated with a milling chamber and agitator made of YSZ and a volume of 550 mL. For production scale a DeltaVita 10,000 (Netzsch) with a YSZ milling chamber as well as YSZ agitator and a chamber volume of 10 L was used. The general design of the DeltaVita machines of different size is similar. Because of the change of the grinding chamber volume, number of pin rows as well as number of pins per row, number of slots in the hollow agitator shaft and distances between pin and grinding chamber wall variate. Comparable geometries (agitator shaft), similar grinding media conditions (type, filling level, size) as well as similar process set-ups (passage or circulation mode, agitator speed) have to be assured to successfully perform a scale-up. Further, the pump speed has to be adapted on the size of milling chamber to get a value for the number of theoretical cycles of the suspension through the milling chamber and – as wet bead milling is a high-energy consuming process – process temperatures have to be well controlled.

All agitator-milling trials were performed using circulation mode for

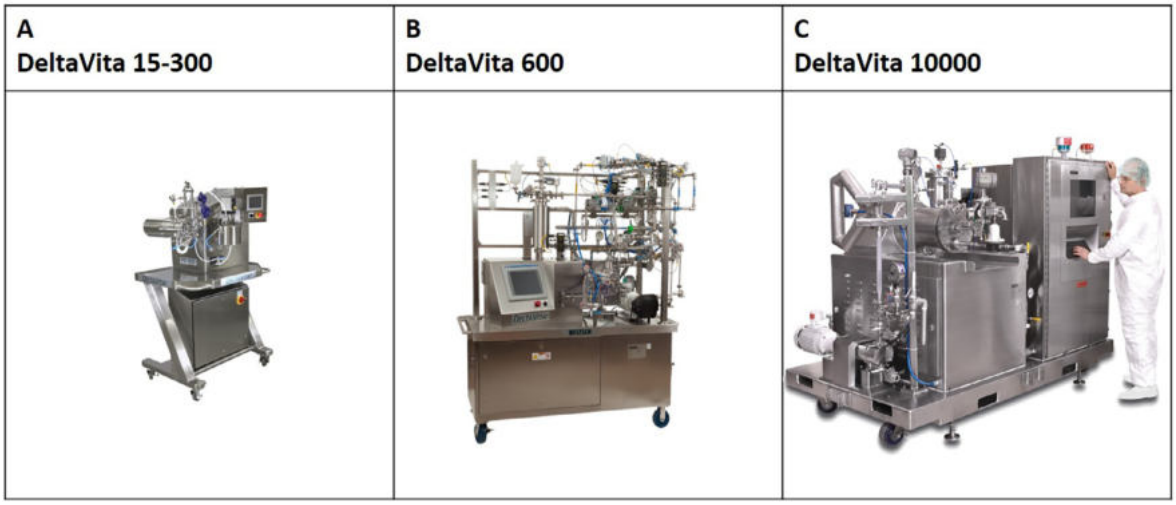

Fig. 3. Milling configurations of used agitator mills. DeltaVita 300 (A), DeltaVita 600 (B) and DeltaVita 10,000 (C).

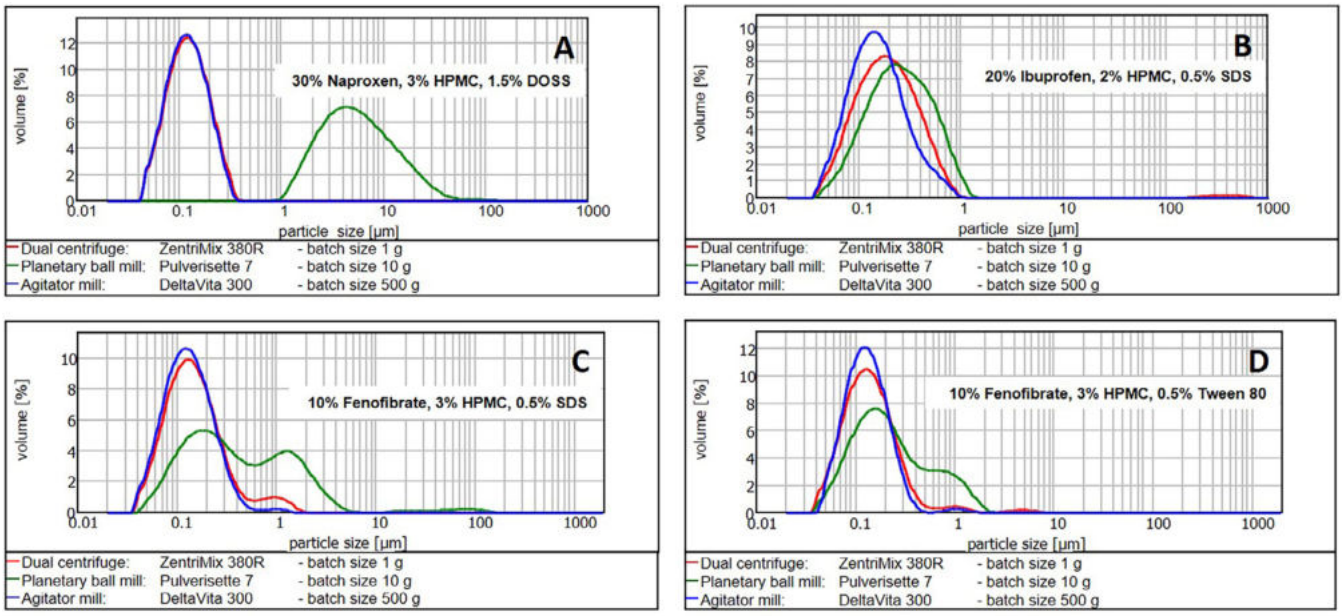

Fig. 4. Comparison of the three milling techniques dual centrifugation-milling (Hettich ZentriMix 380R, red), planetary ball milling (Fritsch Pulverisette 7, green) and agitator-milling (Netzsch DeltaVita 300, blue) using different formulations for the preparation of API-nanosuspensions. Standard milling conditions for each technique as well as typical batch sizes of 1 g, 10 g, and 500 g were used, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

a milling period of 270 min. The milling chambers were filled with 0.3 mm grinding media with a grinding media filling level ϕ_{GM} of 0.8, means 80% of the netto grinding chamber volume is filled with a bulk of milling beads.

Energy consumption correlates with the used scale and increases with an increasing batch size. Thus, the specific grinding energy $E_{M,\,spec}$ can be determined using the following Formula assuming similar operation mode as well as grinding media conditions:

$$Specific\ grinding\ energy\ E_{M,spec} = \frac{Total\ net\ energy\ input\ [kWh]}{Mass\ of\ solids\ in\ suspension\ [kg]}$$

2.3. Analytical methods

2.3.1. Particle size distribution (PSD) by laser diffraction (LD)

Particle sizes were measured by LD (Mastersizer 2000/Hydro 2000S; Malvern Instruments GmbH, Worcestershire, UK). The measurements were performed using purified water as diluent at room temperature. Three runs for each sample were measured with a volume based approach using Mie-Theory calculation. The refractive index of the API and of dispersing medium was set to 1.6 and 1.33, respectively. The average of the three runs is reported.

2.3.2. X-ray powder diffraction (XRPD)

Reflex pattern are obtained with an Stoe Stadi P XRPD (STOE & Cie. GmbH, Darmstadt, Germany) equipped with an Ge-(111)-monochromator using a copper-K-alpha1-radiation. For sample preparation the suspensions were desiccated in a drying cabinet, grinded and applied between two Mylar® foils placed in a rotating sample holder. Transmission was measured.

2.3.3. Scanning electron microscopy (SEM)

SEM pictures of carefully dried nano- and microsuspensions were created by using the scanning electron microscope Jeol JSM 6490 LV (Jeol Ltd, Tokyo, Japan). Sample preparation was performed in a Joel JFC-1200 Fine Coater where the sample was dried under vacuum and sputtered with gold particles prior to SEM imaging.

2.3.4. Cryo-electron microscopy (cryo-EM)

Leo 912 Ω-mega (Leo Elektronenmikroskopie GmbH, Oberkochen, Germany) cryo-EM was used to investigate particle shapes in the suspensions. Pictures were obtained by a Proscan camera (HSC 2 Oxford Instruments, Abingdon, USA). The suspensions are brought onto a grid and the small liquid film is rapidly frozen to 90 K by fluid ethanol, so that ice crystals do not form and the frozen water film remains transparent in an amorphous state. The temperature during the measurements was below −170 °C.

2.3.5. Determination of Zr and Y wear – ICP-OES

For the determination of the heavy metals Zr and Y an ICP-OES system was used (iCAP 7400 DUO equipped with autosampler CETAC ASX-520, Thermo Scientific, Waltham, Massachusetts, USA) applying the following plasma adjustments: plasma 1150 W Nebulizer: approx. 0.5–0.65 L/min; auxiliary gas: approx. 0.5 L/min; pump rate: approx. 50 U/min, time of integration: 15 s.

To ensure similar starting conditions, new and unused milling beads were used for each milling trial. Previous to the addition of milling beads to the milling chamber, they were cleaned and dried to remove possible material adherence caused by manufacturing of the beads.

2.3.6. Zeta potential

For zeta potential measurements a dynamic light scattering system was used (ZetaSizer Nano ZS90, Malvern Instrument GmbH, Malvern, UK). Measurements were performed with diluted sample material at 25 °C by using a disposable folded capillary cell (DTS1070) cell. The refractive index of the API and of dispersing medium was set to 1.6 and 1.33, respectively. The average of three runs is reported.

3. Results

Wet bead milling of different APIs using different milling tools and principles has been investigated and compared. To mill APIs in the lab scale, dual centrifugation (DC, 100–1000 mg batches) and planetary ball milling (PM, 10 g batches) were used. For larger batch sizes in lab-scale, pilot- as well as production-scale, agitator mills of different capacities were investigated (AM, 500 g–30 kg batches). In a first step, the

Table 1
Parameters used for wet ball agitator-milling.

Equipment	DeltaVita 300 (default)	DeltaVita 10,000	DeltaVita 600
Batch size [kg]	1.5	30.0	3.0
Total amount of solids in suspension [kg]	0.30	6.0	0.60
Target Total net energy input [kWh]	1.0	20.0	2.0
Actual Total net energy input [kWh]	1.0	18.2	0.26
Value reached [%]	100	91	13
Target constant energy input (net) [kW]	0.22	4.4	0.44
Actual constant energy input (net) [kW]	0.22	4.0	0.06
Agitator speed [m/s]	8	9	7
Pump speed [L/min]	0.38	14	0.76
Number of passages	76	67	65
Milling time [min]	270	270	270
Particle size d(4,3) before milling [nm]	8,120	8,640	9,370
Particle size d(4,3) after milling [nm]	131	159	213
Actual specific grinding energy (SGE) [kWh/kg solid] EM, spec (target = 3.33 kWh/kg solid)	3.33	3.03	0.43

comparability of milling results after DC and PM and small scale AM were investigated using known API/excipient-mixtures and standard milling conditions. In a second step the influence of milling conditions, especially the specific grinding energy (SGE), was investigated for two aspects – the transfer of DC-milling results to AM as well as for scale-up to pilot-scale and production-scale AM.

3.1. Comparability of DC-milling to agitator bead milling

Fig. 4 shows the particle size distribution (PSD) results of the API-wet ball milling using three fundamentally different mills (dual centrifuge, planetary ball mill and agitator bead mill) and the minimal necessary batch sizes, which was 1 g for DC-milling, 10 g for planetary wet ball milling and 500 g for the agitator bead milling. In each case, standard conditions known from previous studies were used for the milling process. Fig. 4A shows an overlay of the PSDs of Naproxen nanosuspensions (30%) prepared by the different milling techniques. Whereas both, DC and agitator-milling results in almost identical, very small unimodal PSDs, the use of the planetary ball mill results in much larger particles with a D90 > 1 µm. In Fig. 4B the results of nano-milling of a 20% Ibuprofen-suspension using the same milling systems and conditions as before are shown. All PSDs are unimodal and completely placed in the nano-range. The nanosuspension prepared by the agitator bead milling shows the narrowest PSD and the smallest D90 value. DC-milling led to only slightly larger particles whereas the suspension prepared by planetary ball milling again showed in largest API particles.

Fig. 4C and D show the PSDs of two Fenofibrate suspensions containing the same amount of Fenofibrate (10%) and polymer but differ in the type of surfactant (0.5% SDS and 0.5% Tween 80; anionic and neutral). In both formulations, the particles obtained by DC-milling and (AM) are very similar showing low D50 values and narrow unimodal particle size distributions with a very small second peak in the range of 1 µm. However, this second peak was slightly more pronounced for the Fenofibrate suspensions containing the negatively charged surfactant SDS. In contrast to AM and DC, the use of the planetary ball mill resulted in a bimodal PSD, whereby again the second peak is more pronounced when using SDS as surfactant.

Despite the drug load used in the Fenofibrate experiments was lower (10%), the PSDs for DC- and AM are highly comparable to those obtained from the experiments with Naproxen and Ibuprofen, where 30 and 20% API have been used. Moreover, for all three APIs investigated, it could be shown that DC-milling resulted always in particle size distributions which are identical to those resulting from the agitator bead mill applying standard milling conditions. To confirm that PSDs resulted from small batch size DC-milling are also comparable to those resulted from agitator-milling of somewhat larger batches, two suspensions – Naproxen (30%), PVP K25, Tween 80, as well as Fenofibrate (10%), HPMC, SDS were milled again using the same agitator bead mill (DV 300), but with a threefold larger batch size of 1500 g. Using the same milling-parameters (agitator speed, pump speed, milling time) as for milling 500 g batches, milling the threefold larger batches resulted in exactly the same PSDs when prolonging the milling time by a factor of three, which results in the same number of passages as well as the same specific grinding energy (SGE) as for the 500 g batches (Fig. 4E and F).

In a further step an API/polymer/surfactant combination which is known to be not able to stabilize the nanoparticles in the nano-range is used to investigate the prediction capability of small batch size DC-milling to larger agitator mills. Fenofibrate (10%), PVP-K25 (1%), SDS (1.5%)) was processed by DC- and AM (1 g and 500 g, respectively). Fig. 4H shows that both milling procedures resulted in virtually the same but now larger particles showing that DC is predictive also for milling results of API-suspensions which cannot be successfully milled to the nano-range.

3.2. The role of SGE: Scale-up from DC to lab-scale and production scale agitator-milling

To investigate if SGE is also predictive for the PSD during upscale to another and larger AM, a 20% Naproxen-suspension was milled over a standard milling time of 270 min either with the small batch size DV 300 (1.5 kg) as well as with the DV 10000, an agitator mill with a 33-fold larger milling chamber, using a batch size of 30 kg. Based on the energy input during milling of the Naproxen-suspension with the smaller DV 300 (Table 1), the grinding parameters have been calculated for the larger DV 10,000 to reach the same SGE as for the DV 300 (3.33 kWh/kg solid). (Table 1).

However, during the milling trial over 270 min, only 91% of the target energy input could be reached, which resulted in a 10% lower SGE in the 30 kg milling experiment, which in turn resulted in similar but slightly larger particles. Using another AM (DV 600) and a typical batch size for this mill of 3 kg and adjusting the SGE to only 15% (13% reached) of the value necessary to get the very small particles of the 1.5 kg experiment, the resulting Naproxen particles were much larger (Table 1). Fig. 5

Fig. 6, the PSD after milling the 20% Naproxen-suspension using the three different AM as well as DC-milling are compared. PSD after DC-milling was identical to the PSD found after AM with the highest SGE.

3.2.1. Time course of agitator-milling

All milling trials using AM were performed for 270 min. To investigate the time course of the milling processes in the three different AM-types in more detail, samples were taken every 30 min. For each time point, the particles sizes (volume weighted mean d(4,3), De Brouckere mean diameter) were determined and the SGEs achieved so

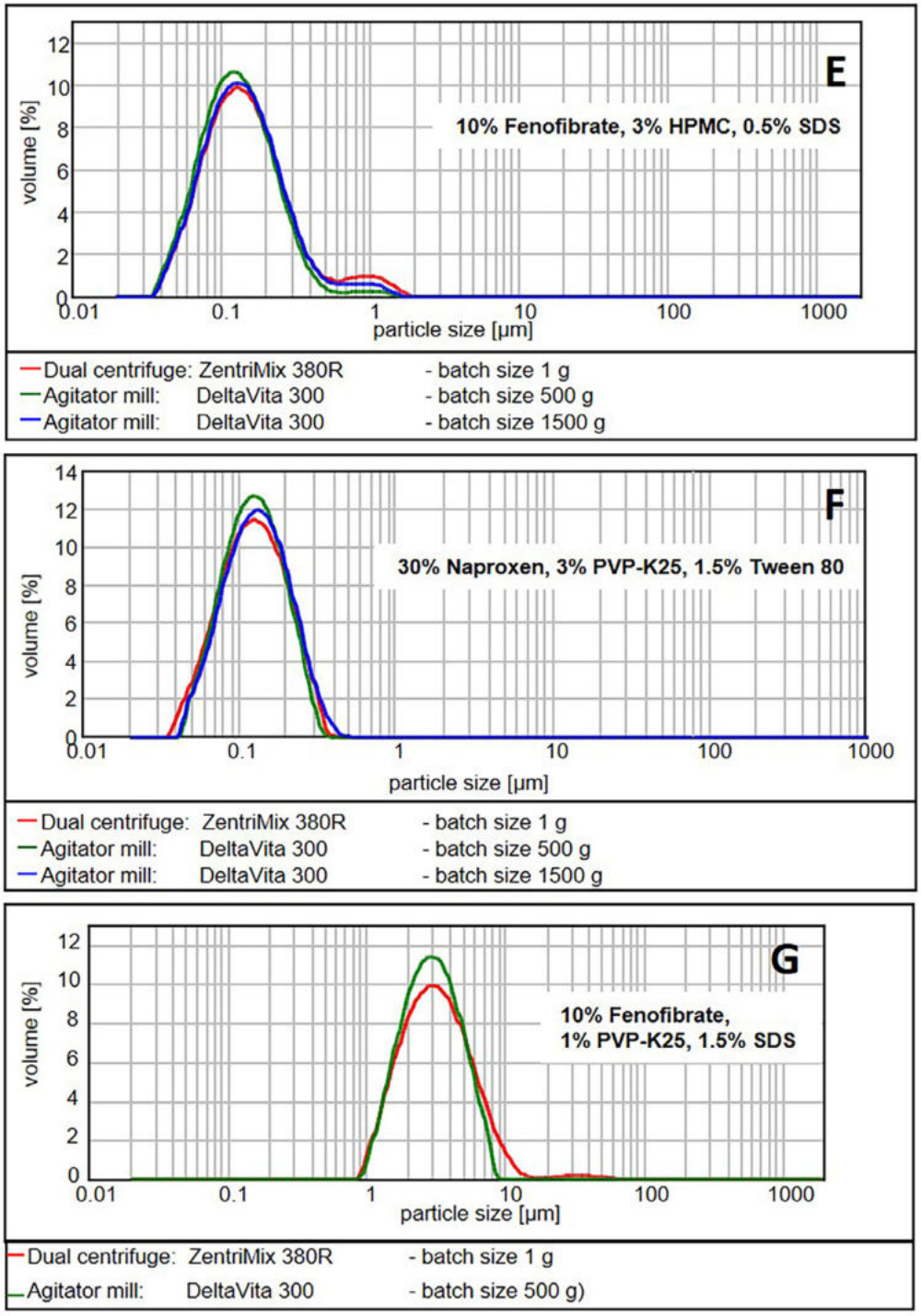

Fig. 5. Comparison of dual centrifugation-milling with 1 g batch size (Hettich ZentriMix 380R, red) and agitator-milling (Netzsch DeltaVita 300, green (500 g batch size) and blue (1500 g batch size)) using different formulations for the preparation of API-nanosuspensions under default milling conditions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

far were calculated.

Applying the highest SGE ($E_{M,\ spec}$ = 3.33 kWh/kg_{solid}; DV 300), a plateau have been reached after 270 min, which appears to be the grinding limit for the tested Naproxen-suspension (Fig. 7). A particle size of d(4,3) = 131 nm could be reached under the used default conditions. Using a 10% lower SGE ($E_{M,\ spec}$ = 3.03 kWh/kg_{solid} ; DV 10000), the time course of the particle size reduction depending on SGE was similar to the time course found for the DV 300 experiment, but could not fully reach the minimal particle size (grinding limit) gaining a value of d(4,3) = 159 nm. It appears possible that an extension of the milling period would have resulted in the minimal particle size of approximately 130 nm.

Using the DV 600 with only 13% of the SGE ($E_{M,\ spec}$ = 0.43 kWh/kg_{solid}) of the DV 300 milling-experiment, the relation between SGE applied and the particle sizes was completely different. After 270 min grinding, a particle size of d(4,3) = 213 nm was achieved. Despite the grinding limit could not be reached, the process appears to be more effective than in the two other tested mills. Compared to the DV 300

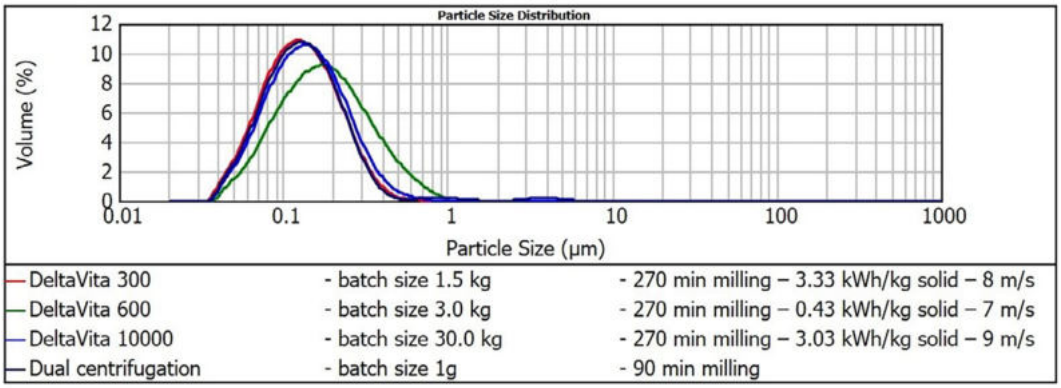

Fig. 6. Particle size distribution of suspensions obtained with DV 300 (red), DV 600 (green), DV 10,000 (blue) as well as DC (purple) using a similar formulation but different SGEs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

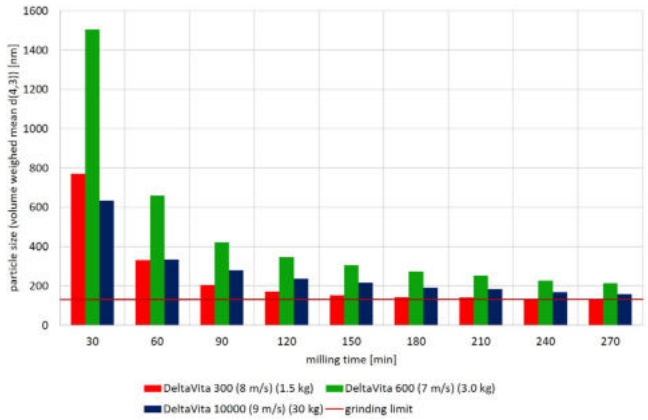

Fig. 7. Volume weighed particle size over grinding time for all manufactured suspensions during scale-up. DV 300 (red), DV 600 (low SGE, green), DV 10000 (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

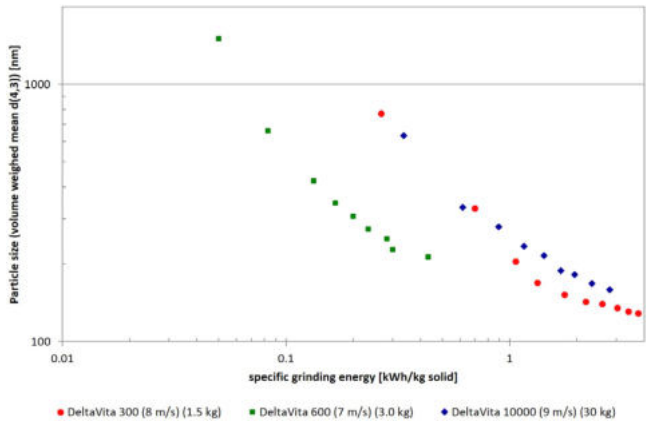

Fig. 8. Volume weighed particle size over specific energy input for all manufactured suspensions during scale-up. DV 300 (red), DV 600 (low SGE; green), DV 10,000 (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and DV 10,000 AMs, in the DV 600 a much lower SGE is sufficient to generate particle sizes which could have been reached in the DV 300 and DV 10,000 only by applying a much higher SGE (parallel shift of the curve, see Fig. 8).

3.2.2. Wear

To investigate the appearance of wear during agitator-milling, residues of zirconium (Zr) and yttrium (Y) in the analysed nanosuspensions have been determined after 180 min milling and compared (Table 2). As expected from the low energy intake, no wear could be found after milling with the DV 600. By milling in the DV 300 and the DV 10,000 with a much higher energy intake, very low amounts of zirconium could be measured. However, despite the energy intake was comparable for both mills, the value for the DV 300 was still low but about 3-fold higher after the DV 10,000 milling trial. The yttrium concentrations were always below 1 ppm, which was the limit of detection.

In contrast to the agitator mills, the “milling chamber” of the dual centrifuge consists of polypropylene, only the beads were made of YSZ. After 90 min of DC-milling, a very low zirconium concentration could be found which was in between the values obtained from the DV 300 and DV 10,000 milling trials.

The investigation of zirconium wear over time showed a roughly exponential increase from 60 min up to 240 min, probably due to the increasing vulnerability of the pre-damaged bead surfaces at later time points (Table 3)

3.3. Analytical and mechanistic investigations

3.3.1. Comparison of the crystalline properties of APIs after DC- and agitator-milling

To investigate if the very effective DC- and agitator-milling processes influence the crystal structure of the APIs, a Naproxen suspension (Naproxen (20%), HPMC (3%), DOSS (0.5%)) was milled using DC and two different agitator mills (DV 300 and DV 10000), and the resulting nano-crystals were investigated by XRPD. In Fig. 9 the diffractograms of the dried suspensions before the milling process as well as after the different milling procedures are shown. In all cases no differences of the crystal structure could be observed by XRPD.

In addition to the XRPD results pictures obtained by cryo–EM measurements of the investigated Naproxen formulation after DC- and the agitator-milling with a batch size of 500 g (DV 300) are shown in Fig. 10. The resulting particle shape looks almost similar for both procedures and it is illustrated that there is no change of the crystal shape and structure. Thus, it can be concluded that the DC milling technique in the described screening scale is not only predictive for the PSD, but also for the shape and structure of the nanoparticles itself in comparison to the larger agitator bead mills.

3.3.2. SEM images initial and after milling

To show and visualize possible differences between the unmilled and milled suspensions, microscopic pictures (SEM) were prepared as displayed in Fig. 11.

By looking at the Naproxen suspension (Naproxen (20%), HPMC (3%), DOSS (0.5%)) before and after milling with the production scale

Table 2
Results of wear determination via ICP-OES; identical YSZ milling beads (0.3 mm) after 180 min (AM) and 90 min (DC).

Milling device	Material of milling chamber	Milling time [min]	Zirconium [ppm]	Yttrium [ppm]
DeltaVita 300	YSZ	180	1.8	< 1.0
DeltaVita 600 (low SGE)	YSZ	180	< 1.0	< 1.0
DeltaVita 10,000	YSZ	180	6.0	< 1.0
ZentriMix 380R	Polypropylene*	90	3.4	< 1

* milling chamber = milling vial.

Table 3
Results of wear determination via ICP-OES; identical YSZ milling beads (0.3 mm) from the DV 300 milling trial with a batch size of 1500 g after 60, 120, 180 and 240 min of milling.

Milling device	Material of milling chamber	Milling time [min]	Zirconium [ppm]	Yttrium [ppm]
DeltaVita 300	YSZ	60	< 1.0	< 1.0
DeltaVita 300	YSZ	120	1.3	< 1.0
DeltaVita 300	YSZ	180	1.8	< 1.0
DeltaVita 300	YSZ	240	3.2	< 1.0

DV 10,000 agitator bead mill in different magnifications of 1000 and 3500 fold (B and D) the arrangement of the particles changed significantly. As the particles in the microparticular suspension (A and C) are undefined in size and shape, the nanoparticles are arranged in circles aligned in a chain like structure, showing very small particles. Thus, the excipients present in the formulation (surfactant and stabilizing polymer) may lead to an ionic charge and occurrence of micellar structures. This ionic charge can be explained with the used negatively charged surfactant DOSS and the zeta potential of the formulation with a value of −14 mV. It is unlikely that this effect has been caused by the drying of the nanosuspensions before measurement.

3.3.3. *Purity profile and assay value initial and after milling*

To prove if a chemical degradation takes place during the milling process, a Naproxen- as well as Ibuprofen-suspension was analysed by HPLC/DAD with regard to probably increasing impurities and a deviation in the assay value. However, all samples show that there were no impurities visible before and after the milling procedure. All samples have shown an unaltered assay value (data not shown). This result is in line with results already mentioned in the literature (Kocbek et al., 2006; Kumar and Burgess, 2014)

4. Discussion

Bioavailability of poorly soluble APIs can greatly be enhanced by milling down the drug crystals to the nano-size, thus increasing the water-crystal contact area and thus solubility. In contrast to common milling processes, nanomilling requires a stabilization of the nanoparticles by API-specific combinations of polymers and surfactants. This helps to enhance the milling process and also prevent reformation of larger crystals from the initially prepared nanocrystals.

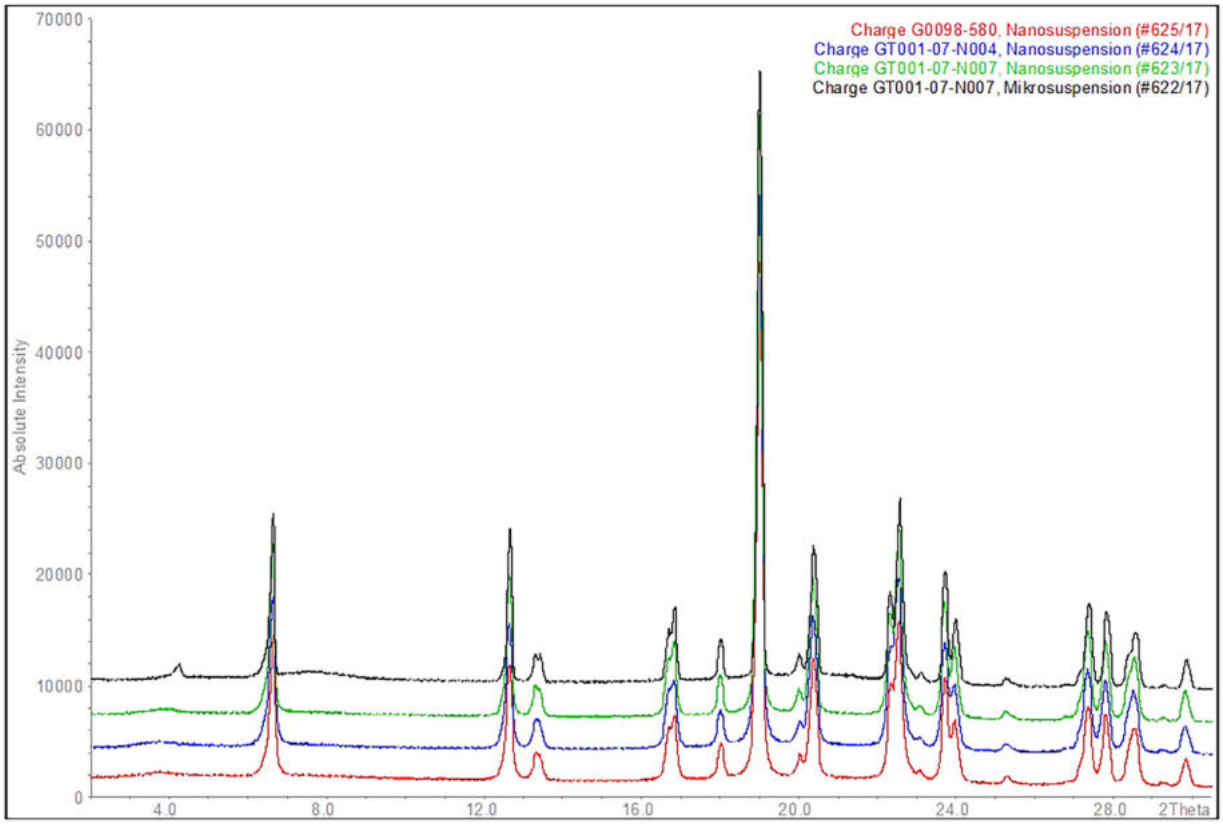

Fig. 9. XRPD measurement of dried Naproxen (20%) suspensions (stabilized by HPMC (3%)/DOSS (0.5%)) before milling (black), after DC-nanomilling (red), after agitator bead milling process with an batch size of 500 g (blue) and after agitator bead milling process with an batch size of 30 kg (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

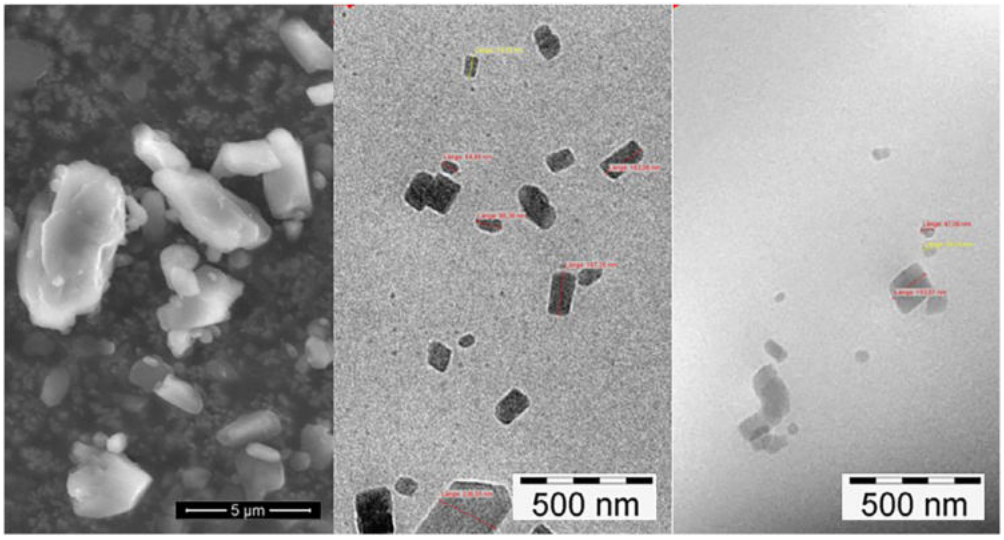

Fig. 10. SEM and cryo-EM Pictures of Naproxen nano-particles (left: unmilled Naproxen (SEM), middle: suspension milled by agitator-milling (cryo-EM), right suspension milled by DC (cryo-EM).

At the very beginning of a clinical drug-development, usually only small amounts of the new APIs are available. This requires small batch size milling to find polymer/surfactant combinations to allow the formation of small and stable nanocrystals. After that, preclinical studies have to be carried out not only to investigate if the respective API is a promising drug candidate *per se*, but also to identify the polymer/surfactant-combination which shows the best preclinical results for the respective API. For the subsequent clinical trials and finally for the use in clinical practice, nanomilling of the optimal API/polymer/surfactant-suspension have to be performed in large batch sizes.

In this study the second challenge, the question if – or to which extent – a certain polymer/surfactant combination found by small batch size nanomilling is also suitable to get very similar or identical API-nanoparticles when running the milling process in larger industrial agitator mills, was addressed. This is a very important aspect, since when the transferability to larger agitator mills is not given, the polymer/surfactant-combination to stabilize and protect the API during milling would have to be changed during the upscale process. This is not only very time expensive, but – and more important – might lead to the situation that the preclinical studies might have to be repeated again with the new API/polymer/surfactant-combination, which is even more time expensive.

It was recently shown that the identification of API/polymer/surfactant-combinations which result in stable API-nanoparticles can be

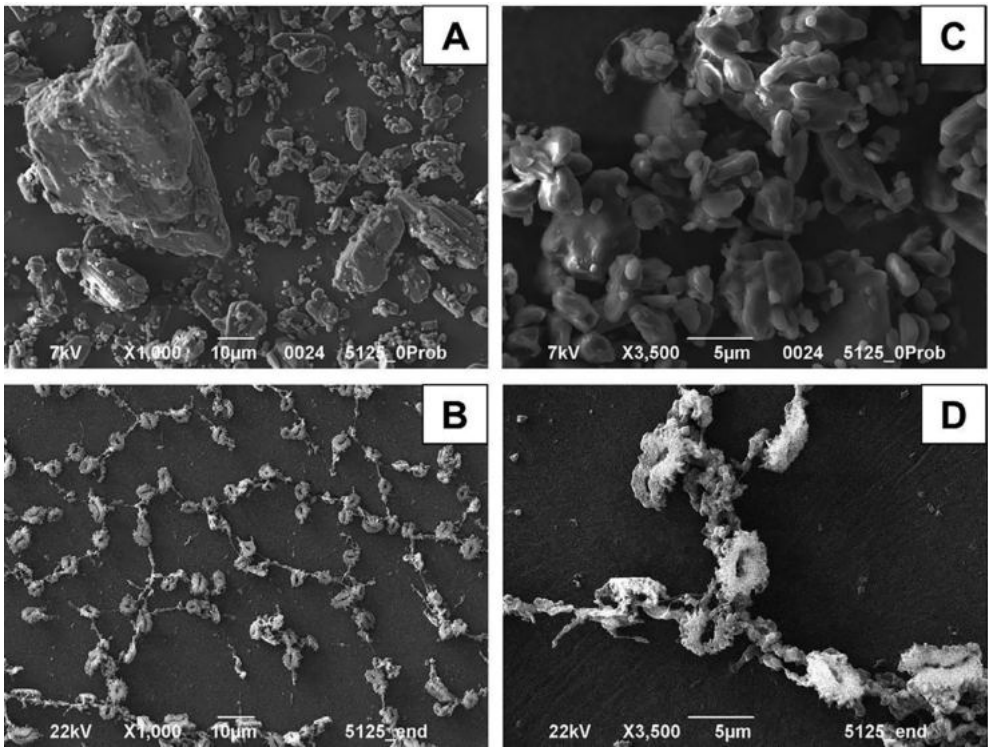

Fig. 11. SEM pictures of the suspension generated in the DV 10,000 agitator bead mill before milling (A, C) and after milling (B, D) in 1000-fold (A, B) and 3500-fold (C, D) magnification.

easily performed by using a new nanomilling approach, dual centrifugation (DC). Up to 40 API/polymer/surfactant-combinations can be milled at once (screening) within 90 min and only 10–100 mg API are necessary to test a certain API/polymer/surfactant-combination. In contrast to that, planetary ball milling, even after much longer milling periods, resulted in larger particles, especially when it comes to higher API-concentrations, as usually used in large batch milling.

Based on that, the standard DC-milling (90 min) with agitator-milling using a rather small mill (DV 300), standard milling conditions and milling time (240 min), were compared. The fact that DC- and AM of four different API/polymer/surfactant combinations resulted in virtually identical and very small particles gives the first hint that the input of the specific grinding energy (SGE) might be identical for both milling approaches. This assumption is supported by the fact that an API/polymer/surfactant-combination known for its poor particle stabilization resulted in much larger particles in DC- as well as AM trials.

Specific grinding energy (SGE) is not measurable during DC-milling. However, since agitator mills develop very high SGEs and since the grinding limit will typically also be reached during agitator-milling over a suitable time, it appeared possible that the SGE applied by DC is at least similar to the SGE applied by agitator mills. This assumption was further supported by the fact that the appearance of wear, despite very low, is comparable between AM and milling by DC, which shows that the impact of the milling beads (bead-bead interactions) during DC must be at least similar to the impact between the milling beads and the milling chamber during agitator-milling. Since the necessary milling time for DC-milling is less than half of the necessary milling time of the agitator mills, one might argue that the “actual constant energy input” during DC-milling is higher than during agitator-milling.

One explanation for the high SGE of DC-milling in a flexible polypropylene tube is that the bead-bead interactions are extremely frequent and powerful. DC-milling based on the rotation of small vials filled with beads and API/excipient-mixtures in a constant and strong field of centrifugal acceleration. Thus, all beads accelerate in the constant centrifugal acceleration field (approx. 1.000 × g) as “one cloud” and clashes at once to the bottom or top of the vials, including the sample material (cloud milling), then the vial turns and the process starts again (Hagedorn et al., 2017). Thus, all beads are constantly involved in the milling process, which is similar to agitator-milling, but not to planetary ball milling. Since disposable and optionally sterile vials can be used for DC, the produced nanoparticles can easily be used in biological studies like cell culture or animal experiments.

That the bead-bead-interaction during DC-milling is comparable or even higher compared to the bead-milling-chamber-interactions during agitator-milling can be illustrated by the finding that the appearance of wear after 90 min of during DC-milling – despite very low – is twice as the values after 180 min of agitator-milling (DV 300).

In contrast to DC milling, during agitator-milling, SGE can be measured over time. Using a Naproxen-formulation which resulted in very small particles by DC-milling, milling were performed using different sized agitator mills and batch sizes, and different milling parameters. The milling trials were performed over a typical time period of 270 min and the respective SGE has been calculated for different time points and correlated with the particle sizes.

The achieved typical size vs time-curves can be explained by the different stages of a grinding process. First, loose agglomerates undergo a deagglomeration process which does not consume much energy (Ghosh et al., 2011). Even the milling trial with the lowest energy input (DeltaVita 600; agitator speed of 7 m/s) shows a significant decrease in particle size within the first 30–60 min.

In parallel, initial cracks in the crystal lattice of particles at potential weak points occur. These two effects rapidly reduce the particle size, especially at the beginning of the milling process and even at low energy inputs. After the elimination of the agglomerates and unstable particles, a particle size reduction can only be achieved by abrasion or destruction of the stable crystal lattice. Significantly more energy is needed as this true comminution of particles is induced by bead impacts, collisions and friction (Engstrom et al., 2013; Hogg, 1999). Besides deagglomeration and true comminution of particles, every grinding process has a specific grinding limit. This minimal achievable particle size depends on material properties (API, used stabilization excipients and bead size) as well as on process parameters influencing the stress intensity (Knieke et al., 2009).

However, the grinding limit could obviously not be reached for the DV 600 trial with an agitator speed of only 7 m/s. In this case, the stress energy on the particles was too low to further significantly decrease the particle size. Next to the formulation of API and excipients, the operating parameters like agitator speed, grinding media diameter and density of the grinding media have a strong influence to the efficiency of the grinding process. Depending on a reasonable target particle size the required specific energy input and therefore the obtained contamination of the product suspension can be reduced by optimization of these process parameters (Breitung-Faes and Kwade, 2008). Overmilling should be avoided because even a prolonged milling time would not have resulted in smaller particles but results in higher wear rates and therefore unnecessary contamination of product. As soon as a crystal is so small that no additional defects can be introduced in the crystal lattice, a further constant energy input is no longer sufficient for further comminution of particles (Knieke et al., 2009).

As can be expected, the highest cumulative SGE of 3.33 kWh/kg results in the smallest particles, probably very close to the grinding limit. Using a much larger AM (DV 10000) and a 20 times larger batch of the Naproxen-suspension, applying of a similar SGE (~10% lower) resulted in similar but somewhat larger particles, which were still close to the proposed grinding limit (Fig. 7, size vs time). This clearly shows that SGE is an independent parameter which is able to predict the particle properties when using different sized agitator mills which have a comparable basic configuration (same type of milling chamber, same chamber materials (here: YSZ)).

Milling the same Naproxen-suspension with a further AM (DV 600) and a cumulative SGE of only 13% of the optimal cumulative SGE of 3.33 kWh/kg resulted in much larger Naproxen-particles, which could be explained in the first instance by the fact that the grinding limit could not be reached during the 270 min milling period (Fig. 8). Thus, the efficacy of the DV 300 and DV 10,000 trials regarding grinding time is significantly higher (Fig. 7).

However, the observed parallel shift of the ‘SGE vs particle size’ curves (Fig. 8) showed that the particle sizes were indeed smaller as expected from the comparison of the reached cumulative SGEs with the respective values gained from the two other AMs. However, since the DV 600 was operated with a slower agitator speed of only 7 m/s (Table 1), the power consumption of the machine was relatively low, leading to much longer required grinding times, which could be shown by comparing the different decreases of the particles sizes over time of the three tested AMs. Further investigations with variation of the stress energy of the grinding media and the power input would be necessary to optimize operating parameters regarding efficiency and production capacity (Mende, 2018).

It is well known that the development of a powerful and robust lab-scale milling approach is essential for the scale-up (Raghava Srivalli and Mishra, 2016). In literature, a couple of lab-scale milling procedures beside the planetary ball mill approach are described (Lestari et al., 2015; Frank and Boeck, 2016; Eerdenbrugh et al., 2009; Salazar et al., 2012; Ahuja et al., 2015). Most of these milling procedures show no correlation with respect to pilot and industrial scale e.g. agitator bead mills. For example, Romero stacked stirring bars (3 bars) plus milling beads in 2 mL glass vials for the milling process and shows afterwards a correlation with respect to agitator bead milling (Romero et al., 2016). However, the chosen milling set-up can handle only drug loads of 5% and needs a milling time of 24 h. Another approach described in literature is the formulation development on the final industrial-scale equipment and therefore the final batch size, is very API- and time

consuming (Singare et al., 2010).

The fact that DC as well as AM are able to develop comparable high SGE to reach the grinding limit of a certain API/polymer-combination, a direct upscale from small batch DC-milling to large batch AM is possible and one can be sure that nanoparticles produced by DC-milling, for example during formulation screening, can also be produced in production scale, which avoids the time intensive scale-up process and – even more important – avoids the danger of doing preclinical experiments again because of a necessary change of the formulation. This finding is further confirmed by the finding that API-suspensions containing excipients which do not show ideal stabilisation (Fig. 5G) resulted in similar but much larger particles, independent if DC-milling or AM have been used. Thus, the finding that the "formulation screening tool" DC-milling is predicting for the results from industrial size AM milling is of high value and might reduce development time and costs.

One important aspect during AM is the appearance of wear, resulting mainly from the impact of the YSZ-milling beads between each other or with the milling chamber walls. Associated therewith, the emergence of wear is dependent on process time and energy input, but also on batch size as well as the filling degree of the milling chamber (Joost, 1994). Since during DC-milling with its proposed similar SGE compared to AM all YSZ-milling beads are strongly accelerated and constantly in use, comparable wear and thus residuals of zirconium have to be expected.

Comparing the wear rates found in this study with wear described by Juhnke (109 ppm Zr and 12 ppm Y) using a somewhat lower SGE of 20 kWh/kg and a grinding media filling level ϕ_{GM} of 0.8, wear generation was in the same range in relation to the required specific energy input (Juhnke et al., 2012). Even compared to the worst case trial (DV 10000) in the set-up of this work with values significantly lower than 10 ppm for zirconium and < 1 ppm for yttrium were found. Also compared to the investigations of Hennart the gained wear rates are lower (Hennart et al., 2010). Using comparable tip speeds, filling levels, grinding time as well as milling chamber volumes Hennart generates 7.9 ppm of Zr wear whereas only 1.8 ppm residues were generated within the DeltaVita 300 after 180 min. This investigation shows, that for reasonable target particle sizes with limited specific energy inputs a production of nanosuspensions with low wear rates and obtained residual values for zirconium and yttrium below 10 ppm is possible.

An important aspect is that appearance of wear is not linear, but showed a rather exponential increase over time (Table 3) which led to the assumption that the bead-bead interactions will lead to minimal damages of the bead-surfaces and that pre-damaged YSZ-beads which are in use for a while are more sensitive to further abrasion. Thus, multiple use of even YSZ-milling beads may not be advisable.

The consequent use of a mill equipped with a grinding chamber inner liner and an agitator made of ceramic does not lead to lower wear rates with regard to the grinding media. Nevertheless, it completely avoids a contamination with stainless steel which consequently leads to a huge reduction of the contamination of the produced nanosuspensions. An interesting grinding chamber inner liner material for further investigations is silicon carbide (SiC). SiC is characterized by extremely high hardness and wear resistance as well as excellent head capacity and therefore heat transfer which is an important advantage with regard to this very energy intensive technology.

During DC-milling, similar wear rates compared to AM clearly showed that the energy input has to be very similar to that of AM, which again explains the very good agreement of the DC- and AM results.

An additional parameter to compare DC- and AM is the potential change of the API-crystal structure. However, even using AM at maximum SGE or DC-milling, no change of crystal structure could be found showing that at least DC-milling is also in that respect predictive for the results from AM.

5. Conclusion

Using well known API/excipient suspensions, it could be shown for the first time that nanomilling by dual centrifugation (DC-milling), a recently developed milling tool for very small batches, results in fully comparable nanoparticles compared to large batch size agitator-milling. It appears highly probable that DC-milling – despite performed in small disposable polypropylene vials – introduced very high specific grinding energy to the suspensions which is comparable to that of agitator-milling. Nanoparticles obtained by the new technique turned out to be much smaller than those obtained by planetary ball milling, a technique which is widely in use for early stage API-milling tasks.

However, the now simple prediction of the results of production scale milling (agitator-milling) by small scale DC-milling might improve formulation development in the future. After the selection of promising prototypes by using DC as screening tool (40 samples in parallel), promising formulations can be used for scale-up trials using agitator mills of increasing size. As also found in this study, the parameter "SGE" (specific grinding energy) allows an independent down-scale and scale-up between agitator mills, at least when the milling chambers consist of the same material and the same milling beads will be used.

For a better understanding of the milling processes, further studies focussing on the specific grinding energy during DC-milling are necessary.

Conflict of interest

None declared.

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