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Simple low-cost miniaturization approach for pharmaceutical nanocrystals production

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ABSTRACT

Systematic screening for optimal formulation composition and production parameters for nanosuspensions consumes a lot of time and also drug material when performed at lab scale. Therefore, a cost-effective miniaturized scale top down approach for nanocrystals production by wet bead milling was developed. The final set-up consisted of 3 magnetic stirring bars placed vertically one over the other in a 2 mL glass vial and agitated by a common magnetic stirring plate. All of the tested actives (cyclosporin A, resveratrol, hesperitin, ascorbyl palmitate, apigenin and hesperidin) could be converted to nanosuspensions. For 4 of them, the particles sizes achieved were smaller than previously reported on the literature (around 90 nm for cyclosporin A; 50 nm for hesperitin; 160 nm for ascorbyl palmitate and 80 nm for apigenin). The "transferability" of the data collect by the miniaturized method was evaluated comparing the production at larger scale using both wet bead milling and high pressure homogenization. Transferable information obtained from the miniaturized scale is minimum achievable size, improvements in size reduction by reduction of beads size, diminution kinetics and potentially occurring instabilities during processing. The small scale batches also allow identification of optimal stabilizer types and concentrations. The batch size is 0.5 mL, requiring approximately 50 mg or 5 mg of drug (5% and 1% suspension, respectively). Thus, a simple, accessible, low-cost miniaturized scale method for the production of pharmaceutical nanocrystals was established.

1. Introduction

Nanocrystals are meanwhile well established for the formulation of poorly soluble actives, preferentially for oral (Keck and Müller, 2006) and dermal delivery (Shegokar and Müller, 2010). Products are on the market in pharma (e.g., Rapamune, Tricor), but also in cosmetics (e.g., platinum rare by la prairie). The typical size is above 100 nm (but below 1000 nm), thus, they are no nanomaterial/nanoparticle product according to e.g., European Commission recommendation on the definition of nanomaterial (2011/696/EU) and FDA guidance "Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology". This fact eases product registration. In addition, this "submicron" size range is more easily accessible by the various production methods used. The most important industrially used methods are bead milling (Alkermes, prev. élan/Nanosystems) (Liversidge et al., 1991) and high pressure homogenization (HPH) (SkyePharma PLC) (Kruss et al., 1996; Parikh and Selvaraj, 1999). There are also combination methods described consisting of a pre-treatment step followed by a main step of crystal disintegration, e.g., NANOEDGE technology by Baxter (Kipp et al., 2006); H69 process: spray drying and subsequent HPH (Müller and Möschwitzer, 2009); H96 process: lyophilisation and subsequent HPH (Lemke and Moeschwitzer, 2007). Here often one aim is to access also the particle size range below 100 nm (H69, H96).

For assessing the comminution ability of crystals, formulation screening (e.g., type and concentrations of stabilizers, stabilizer mixtures) and first physical stability investigations, a downscaling to small volumes is desirable. This saves time, and often very important, saves active. This is especially important in case of new chemical entities with potentially very limited amount available (often rather milligrams than grams). Commercial bead mills in their small volume version have suspension volumes rather in the range 50–100 mL (e.g., Bühler PML-2, 200 mL chamber and required suspension volume about 120 mL). Assuming a 5% concentration of active, a density of 1.5 g/mL, about 10 g of active are required. Most of the high pressure homogenizers require

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40–200 mL minimum volume, i.e., at least about 3 g of active (e.g., Micron LAB 40, APV Deutschland, Germany: 40 mL). There are also homogenizers with smaller volumes (e.g., 3–5 mL), but the process parameter pressure cannot be controlled very precisely (e.g., Avestin EmulsifFlex-B15, minimum volume 3 mL). One approach could be to reduce the drug concentration (e.g., to 1%), but this makes the milling process less effective. The drug crystals move against each other during the milling process, which contributes to the comminution efficiency. Thus rather about 20% suspension concentration is ideal. Independent on the concentration aspect, all these instruments require a certain processing time, which also might be shortened by using a small scale "multiple" system (i.e., small scale and running many samples in parallel). Thus, there is clearly a need for effective down scaling, i.e., having a small volume and being at the same time cost-effective.

A very simple approach in bead milling is filling of a 20 mL injection vial with milling beads, adding a magnetic stirrer bar and the suspension and placing it on a magnetic stirrer plate. However, this milling process is not very controlled, sometimes not effective due to uncontrolled movement of the stirring bar. In case of coated stirrer bars, erosion from the polymer coat can take place. To have a more controlled process, e.g., a glass-vial-based system has been described, where vials with different volumes were placed in a Retsch PM 400 MA planetary mill (Van Eerdenbrugh et al., 2009). However, this requires the investment of a mill, or a few mills (20 vials per holder in the mill). In parallel, milling in a 96-well plate was investigated, fixed on a shaker (25–340 μL/well) (Van Eerdenbrugh et al., 2009). In case of plates made from plastic material, erosion from the walls cannot be excluded. The suspensions used were about 16%, and drug amounts down to 1 mg could be processed.

In this study, a down scaled system was developed based on the preferable glass vials, having a special optimized arrangement of the stirrer bars, and avoiding the use of a planetary mill by using a multiple magnetic stirring plate. Systematically investigated were the effect of number and arrangement of stirring bars, the effect of the type of active to be diminuted, and effect of milling bead size in this miniaturized system. The results obtained from the small scale were compared to data from larger scale high pressure homogenization and the Bühler bead mill PML-2, to judge the transferability of data from miniaturized to larger scale.

2. Materials and methods

2.1. Materials

Resveratrol, hesperitin, ascorbyl palmitate, apigenin and hesperidin were purchased from Denk Ingredients GmbH (Germany). Cyclosporin A was a donation from PharmaSol GmbH (Germany). Vitamin E polyethylene glycol succinate (TPGS) (trade name Kolliphor® TPGS), alkyl polyglucoside C8–C10 (trade name Plantacare® 2000 UP) and poloxamer 188 (trade name Kolliphor® P 188) were donations from BASF SE (Germany). Double distilled and ultrapurified water was obtained from a Milli-Q apparatus (Millipore GmbH, Germany). All other reagents were from analytical grade.

2.2. Nanosuspensions production

2.2.1. Miniaturized wet bead milling using a magnetic stirring plate

The production principle was a top-down approach, in this case wet bead milling with stirring bars in a super reduced scale. The milling chamber consisted of a 2 mL glass vial (i.e., 10× lower than the namely used 20 mL injection vials). The final set-up was composed of 3 cylindrical stirring bars (9.5 × 6 mm) (VWR International, Germany), disposed vertically one over the other (Fig. 3).

The volume of the 3 stirring bars was 0.7 mL (0.233 mL/stirring bar). From the remaining space (1.3 mL), 1 mL was destined for the milling beads and the suspensions of the raw powder of the actives (i.e., 0.3 mL headspace). The milling beads possessed various sizes (diameters of 0.05, 0.1, 0.2 and 0.4–0.6 mm) and were yttria stabilized zirconium oxide beads (Hosokawa Alpine, Germany).

All the formulations investigated were aqueous suspensions and contained 5% active and 1% stabilizer (all w/w). The proportion of beads to suspension was 1:1 (volume), in other words, 0.5 mL (1.9 g) of milling beads (density 3.8 kg/L) and 0.5 mL of the suspension. The final 0.3 mL was left empty as headspace. The actives used and their respective stabilizers are shown in Table 1. The stabilizer type for each active was selected based on previous experiences producing nanosuspensions.

The vials were stirred on a magnetic stirring plate RCT basic (IKA-Werke GmbH & Co. KG, Germany) at 1200 rpm and 5 °C. Samples were drawn after defined intervals up to 120 h.

2.2.2. Bench scale wet bead milling using a Bühler PML-2

The bench scale production by wet bead milling was performed using a bead mill PML-2 (Bühler, Switzerland). Milling beads of 0.2 or 0.4–0.6 mm identical to the ones described in 2.2.1 were used. The milling time was up to 60 min, at a speed of 2000 rpm and 5 °C. Samples were drawn after 10, 20, 30 and 60 min of processing.

2.2.3. Bench scale high pressure homogenization with a Micron LAB 40

The bench scale production by high pressure homogenization was performed using a homogenizer Micron LAB 40 (APV Deutschland GmbH, Germany). The suspension was first pre-processed by 2 high pressure homogenization (HPH) cycles at 150, 500 and 1000 bar, respectively, followed by 20 cycles at 1500 bar.

2.3. Particle characterization

2.3.1. Photo correlation spectroscopy

The particle size of the nanocrystals was analyzed by photon correlation spectroscopy (PCS), using a Zetasizer Nano SZ (Malvern Instruments, UK). The results are the hydrodynamic diameter (z-average, z-ave), which is the intensity weighted mean diameter of the bulk population, and the polydispersity index (PdI), which is a measure for the width of the size distribution. Samples were diluted in water to a suitable concentration and the mean values were calculated from 10 single measurements.

2.3.2. Laser diffraction

Potential larger particles or aggregates (>3–5 μm) which cannot be detected by PCS were investigated by laser diffractometry (LD) using a Mastersizer 2000 (Malvern Instruments, UK). Samples have

Table 1
Actives and their respective stabilizer processed by the miniaturized milling method (alkyl polyglucoside C8–C10 = Plantacare® 2000 UP).

Active	Stabilizer
Cyclosporin A	TPGS
Resveratrol	Alkyl polyglucoside C8–C10
Hesperitin	Alkyl polyglucoside C8–C10
Ascorbyl palmitate	Alkyl polyglucoside C8–C10
Apigenin	Alkyl polyglucoside C8-C10
Hesperidin	Poloxamer 188