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smartCrystal combination technology – scale up from lab to pilot scale and long term stability

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The production of nanocrystals was scaled up from lab scale (20 g) to pilot scale (3 kg), scale up factor 150. The flavonoid apigenin was used as model compound, with potential for pharma, cosmetic and nutraceutical products. Lab scale production was performed by high pressure homogenization (HPH), pilot scale by applying the smartCrystal combination technology (CT), combining pearl milling and a subsequent HPH (1 cycle, 300 bar). The obtained particle sizes were compared on the basis of photon correlation spectroscopy (PCS), laser diffractometry (LD) and light microscopy. The results showed, that assessment of successful scale up depends on the characterization method used, e.g., PCS covering only a part of the particle size range (3 nm-3 μm) of the population, or LD the full size distribution. Long-term stability was predicted on zeta potential (ZP) measurements. Lab and pilot scale possessed sufficiently high ZP values (> 30 mV) for a stable dispersion, but the ZP values were different (5-7 mV). This was explained by differences in the Stern/Nernst potential of the nanocrystals, potentially due to different levels in the crystals where they break in a high energy process (HPH) versus a low energy size reduction (pearl mill). Independent on the production method and batch size, the nanosuspensions proved to be physically stable for 6 months at storage temperatures 4 °C, room temperature and 40 °C.

1. Introduction

According to the biopharmaceutical classification system (BCS), poorly soluble drugs are classified under class II (poor solubility and high permeation) and class IV (poor solubility, poor permeability), which generally have oral bioavailability problems (Müller et al. 1999; Rabinow 2004; Stegemann et al. 2007). One of the several approaches used to overcome poor solubility is the production of nanocrystals. Nanocrystals are well studied and are one of the safest and also most practical, industrially feasible ways to enhance the dissolution velocity (Buckton and Beezer 1992; Müller et al. 2000; Hecq et al. 2005; Jinno et al. 2006; Keck and Müller 2008). Rendering the drug particles into nanocrystals not only increases the dissolution velocity, but also increases the saturation solubility. Having higher saturation solubility and dissolution velocity in turn can significantly improve the bioavailability of the drug. Nanocrystals, generally produced on lab scale, were thoroughly studied for various administration routes, e.g., oral administration (Van Eerdenbrugh et al. 2008; Lai et al. 2009; Mauludin et al. 2009), intravenous injection (Gao et al. 2008; Ganta et al. 2009), targeted drug delivery (Schöler et al. 2001; Kayser and Kiderlen 2003; Keck 2008), ocular drug delivery (Kassem et al. 2007; Agnihotri and Vavia 2009) and dermal delivery (Teeranachaideekul et al. 2008; Mishra et al. 2009).

Nanocrystals can be produced in two different ways, the "bottom-up" and "top-down" technologies. In the "bottom-up" technology the production starts from molecules dissolved in a solvent followed by precipitation, i.e., adding the solution to a non-solvent (e.g., Nanomorph). Scaling-up the "Bottom-up" technology can be relatively easy when using e.g., static blenders. However, major disadvantages of this technology are the use organic solvents (costs for removal, potential residues in the product), and the difficulty to avoid crystal growth to the μm range, and long-term preservation of the crystal size in the nanosuspensions. Therefore all the nanocrystal products on the pharmaceutical market (about 10) are based on top-down technology.

Top-down technologies are better applicable in pharmaceutical industry. Nanocrystals can be produced using two different wet milling methods, low energy milling technology (Nanosystems/élan (Liversidge 2003)) or high pressure homogenization (Dissocubes® technology (Müller et al. 2008)). In low energy milling the grinding of the particles takes place by beads or pearls. High pressure homogenization (HPH) forces the particles to pass through a tiny gap (5–10 μm, depending on the applied pressure) with high velocity causing the particle size reduction by cavitation, particle collision and shear forces. A second generation of drug nanocrystals (smartCrystals) prepared by a combination of both technologies was introduced. The particles are firstly pre-milled by pearl milling, and subsequently passed through a high pressure homogenizer (Petersen 2006; Keck et al. 2008). There is only limited data published about the scale up of this nanocrystals production technology. The reasons are that the technology is relatively young (at beginning of the 1990ies), and research has been less performed in academia, most in industry and the data are held confidential.

As an active compound the poorly soluble flavonoid apigenin was chosen, because it has the potential to be used in products. Flavonoids comprise a large group of polyphenolic compounds

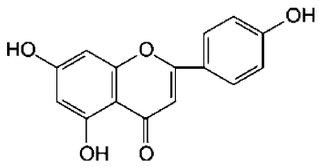


Fig. 1: Structure of apigenin (5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one

that are characterized by a benzo-y-pyrone structure. They have a wide range of applications in food industry, healthcare, pharmacology and even in cultural heritage (Cornard et al. 2001; del Valle 2006; Favaro et al. 2007). Pharmaceutical, cosmetic and food industry have focused their interest in the large spectrum of biological and pharmaceutical activities of flavonoids (VanAcker et al. 1996; Perez-Vizcaino et al. 2006).

Apigenin (APG, Fig. 1) is an active bioingredient found naturally in many citrus fruits (Yi et al. 2008) as well as vegetables like basil, oregano, tarragon, cilantro and parsley (Peterson and Dwyer 1998; Siddique and Afzal 2009). It is used as traditional or alternative medicine and possesses anti-inflammatory, analgesic (Datta et al. 2004; Jeong et al. 2009), free radical scavenging (Romanova et al. 2001), anti-carcinogenic activity (Kim et al. 1998) and antihistamine action. It has been shown to possess growth inhibitory properties in breast, colon, skin, thyroid leukemia and pancreatic cancer cell lines (Yin et al. 1999; Wang et al. 2000). However, the usage of flavoniods is still limited due to their low water solubility especially when they are poorly soluble in both aqueous and organic solvents (e.g., APG).

In this study, the scale up ability from lab sale (20 g batch) to pilot scale of 3 kg batch is investigated, that means a scale up factor 150. Due to the discontinuous mode arrangement of the smartCrystal process used, the pilot scale data will be transferable to production scale (a few hundred kg). The nanocrystals from both production processes are comparable in size, but also crystallinity. In addition, physical long-term stability was studied to assess, whether the production method affects the stability (e.g., differences in remaining large microcrystals with the potential to cause Ostwald ripening).

2. Investigations, results and discussion

2.1. Production of aqueous nanosuspensions of Apigenin

2.1.1. Lab scale production

High pressure homogenization (HPH) as a single step reduction process is very convenient for lab sacle batches, e.g., using the Micron LAB 40. The sample container has a volume of 40 ml, after completion of the production process with a certain loss of suspension, a minimum of 20 g nanosuspension is obtained. Therefore HPH was used for lab scale production in this study. The HPH process is more tedious, when producing on production scale (e.g., 100–200 kg), because the production technology requires often 20 passages through the homogenizer. In addition, solid concentrations able to be processed are typically 10–20% (w/w), and seldom higher (depending on the viscosity of the macrosuspension to be processed). Therefore the smartCrystal combination process of pearl milling followed by HPH is more convenient (cf. 2.1.2)

Production of nanocrystals by HPH takes place in two homogenization steps, the so called pre-milling (stepwise increasing pressure), and the actual homogenization process (1,500 bar) for reduction to the final nanocrystals size. In HPH the suspension is pressed through a tiny gap, which according to the Bernoulli equation leads to an increase in the dynamic pressure and a reduction of the static pressure on the liquid (Fig. 2). The

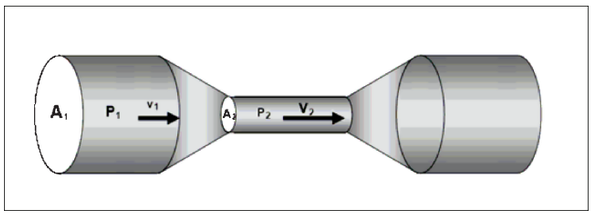


Fig. 2: A 3-dimensional drawing showing the principle of the high pressure homogenization. The cross-section narrowing of the tubes (A2 < A1, = gap in homogenizer) results in a decrease in the static pressure (P2 < P1) and an increase in the streaming velocity/dynamic pressure (v2 > v1). The static pressure falls below the vapour pressure of water, leading to boiling and cavitation. After widening of the cross section, the static pressure increases, the gas bubbles collapse

static pressure falls below the vapour pressure of the dispersion medium water, the water starts boiling leading to cavitation. The width of homogenization gap is a function of the applied pressure being about 5 μm at 500 bar and 3 μm at 1,500 bar (Müller and Junghanns 2006). The premilling is essential to diminute very large crystals being present when coarse suspensions are processed. Premilling avoids blocking of the homogenization gap by large crystals that are present in the macrosuspension. By applying pre-milling, also very coarse powders can be homogenized, no prior micronization of the powder is required. This is important when only small amounts of drug are available, because in micronisation (i.e., jet milling) the loss of drug is relatively high.

Based on previous experiences, as production pressure 1,500 bar were used. Further increase in pressure to 2,000 bar has little effect on reducing the particle size (Fichera et al. 2004). Generally the wearing of the machine is more at such very high pressure and this is not acceptable in pharmaceutical production. Thirty passages (homogenization cycles) were employed, to be sure to reach the maximum dispersitivity (smallest particle size). The decrease in particle size is a function of the pressure applied and the number of cycles, till the lowest size reaches a minimum. At which cycle number this lowest size is reached depend upon the physical properties of the drug (e.g., hardness, number of imperfections in the crystal, amorphous fraction).

2.1.2. Pilot scale production by the smartCrystal combination technology

The smartCrystal technology is actually a family of different combination processes, a pre-treatment step followed by a HPH step. The aims are to accelerate nanocrystals production or to obtain smaller nanocrystals sizes, ideally a combination of both. For example, the H96 process consists of lyophilisation of an organic solution of the poorly water soluble drug, the lyophilisate is then dispersed in aqueous surfactant solution and homogenized. Lyophilisation makes the drug material more fragile, and nanocrystals <100 nm are obtained, a size range normally not accessible by HPH alone. In the CT (combination technology) process, the first step consists of pearl milling, followed by HPH. This process has the advantage, that more viscous macrosuspensions with higher drug concentrations can be processed by the pearl mill compared to a homogenizer, as a rule of thumb 2–3 times higher. Less total volume needs to be processed in large scale production. In addition, it was found that the subsequently, in the seond step HPH treated nanosuspensions possessed a higher electrolyte stability and a improved physical long-term stability for a number of drugs. For these reasons the smartCrystal (CT) process was chosen in this study. Figure 3 shows the basic set up for the production of large scale batch. A macrosuspension was prepared and placed in the supply container. The suspension passes the mill with the pearls/beads

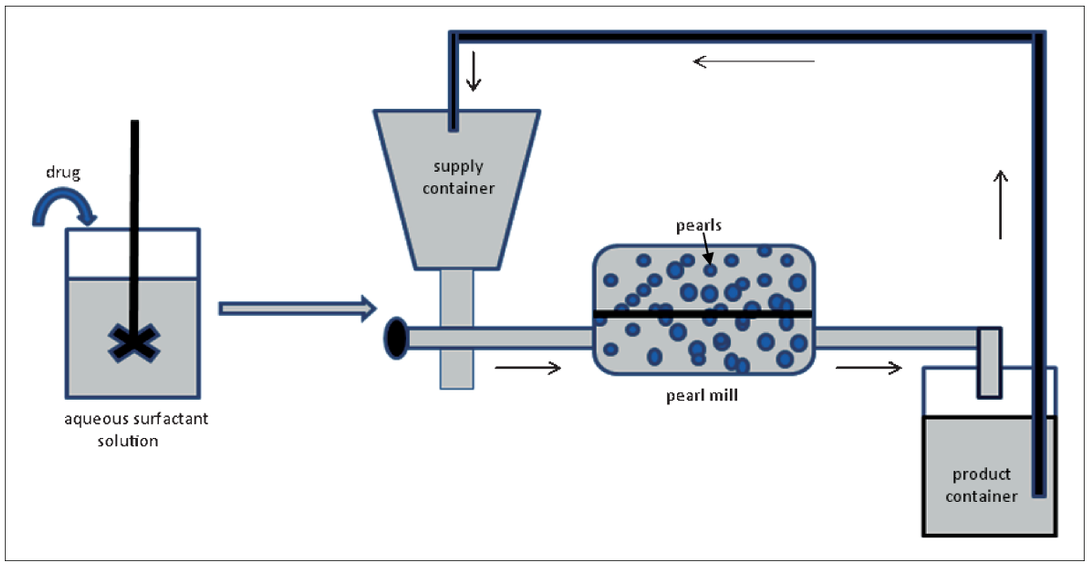


Fig. 3: Schematic diagram for production of APG nanosuspension

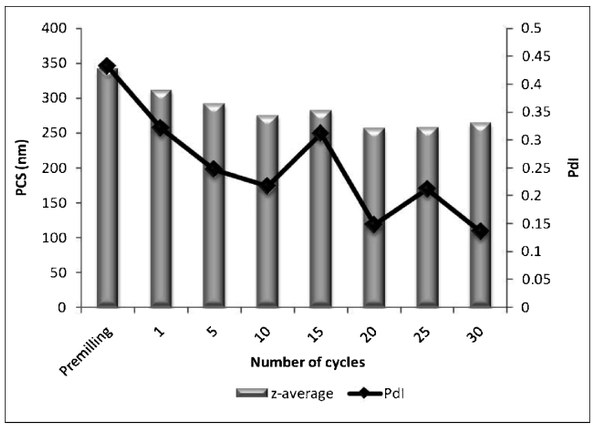
to the product container (= 1 passage). After completion of the passage, the product is transferred again to the supply container for the next passage. In this study 7 passages were applied, to be sure to reach the maximum dispersivity. A batch size of 3 kg was produced, but the set up makes clear, that it makes no difference if in one passage for 3 kg or 100 kg are processed. That means the established production parameters for this pilot scale can identically be applied also for production batches. In pearl milling the particle size reduction mainly depends on the rotating speed of the agitator, the pump through velocity of the suspension and number of passages. The final parameters were selected on basic screening studies, varying all these parameters.

2.2. Physical characterization of nanocrystals and stability

2.2.1. Photon Correlation Spectroscopy (PCS)

PCS yields the mean diameter of the bulk population (z-average), and a polydispersity index (PdI) as measure for the width of the size distribution. Particles larger than 3-5 μm (depending on the density of the particles) are outside the measuring range, for analysis of these particles laser diffraction was employed. In the lab scale production using HPH only, the PCS diameter decreased until cycle 20 (256 nm). Further increase in cycle number did not further decrease the size, that means maximum dispersivity was reached after 20 cycles. In parallel, the polydispersity index decreased indicating removal of particles larger than the bulk population and a narrowing of the size distribution. Apigenin nanosuspension showed a mean particle size of 264 ± 5 nm after 30 cycles with narrow polydispersity index of 0.136 ± 0.05 (Fig. 4). The results were reproducible (n = 3, the diameters for the other 2 productions were 247 nm and 255 nm).

For a pharmaceutical or cosmetic product, one does not have necessarily the smallest achievable nanocrystals size. A size of apigenin of about 300 nm after pre-milling and 1 production cycle at 1,500 bar might be sufficient. Of course this would reduce clearly the production costs. In case maximum solubility enhancement is required, the smallest achievable size should be chosen. In addition, when the final product is liquid (e.g., oral suspension), the PdI should be as low as possible, to minimize the content of remaining larger crystals which can cause Ostwald ripening. This requires 20 homogenization cycles.



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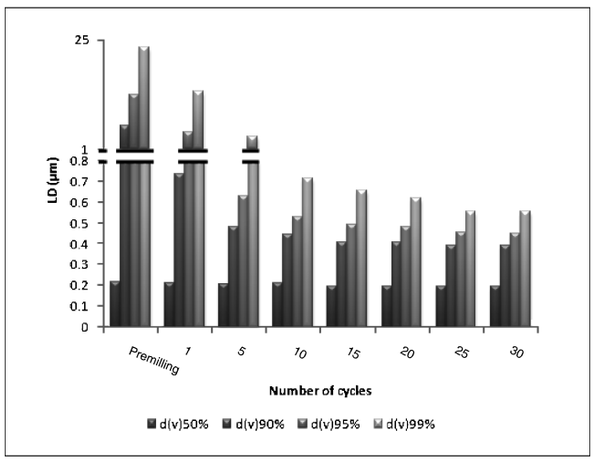
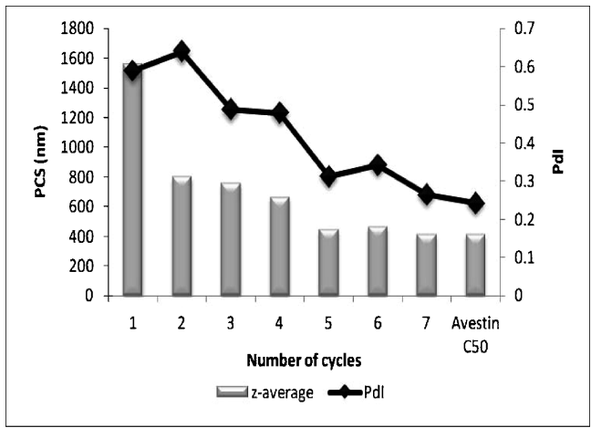


(b)

Fig. 4: PCS diameters (z-average) and polydispersity indices (PdI) (upper) and LD diameters 50% to 99% of apigenin suspensions produced on lab scale (high pressure homogenization) after pre-milling and as function of homogenization cycles (1 to 30) (lower) on day of production



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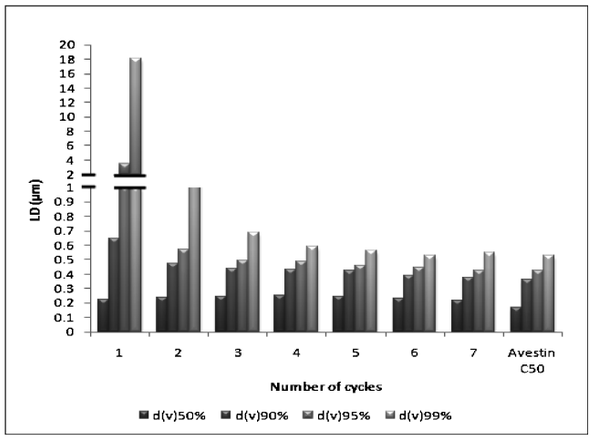


(b)

Fig. 5: PCS diameters (z-average) and polydispersity indices (PdI) (upper) and LD diameters 50% to 99% of apigenin suspensions produced on pilot scale as function of passages through the pearl mill (1 to 7) and after final passage through the Avestin C50, 1 cycle at 300 bar (lower) on day of production

In the smartCrystal (CT) process, the pearl milling is a low energy process – in contrast to the high energy process HPH. After the first passage the PCS diameter is about 1,550 nm, compared to about 350 nm after pre-millling in the HPH process. Applying a second passage leads to a very distinct decrease to about 800 nm, followed by a slower decrease until completion of passage 5. This is the smallest size, further passages until 7 have practically no effect on the size, and little effect on the PdI. After 7 passages, the mean particle size was 440 nm with a narrow polydispersity index of 0.265 (Fig. 5). A sharp decline in polydispersity index was observed from 0.588 to 0.265 from the 1st passage to the 7th passage.

It was found that in the subsequent homogenization step, processing with lower pressures (e.g., 100–500 bar) yields smaller and more homogenous nanocrystal populations than applying 1,000–1,500 bar (Petersen 2006). The size distribution gets narrower, the PdI decreases. The hypothesis is, that the observed increased physical stability can at least partially be attributed. This is the reason, why 300 bar were applied. Using 100 bar is difficult, because the pressure adjustment is not precisely possible. In some cases, a further decrease in size was observed in the final HPH step, sometimes the size stayed unchanged and only the PdI decreased. The latter was the case with the apigenin nanocrystals. The mean PCS particle size stayed practically unchanged, but a further slight decrease in polydispersity index was observed.

2.2.2. *Laser Diffractometry (LD)*

The laser diffractometry results of the lab scale nanosuspensions produced by HPH were well in agreement with the results obtained by PCS. The LD diameters d(v)90% to d(v)99% decreased continuously until cycle 20, at which maximum dispersitivity was found based on the PCS data. The most pronounced decrease took place between pre-milling and cycle 10, while little decrease was noticed up to cycle 30. These diameters are a measure for larger remaining particles in the population. Their decrease is in parallel to the decrease in PdI in the PCS measurements. In contrast, the diameter 50%, representing the bulk population, should decrease little (Fig. 4). This is also in agreement with the PCS data, all PCS diameters were in the range of about 350 nm (after pre-milling) to 265 nm (after 30 cycles), a difference.

In the pearl mill process, the d(v)99% decreased constantly till the 5th passage. Between the 5th and the 7th passage there was no significant change. This confirms the PCS data that maximum dispersitivity has been reached after 5 passages. The diameter 50% of the raw material was 9.297 µm. It dropped sharply to 0.223 µm after the first passage, and then remained unchanged till the seventh passage, i.e., 0.231 µm. This is identical to the observation with d(v)50% in the lab scale production. However, a further reduction in d(v)50% to 0.165 µm was obtained after homoginizing the pearl milled nanosuspension with the Avestin C50, which is according to theory (Petersen 2006).

2.2.3. *Comparison of PCS and LD results*

In lab scale production, the PCS diameter after 30 cycles was 264 nm with a narrow polydispersity index of 0.136. The PCS diameters were 413 nm after 7 passages with the pearl mill, and remained unchanged after the subsequent passage through the Avestin C50 homogenizer. The PdI values were 0.270 and 0.200, indicating a narrowing of the size distribution and removal of larger particles in the final homogenization step in the pilot scale. In lab scale production, the LD diameter 50% was 0.193 µm after 30 homogenization cycles. In the pilot scale with the smartCrystal process it was 0.215 µm after passage 5 with the pearl mill, and 0.165 µm after the final homogenization step.

Based on the PCS data, there is a clear difference in the bulk diameter (264 nm versus 413 nm). However, one needs to keep in mind, that PCS analyses only a part of the size distribution, from a few nm to about 3 µm, the larger sized particles are excluded. That means the two productions are not comparable regarding their bulk populations in the size range 1 nm to 3 µm. In contrast, LD covers the range from 40 nm until 2,000 µm, that means the calculated diameters are based on the total size distribution, all crystals are considered. In LD, the diameters 50% are 0.193 µm in lab scale and 0.215/0.165 µm in pilot scale, that means close together when considering the sensitivity of LD (Keck and Müller 2008). That means looking at the overall distribution, the two productions are comparable in size.

2.2.4. *Light microscopy*

Precise size information about the nanocrystals size cannot be obtained using light microscopy because of the lower detection limit (approx. 500 nm, 1,000 fold magnification). However, the presence or absence of large crystals or aggregates >1 µm can be easily verified under microscope, even the presence of a few large crystals being below the detection limit of the laser diffraction. Therefore light microscopy is a standard complementary sizing method to LD. To increase the likelihood of finding even a few large particles, the samples need to be investigated undiluted, a standard method used since the 1950's for analysis of o/w emulsions for parenteral nutrition (Müller and

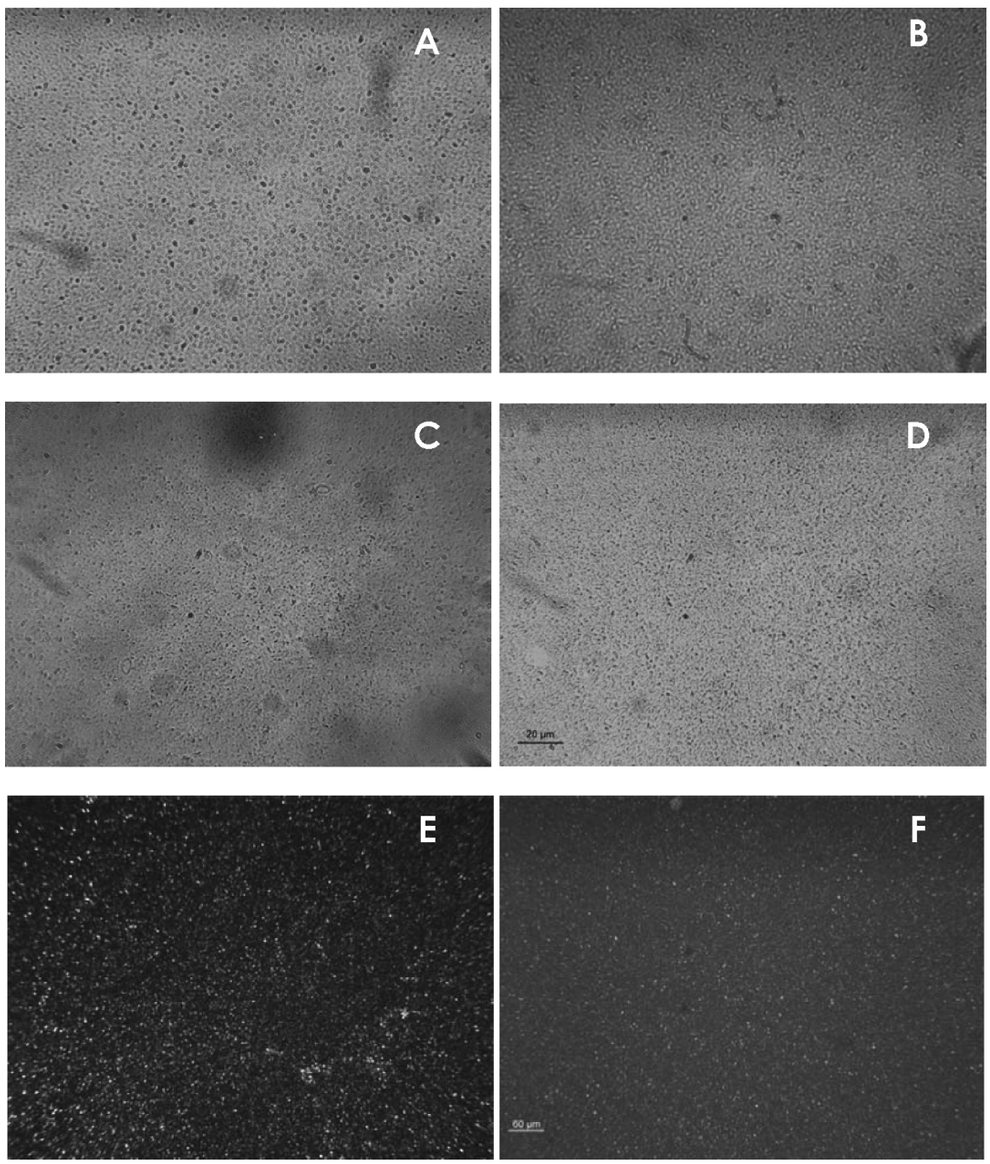


Fig. 6: Light microscopic pictures of Apigenin nanosuspensions on day of production (A, B), after six months of storage at room temperature, under non-polarized (C, D) and under polarized light (E,F) for lab scale (left column) and pilot scale (right column) at 600 magnification, respectively

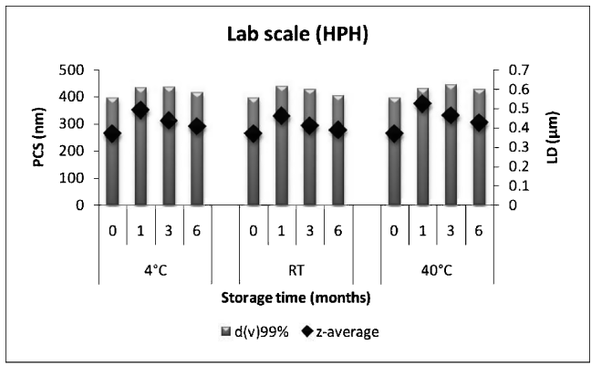
Heinemann 1993a,b). In addition, for finding the large crystals, magnification of 160 and 600 are best suited. They provide large field sections in the microscope.

Fig. 6 shows the light microscopic pictures of the apigenin nanocrystals from lab and pilot scale using 160 (A, B) and 600 magnification, with non-polarized and polarized light, respectively. No clearly visible aggregation and no large crystals were seen for the formulations on the day of production, as well as at the end of six months stability study at room temperature. The pictures remained unchanged. This confirms previous reports that Plantacare 2000 at 1% concentration provides optimal stability to the apigenin nanosuspensions (Kerč et al. 2009).

2.2.5. Zeta potential (ZP) measurements

Plantacare stabilized nanocrystals showed excellent stability avoiding aggregation of fine particle during long term storage. The charge of the articles, expressed as zeta potential (ZP), is an important stability determining parameter. Measurements were performed in distilled water and in the original dispersion medium of the suspension. Zeta potential measured in distilled water is close to the Stern potential which is related to the charge of the particle surface (Nernst potential). The higher the measured Stern potential, the higher is the Nernst potential (= surface charge). A high surface charge leads to a high related high zeta potential in the original dispersion medium, thus promoting stability to the particle dispersion.

Plantacare stabilized apigenin nanosuspensions produced with the LAB 40 showed a ZP of −38 mV in water, indicating a well charged surface and related stability. A zeta potential measurement in original dispersion medium is a measure for the thickness of the diffuse layer. The higher the zeta potential, the thicker is the diffuse layer and the more stable is the suspension. Plantacare 2000 possesses a ZP of about −37 mV in the original dispersion medium. This is well above the critical 30 mV (Riddick 1968) and indicates a good physical stability during storage. Theoretically for physically stable suspensions stabilized by electrostatic repulsion, a zeta potential of



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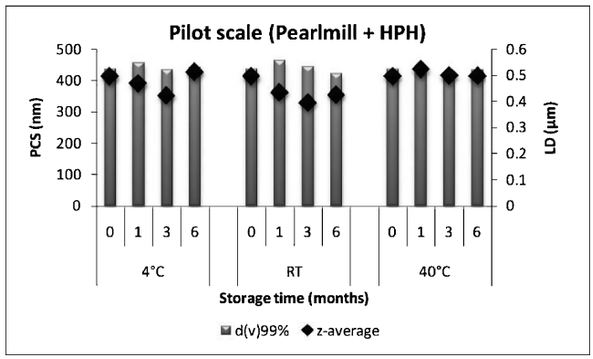

(b)

Fig. 7: Long term stability data (PCS mean diameters, LD diameters 99% as measure for occurring of large particles) of Apigenin nanosuspensions produced on lab scale using high pressure homogenization only (upper) and pearl milling with subsequent HPH (lower) stored at 4 °C, room temperature (RT) and 40 °C over 6 months

approximately ± 30 mV is required as minimum. Whereas, in combined electrostatic and steric stabilization (as in case of Plantacare), ±20 mV is sufficient. Thus, based on the ZP data, Plantacare 2000 at 1% (w/w) should be sufficient to provide a good long term stability for apigenin nanosuspensions. The theoretical assumption was confirmed by the long term stability data (PCS, LD and light microscopy).

The apigenin nanocrystals showed slightly higher ZP values, i.e., −45.0 mV in water and −42.5 mV in the original dispersion medium. The composition of the dispersion media (water, surfactant solution) was exactly the same during the measurements. Therefore the higher ZP by 5-7 mV can only be explained by a difference in the surface potential (Nernst potential). The surface charge depends at which interfaces the crystals break. There might be a difference between the high energy (HPH) and low energy milling process (pearl mill). Independent on the reason for this, also the nanocrystals form the pilot scale are well electrostatically stabilized.

2.2.6. Long term stability

Nanocrystals are provided as aqueous concentrates as intermediate products, or are suspensions for oral use (e.g., Megace® ES). For reasons of safety it is essential to ensure the long term stability. Three batches of both the lab scale and pilot scale production were stored as a function of storage time at various stress conditions viz. refrigeration, room temperature and 40 °C, storage time 6 months. According to PCS and LD data, it can be seen that there was neither significant increase in particle size nor change in particle size distribution. There is no significant change in PCS diameters (Fig. 7) and d(v)50% values (data not shown), being characteristic for the bulk population. The LD diameters 99% was also remained unchanged, proving that there is no aggregate formation or crystal growth (Fig. 7). Light microscopy confirmed the obtained results (Fig. 6).

2.3. Conclusions

Scaling up from lab to pilot scale was performed using the smartCrystal combination technology (CT). To judge the success of the scale up, i.e. if obtaining the same particle sizes, depends very much on the characterization methods employed to compare the products from different scale. PCS yielded clear differences, within the covered size range of this method (few nm-3 µm). This represents the bulk population, but not the overall size distribution including larger particles. Looking at the complete distribution using LD, revealed e.g., practically identical diameters 50%. In addition, it needs to be considered, that the mean diameters calculated are intensity-weighted in PCS, but volume diameters in LD. In the latter case larger particles are more weighted than small nanocrystals, which might explain the identity of the LD 50% diameters – despite differences in the small particle bulk population. To summarize, for the full picture, PCS and LD, but also light microscopy should be performed in parallel.

To obtain also identical PCS diameters, the option is to reduce the size of the milling pearls in the CT process. As a rule of thumb, the obtained particle size is a factor 1,000 smaller than the milling beads used, i.e., 0.4 mm pearls (400 µm) yield 400 nm sized milled particles. This rule was confirmed in the present study. To reduce the PCS size, one could employ 0.2–0.4 mm pearls. However, separation of pearls from the product is easier when the larger pearls are used. This is more production friendly in an industrial process. Therefore it needs to be carefully considered, which size of nanocrystals is required to "do the job", because smaller nanocrystals are more tedious and costly to produce on industrial scale.

3. Experimental

3.1. Materials

Apigenin was purchased from Exquim, S.A. (Spain) and the stabilizer Plantacare 2000® (alkyl polyglycoside) was provided by Cognis GmbH (Germany). As dispersion medium, freshly prepared double distilled and ultra purified water (milli-Q, Millipore GmbH, Germany) was used.

3.2. Methods

3.2.1. Lab scale production of nanosuspensions

Production of APG nanocrystals on lab scale was performed by Micro LAB 40 (APV Deutschland GmbH, Germany) having a capacity of 40 ml in discontinuous mode (number of cycles), Fig. 2. The stabilizer Plantacare 2000 (1% w/w) was dissolved in water and apigenin powder (10.0% w/w) was then dispersed in the prepared solution. Premixing was performed with an Ultra-turrax T25 (Janke and Kunkel GmbH, Germany) for 1 min at 10,000 rpm followed by pre-milling, i.e., 2 cycles each at 200, 500 and one cycle at 1000 bar. Final milling (actual homogenization) was performed by applying 30 cycles at 1,500 bar. Sampling was done after pre-milling, and after 1, 5, 10, 20 and 30 cycles and characterized regarding size.

3.2.2. Pilot scale production of nanosuspensions

Aqueous nanosuspensions of APG were produced with agitating pearl mill Bühler PML-2 (Bühler AG, Switzerland) in discontinuous mode, Fig. 3. Yttria stabilised zirkonia milling pearls of size 0.4–0.6 mm were used as a milling medium. In short, Apigenin 10.0% (w/w) powder was dispersed in 1.0% (w/w) aqueous surfactant solution by using an Ultra-Turrax T25 (Janke and Kunkel GmbH, Germany) for 1 min at 10,000 rpm, followed by milling using the pearl mill. Seven passages through the pearl mill were applied. In the second subsequent step, the milled nanosuspensions were diluted with 1.0% (w/w) aqueous surfactant solution and passed through an Avestin C50 piston-gap homogenizer (Avestin Europe GmbH, Mannheim, Germany) at

300 bar (1 cycle only). Sampling was performed after each passage through the mill and after homogenization.

3.3. Characterization of nanosuspensions

3.3.1. Photon Correlation Spectroscopy (PCS)

Photon correlation spectroscopy (PCS) was performed using a Zetasizer Nano ZS (Malvern Instruments, UK). The analysis yields the z-average of the sample, which is the intensity weighted mean diameter of the bulk population. The polydispersity index (PdI) is a measure of the width of the size distribution. The nanosuspension samples were diluted in distilled water and measurements were performed at 25 ° C temperature.

3.3.2. Laser diffractometry (LD)

Laser diffraction, also known as static light scattering, was performed using the Mastersizer 2000 (Malvern Instruments, UK) in deionized water as dispersion medium. The instrument was operated with the Hydro S sample dispersion unit. LD yields volume weighted diameters d(v)50%, d(v)90%, d(v)95% and d(v)99% as characterization parameters. All parameters have been analyzed using the Mie characterization mode with optical parameters 1.59 for the real refractive index and 0.01 for the imaginary refractive index.

3.3.3. Light microscopy

Light microscopy (Ortophlan, Germany) was performed to detect potential particles > 1 μm. Polarized light was used to make observation easier for the presence or absence of larger crystals (aggregates) using the magnifications of 160-fold and 600-fold with and without polarized light.

3.3.4. Zeta potential (ZP) measurements

The surface charge of the particles was assessed by zeta potential measurements using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK) applying a field strength of 20 V/cm at 25 °C. The Helmholtz–Smoluchowski equation was used for the ZP calculation built into the Malvern Zetasizer software. The zeta potential of particles depends on the dispersion medium; therefore, the surface charge has been measured in Milli-Q water adjusted to 50 μS/cm using 0.9% NaCl solution and in the original dispersion medium.

3.3.5. Long term physical stability

Samples were stored at three different temperatures, fridge (4 °C), room temperature (RT) (25 °C) and 40 °C. Particle size analysis was performed for 1, 3 and 6 months after the production.

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