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Production & stability of stavudine solid lipid nanoparticles—From lab to industrial scale

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ABSTRACT

The production of stavudine-loaded solid lipid nanoparticles (SLN) for intravenous injection was scaled up from lab scale (40 g) to medium scale (10 kg) and large scale (20/60 kg). The SLN were produced by high pressure homogenization of stavudine lipid melt dispersed in hot surfactant solution (pre-emulsion) applying 800 bar pressure. Employed were piston-gap homogenizers with increasing capacity (APV Gaulin products LAB 40, LAB 60 and Gaulin 5.5, and Avestin C50), using them in the continuous (circulation) and discontinuous mode. Size analysis was performed by photon correlation spectroscopy (PCS), laser diffractometry and light microscopy. At lab scale a PCS size of 53 nm was obtained. At the same pressure, all homogenizers on larger scale yielded a size in the range of the lab scale product (35–70 nm). Differences were found in the size as a function of circulation time (size increase or size reduction with time) and the number of cycles required (1 or 5) for the optimal product. The stavudine SLN formulation (2% lipid content, high surfactant to lipid ratio) showed a different behavior to conventional higher concentrated SLN suspensions or nanoemulsions (10% or 20% lipid/oil, low surfactant to lipid ratio). In general, smallest sizes were obtained in the discontinuous mode after just one homogenization cycle. The continuous production mode was only efficient with a 10 kg batch size using the LAB 60. In addition, the long-term stability over 1 year was monitored at refrigeration, room temperature and at 40 °C to assess a potential effect of the homogenizer type on stability. All batches at room temperature and below were stable, only a negligible increase in size was observed.

1. Introduction

A prerequisite for the introduction of particulate or nanoparticulate drug carriers into the pharmaceutical market is the availability of a large-scale production method. The method itself needs to be able to be qualified and validated, to be accepted by the regulatory authorities—apart from being cost-effective. In addition, it should yield a product of a quality also acceptable by the regulatory authorities, e.g. regarding accepted status of excipients and nanotoxicology. Large-scale production methods are established for microparticles, but there are still major problems in establishment of large-scale production methods for many nanoparticles (Muchow et al., 2008; Müller et al., 2002). A large-scale nanoproduction method in pharmaceutical industry is high pressure homogenization (HPH), e.g. for intravenous nanoemulsions (e.g. Intralipid, Lipofundin). The same production method is employed to produce solid lipid nanoparticles (SLN).

SLN are solid nanoparticles made from lipids being solid at body temperature (Hou et al., 2003; Manjunath et al., 2005; Müller et al., 1995, 2000b; Wissing et al., 2004) and are an alternative nanocarrier system to polymeric nanoparticles, liposomes and emulsions (Bullock et al., 1992; Deshpande et al., 2009; Krohn and Koletzko, 2006). One major advantage is, that SLN can be produced for different application routes (dermal, oral, i.v., etc.) using lipids and surfactants already accepted, or lipids being made from physiological compounds (e.g. glycerides of fatty acids available in the body, and fatty acids present in oils in parenteral nutrition). Many researchers studied SLN over the time for various applications (Abdelbary and Fahmy, 2009; Almeida and Souto, 2007; Basaran et al., 2010; Bhalekar et al., 2009; Bondi et al., 2009). Ability of production of lipid nanoparticles at lab scale, pilot and large scale is essential, the latter should provide not only the quantity but also lipid nanoparticles with long-term stability comparable to the lab scale (Weyhers et al., 2006). Some reports are available in the literature about the long-term stability of lipid nanoparticles from lab scale, e.g. till 12 months (Freitas and Müller, 1999) and 24 months (Schwarz, 1995). However, there is limited long-term stability data available when produced on large scale.

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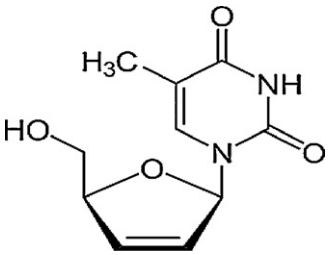

Fig. 1. Chemical structure of stavudine.

High pressure homogenization (HPH) is used since the 1950s for the production of parenteral emulsions. The first publications about the use of HPH for large-scale production of lipid nanoparticles date back to around 2000 (Müller et al., 2000a; Schneppe, 1998). Meanwhile three large-scale production lines – based on the published concept – are running in Germany (e.g. CLR GmbH, Berlin, www.clr-berlin.de, Dr. Rimpler GmbH/near Hannover, www.rimpler.de) and one in Nanjing/China. However, by now, never a systematic study was published comparing homogenizers with stepwise increasing capacity (e.g. 40 g, 2–10 kg, 50 kg and up to half a ton and more). This is realized in the present study, moving from small- to large-scale homogenizers using APV Gaulin types, i.e. LAB 40, LAB 60 to a Gaulin 5.5. In addition an Avestin C50 was included, same piston-gap principle, but different producing company with different design of the homogenization chamber.

Various nanoparticles are explored for HIV therapy (Asasutjarit et al., 2007; Baert et al., 2009; Bender et al., 1994; Berry, 2008; Chattopadhyay et al., 2008; Govender et al., 2008; Rohan and Sassi, 2009; Shah and Amiji, 2006) with promising results but very limited data is available on scale up of these nanoparticles on industrial scale. In addition, some of them are made of regulatory not accepted excipients. In this study – to have potential for clinical studies and finally a marketed product – lipid nanoparticles were employed. They provide scale up potential under GMP and acceptance of the excipients. Stavudine (nucleotide reverse transcriptase, Fig. 1) was selected as drug, because it showed a very promising potential to various organs in previously performed in vivo biodistribution studies with gamma scintigraphy (Shegokar and Singh, 2008). In addition, the treatment proved to be efficient in rat studies (Shegokar, 2010; Shegokar et al., 2009a,b). The stavudine-loaded SLN were produced and then investigated regarding their long-term stability. Ideally a ready-to-use product should result, i.e. ampoules with aqueous SLN suspension ready for injection, avoiding a tedious reconstitution in the clinic by the physician. However, even when different capacity homogenizers might yield a similar bulk size, the suspensions do not need to possess necessarily the same long-term stability. Stability differences can occur due to differences in the content of few large particles, or different arrangements of surfactants in the interfacial layer affected by the flow and dispersion profile in differently shaped homogenization chambers, etc. Therefore the long-term stability was investigated as second important parameter, apart from the essential fact of similarity in achievable sizes when scaling up.

2. Materials and methods

2.1. Materials

Stavudine, a nucleoside analog reverse transcriptase inhibitor (NRTI) of pharmaceutical grade purity was provided as a gift sample from Alkem Laboratories, Mumbai, India, Dynasan 114 (Trimyristin) from Sasol GmbH (Germany), Plurol® Oleique CC 497 from Gattefosse (France), Solutol HS 15 and Poloxamer 188 from BASF (Germany), and Tween 80 from Unichem, Mumbai.

2.2. Lab scale production

Stavudine SLNs were prepared by hot high pressure homogenization. Drug was dissolved in the melted lipid Dynasan 114 (2%, w/w total lipid phase = 0.150% drug and 1.850% lipid) in the presence of surfactants at 80 °C (Solutol 1%, Tween 80 1%, Plurol Oleique 1%, w/w). The hot lipid mixture was dispersed in an aqueous surfactant solution (Poloxamer 188 0.5%, w/w) at the same temperature. The formed coarse microdroplet emulsion was then homogenized using different capacity homogenizers in the continuous (CNT) and discontinuous (DIS) mode at 800 bar.

An APV Micron LAB 40, a piston-gap homogenizer (APV Systems, Unna, Germany) was employed for lab scale production of stavudine lipid nanoparticles. Minimum 20 mL and maximum 40 mL can be produced using the LAB 40 (Fig. 2, left), the pressure can be varied from 100 to 1500 bar. The aqueous dispersion is pressed by a piston through a small homogenization gap. A pressure of 800 bar and 5 homogenization cycles (passages) were applied. A sample was drawn from the product after each cycle for size analysis.

2.3. Medium-scale batch production

For production of the medium-scale batch, a Micron LAB 60 modified and equipped with special features was used (Fig. 2, right). It is equipped with a 10 L supply container and product container, double-walled for temperature control, including the pipes. It can be placed under an LAF unit for aseptic production. The drug containing lipid melt was added to the supply container containing hot aqueous surfactant solution and the coarse pre-emulsion produced with a dissolver disk. The supply container (and in discontinuous mode also the product container), and the pipes were maintained at 80 °C (water bath). This pre-emulsion was then circulated in the continuous mode for 30 min, and in the discontinuous mode 5 cycles (passages) were performed. The production pressure was 800 bar for both modes. In the circulation mode and in the discontinuous mode, the batch size was 10 L (appr. 10 kg). In the continuous mode, samples were drawn at the outlet pipe of the homogenizer at 2, 5, 10 min, etc. At the end of the production at 30 min a sample was drawn from the well mixed product. In the discontinuous mode, a sample was drawn from the product in the collection container. The samples were analyzed for size.

2.4. Large-scale production with the APV Gaulin 5.5

A Gaulin 5.5 was employed for production of the large-scale batch (Fig. 3, left). The Gaulin 5.5 has three pistons and two homogenization valves; the maximum capacity is 160 L/h (approximately 160 kg). The final product is passed to a product container, cooled in a controlled way, and filled into primary containers for transport. The connecting tubes were all insulated allowing temperature control of pre-emulsion and product. This unit allows also aseptic production. The homogenizer and supply and product containers can be sterilized by streaming steam. The containers are provided with sterile filtered air or protective gassing (e.g. nitrogen). The metal surfaces of all equipment were electro-polished. The stavudine containing lipid melt and the surfactants were added to the supply container, sterile hot water added and the pre-emulsion produced using a dissolver disc (Fig. 3, right). The stirring rate of the dissolver disk in the feeding container was kept constant at 220 rpm throughout the experiment and optimized prior to running batches. The temperature of the supply container was maintained at 80 °C. When using the Gaulin 5.5 in the continuous (circulation) mode, the pre-emulsion was passed through the non-temperature controlled homogenization unit and circled back to the supply container. The batch size was 20 kg, maximum possible with this arrangement was 60 kg (=container capacity). In the discontinuous mode, pas-

Fig. 2. APV Micron LAB 40 for lab scale production unit used for 40 mL batch (left) and LAB 60 for 10 kg batch production (right).

sages (cycles) were run, i.e. after passing the homogenization unit the complete batch was collected in the product container. Then the product was passed 4 more passages through the homogenization unit to the respective other container, giving a total of 5 passages. Finally the product (hot nanoemulsion) was cooled to room temperature. In the discontinuous mode, the batch size was only 20 kg, but with exactly the same set up, larger quantities can also be run—depending upon the size of the containers (e.g. half a ton in about 3 h). The obtained data are also valid for such larger batches, therefore represent a full scale up. Samples for size analysis in the continuous and the discontinuous mode were drawn as described in Section 2.3.

2.5. Large scale with the Avestin C50

The Avestin C50 (Avestin Inc., Ottawa, Canada) homogenizer has a capacity for larger scale production from 15 to 50 L/h (high/low pressure). It has a pneumatically controlled, dynamic homogenizing valve with a standard plunger back flush system. The entire homogenizing valve can be easily disassembled for clean-

Fig. 3. APV Gaulin C 5.5 high pressure homogenizer with capacity: 150 L/h (left) and 60 kg supply/product container (right) with open lid showing the dissolver disk for preparing the pre-emulsion.

ing and inspection. All other seals are precision metal/ceramic and ceramic/ceramic face seals without gaskets. There are no "O"-rings or gaskets in the entire path of the product. The only plastic seal is the plunger seal which is UHMWPE (Ultra High Molecular Weight Polyethylene). The flow rate depends on the homogenizing pressure selected, it decreases with increasing pressure. At high pressure of 2,000 bar it goes down from 50 L towards 15 L/h. The minimum sample volume is about 25 mL (hold back volume < 4 mL) at homogenization pressure ranging from 500 to 30,000 psi/3.5–207 MPa/35–2000 bar. All wetted parts are autoclavable and are FDA approved. The pump inlets can be connected easily to steam (120–130 °C for steam sterilization in place). The coarse pre-emulsion was produced using an ultra-turrax (Janke und Kunkel GmbH, Staufen, Germany) for 1 min at 9,500 rpm, and homogenized at 800 bar in the continuous (circulation) and discontinuous mode (passages). The batch size was 10 kg for the continuous mode, and for the discontinuous mode. The results for the continuous mode are only valid for this batch size, because the time for 99.9% of the droplets having passed the homogenization unit at least once increases tremendously with the size of the batch. The discontinuous data are also valid for larger batches, depending again on the size of supply and product container. Due to the lower capacity per hour at higher pressures compared to the Gaulin 5.5, the Avestin C50 is less suited for larger batches, but fully suited for smaller batches of potent actives. Again samples for size analysis in both modes were drawn as described in Section 2.3.

2.6. Characterization

2.6.1. Photon correlation spectroscopy (PCS)

PCS for size analysis of the bulk population was performed using a Zetasizer Nano ZS (Malvern Instruments, UK). The analysis yields the *z*-average of the particles, 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. The measuring range of the Zetasizer is from approximately 0.6 nm to 3 μm. The SLN samples were diluted in distilled water and measurements were performed at 25 °C temperature.

2.6.2. Laser diffractometry (LD)

LD was performed using a Mastersizer 2000 (Malvern Instruments, UK) using deionized water as dispersion medium. The instrument was operated with the Hydro S sample dispersion unit. LD yields a volume distribution. The diameters $d(v)50\%$, $d(v)90\%$, $d(v)95\%$ and $d(v)99\%$ were used as characterization parameters. For example, $d(v)50\%$ means that 50% of the particles are below the given size. Five consecutive measurements were performed, to ensure that no change in the sample occurred during measuring. Values given are the mean of $n = 5$.

2.6.3. Zeta potential (ZP) measurements

The surface charge of the particles and electrostatic stabilization was quantified by zeta potential measurements using the Malvern Zetasizer Nano ZS (Malvern Instruments, UK). The Zetasizer Nano measures the electrophoretic mobility of the particles, which was converted into the zeta potential using the Helmholtz–Smoluchowski equation built into the Malvern Zetasizer software. The measured zeta potential of particles depends on the dispersion medium; therefore, the surface charge has been measured in Milli-Q water with conductivity adjusted to 50 μS/cm using 0.9% NaCl solution (=Stern potential, related to the surface potential (charge), i.e. Nernst potential) and in the original dispersion medium (stability criterion for electrostatic stabilization).

2.6.4. Long-term stability measurements

The long-term stability was determined for all the formulations produced using the Micron LAB 40, LAB 60, Gaulin 5.5 and Avestin C 50 for both continuous/discontinuous mode at refrigeration (4–8 °C), room temperature and 40 °C. The SLN dispersions were filled into 20 mL silanized glass vials, sealed with rubber stopper and metal clip. The samples were analyzed for particle size using PCS and LD. In addition, light microscopy was performed to check the cause of potential instability (aggregates versus crystal growth (e.g. Ostwald ripening) or drug expulsion and related crystal formation). Measurements were performed on 1, 7, 14, 30 days, and 3, 6 and 12 months (data is only given for 1, 6 and 12 month).

3. Results and discussion

3.1. Lab scale production with LAB 40

The scaling up was performed with SLN loaded with a drug, because not only the scale-ability should be investigated, but at the same time a production process specifically for stavudine SLN should be established. This would open the perspective to introduce this in animal studies promising formulation into clinical trials. The advantage of the LAB 40 is the batch volume of 20–40 mL. This minimizes the amount of drug, in case of expensive or limited availability of drugs, when performing a formulation screening (lipid composition, surfactant type and concentration). The 40 mL is still sufficient to obtain 3 samples for preliminary stability studies at 3 temperatures. As the process is discontinuous, the system needs to be dismantled and the dispersion poured back into the central feeding cylinder for the next homogenization cycle. This is somewhat time consuming but the advantages outlined above compensate easily for this slight inconvenience.

In a previous study, the optimal homogenization pressure for stavudine SLN was investigated for the LAB 40, being 800 bar (Shegokar et al., 2009a,b). The same pressure was applied in this study when comparing the small-, medium- and large-scale industrial batch production. To assess the effect of cycle numbers, 1–5 cycles were run with the discontinuous homogenizers, and the size analyzed by PCS and LD after each cycle. A circulation time of 30 min was selected in the continuous homogenizers, samples taken in between to assess the effects of circulation time.

In previous studies, most SLN suspensions were typically composed of 1–2% surfactant/s and 10% or 20% lipid phase (ratio surfactant to lipid around 1:10 to 1:20). The rational behind selecting high lipid (particle) content was to have higher concentrated suspensions for further processing, e.g. admixture to creams. These concentrations yielded particle sizes around 200–300 nm, the minimum size typically obtained after 2–3 homogenization cycles with the LAB 40 (Souto et al., 2005). No further size reduction occurred when running additional cycles, i.e. the maximum dispersitivity was reached. Smaller particle sizes can be obtained when reducing the lipid phase (lipid and drug) content and increasing the ratio surfactant to lipid, e.g. sizes below 100 nm (Westesen et al., 1997). To obtain very small injectable SLN, in this study the lipid content was reduced to 2% and the surfactant to lipid ratio increased to 35:10. This resulted in a size of 53.1 nm with a PDI of 0.213 after just 1 homogenization cycle (Fig. 4a, Table 1). The LD diameter 99% was 0.238 μm, well below the critical 5 μm for i.v. injectables. The stavudine suspensions, independent on the type of homogenizer used, were bluish transparent in color and the pH was in range of 6.7–7.1.

Increasing the number of cycles to 5 did not lead to a further decrease in the bulk (PCS) size—in contrast to higher concentrated SLN suspensions. There was even a clear increase in PCS size, most pronounced from cycle 3 onwards. This can be explained as fol-

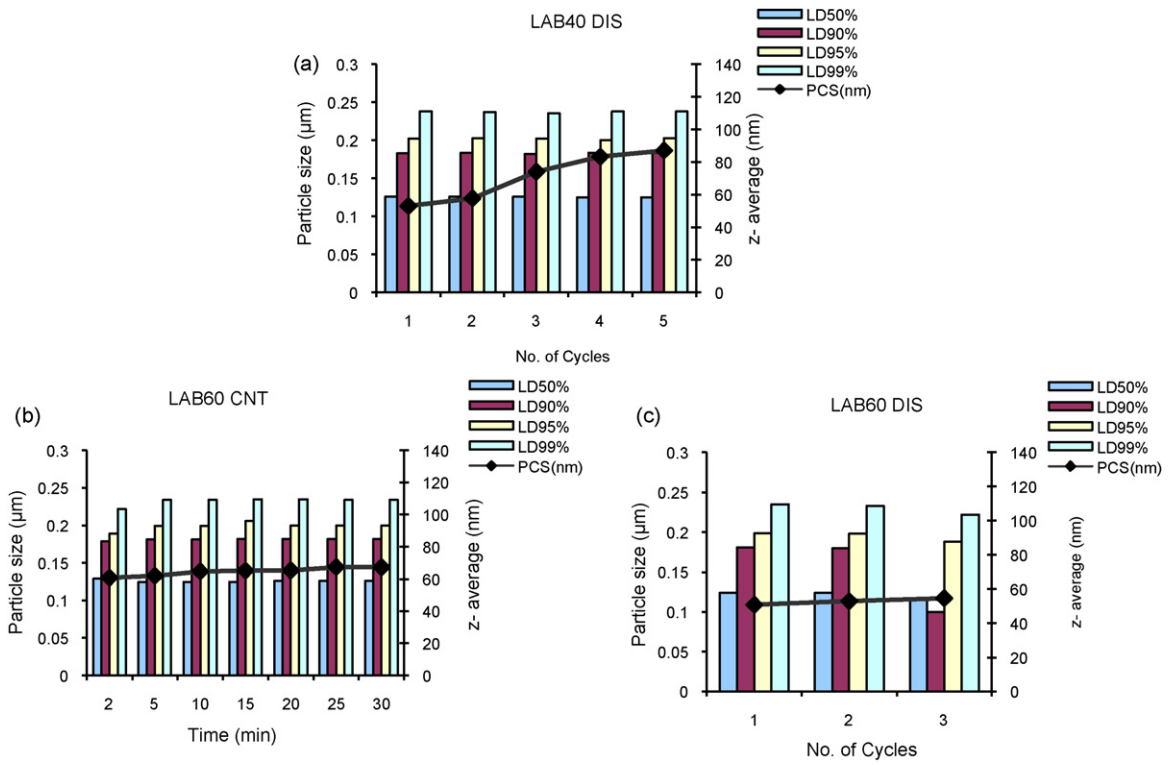

Fig. 4. PCS size and LD diameters 50%, 90%, 95% and 99% of SLN as a function of cycle number (discontinuous production mode (DIS)) or as a function of circulation time (continuous mode (CNT)), (a) the LAB 40 in discontinuous (LAB 40 DIS), (b) the LAB 60 in continuous (LAB 60 CNT) and (c) in discontinuous mode (LAB 60 DIS).

lowed: the additional energy input led to higher kinetic energy of the droplets with subsequent coalescence. The maximum dispersitivity was already reached at 1 cycle. Additional energy put into the system cannot be used for further dispersion of lipid. The energy put in can only accelerate the velocity of the particles, giving them sufficient kinetic energy to overcome the stabilization by the surfactants, leading to aggregation and increase in mean size. The size increase affected only the bulk population of 50 nm droplets, there was little change in the larger sizes detected by LD, e.g. diameters 90% to 99%. In summary, applying 1 cycle at 800 bar are the optimal production conditions on lab scale with the LAB 40.

The zeta potential (ZP) observed was practically the same, independent on the homogenizer used, i.e. in range of about −20 to −24 mV in water and around −17.0 mV in the original dispersion medium (Table 1). This is logic, because it is mainly influenced by the formulation composition.

3.2. Medium-scale production with the LAB 60

The Micron LAB 60 homogenizer (production capacity of 60 L/h) can be operated in a continuous and a discontinuous production mode. In contrast to the LAB 40 as a single punch machine, it consists of two pumps yielding a product flow with minimized fluctuations in the homogenization pressure. The dispersion is subsequently passed through two homogenization valves: a first main homogenization valve, and a second valve that creates a certain reverse pressure. It is also in charge of re-dispersing potentially coalesced droplets or aggregates formed after passage of the 1st valve, when the droplets have a high kinetic energy. As a general rule, the homogenization pressure of the second valve should be about one-tenth of the pressure used in the first valve. In other words, this means that using a pressure of 800 bar for the first valve results in a pressure of 80 bar for the second valve.

For small batches the discontinuous mode is not sensible, because of the dead volume of the machine being around 0.5 L. That means in the discontinuous mode with a 1 kg batch, about half of the product would still remain in the machine after each passage, and not being properly homogenized. For smaller batches the continuous (circulation) mode is preferred, e.g. 2 kg. It has the disadvantage, that homogenized product is mixed with non-homogenized product when circulating back to the emulsion container, which feeds the homogenizer. Statistically a certain circulation time is required,

Table 1
Production parameters of the different homogenizers for the stavudine lipid nanoparticles batches, which yielded a product comparable to the lab scale with the LAB 40, plus characterization parameters size and zeta potential (DIS, discontinuous mode; CNT, continuous production mode, cycle number (1, 5) or circulation time (min) is given; PCS, photon correlation spectroscopy; LD, laser diffraction diameters; zeta potential was measured in water and original dispersion medium = surfactant solution).

Homogenizer type/production details		PCS		LD (µm)		Zeta potential (mV)	
		Size (nm)	PDI	$d(v)$90%	$d(v)$99%	Water (50 µS/cm)	Original surfactant solution
APV LAB 40	DIS/1 cycle	53.1	0.213	0.183	0.238	−23.3	−17.4
LAB 60	CNT/30 min	70.2	0.196	0.182	0.234	−24.3	−17.1
	DIS/1 cycle	54.8	0.195	0.170	0.224	−21.5	−15.3
Gaulin 5.5	CNT/30 min	115.3	0.132	0.211	0.230	−19.5	−15.4
	DIS/5 cycle	63.3	0.158	0.152	0.203	−20.6	−16.5
Avestin C50	CNT/30 min	107.5	0.154	0.190	0.235	−22.6	−16.8
	DIS/1 cycle	35.8	0.150	0.179	0.231	−22.8	−16.3

to be sure that 99.9% of the droplets have passed at least twice the homogenization gap. There is an equation to calculate the required homogenization time as function of batch size and the number of equivalent passages (Dingler, 1998; Jarchau, 1997). To be sure that 99.9% of the droplets have at least 2 passages through the homogenization gap, approx. 18 min are required for a 2 kg batch, and theoretically about 90 min for a 10 kg batch (i.e. 45 min for one passage).

For reasons of direct comparison, 10 kg batches were produced in this study with both modes. In the continuous mode only 30 min instead of the theoretical 90 min were run, because 90 min are not a "production friendly" time, it is too long. It should be investigated, if a shorter time is sufficient for this specific product. In the continuous mode (LAB 60 CNT), after 2 min homogenization, the mean PCS diameter was 67.4 nm with a PDI of about 0.193, the LD diameter $d(v)$99% 0.222 μm (Fig. 4b, Table 1). A narrow particle size distribution was obtained when production was performed in continuous mode. During the circulation time of 2–25 min, the size of the product leaving the homogenizer stayed practically unchanged. At 30 min, the size of the product in the container was analyzed, being 70.1 nm PCS diameter. Within less than the theoretical time of 45 min required for a first passage of 99.9% of the particles, the particle size obtained was practically the same as that in lab scale production with one homogenization cycle (58.1 nm). The PDI and LD values were also the same (Table 1).

In summary, scale up to medium size with the LAB 40 in the continuous mode was achieved, optimal production conditions are 800 bar/80 bar (1st and 2nd valve) and 30 min circulation time. Surprisingly a good product was obtained, despite the homogenization time being below the theoretically calculated time of 45 min for 99.9% of the droplets having passed the homogenization gap at least once. An explanation might by that the formula was derived for rather larger nanoemulsions (>200 nm) of normal composition (e.g. 20% lipid content), and not for such a low concentrated system with very high surfactant to lipid ratio.

In the discontinuous mode, the 10 kg batch was produced at identical pressures (800 bar/80 bar) and temperature running three homogenization cycles, instead of 5 with the LAB 40. A reduced number of cycles was selected, because the LAB 60 has 2 homogenization valves, instead of one in the LAB 40. This makes the process of dispersing more efficient, and from our experiences about half the cycle number is required to obtain the same size.

Application of just one homogenization cycle with the LAB 60 was sufficient to reach a size of 54.7 nm, the LD diameter 99% was 0.224 μm (Fig. 4c, Table 1). These are identical data as obtained on lab scale with the LAB 40 (53.1 nm). With both machines, just 1 cycle proved to be sufficient for the present formulation composition. Applying additional cycles had practically no effect on the size, there was no size increase as observed with the LAB 40. In summary, without changing homogenization pressure and temperature, a medium-scale batch can be successfully produced with the LAB 60, applying only 30 min circulation time or just 1 cycle. The discontinuous mode can be used also for larger batches than the investigated 10 kg, because it is only a function of the size of the emulsion containers. Of course, too large batches should not be produced with the LAB 60, because the pre-emulsion in the supply container might be exposed for a too long time to elevated temperature. For example, 10 kg product takes in the LAB 60 about 10 min, a 60 kg batch requires 1 h. In such cases higher capacity homogenizers as the Gaulin 5.5 are preferable (homogenization time for 60 kg only about 23 min).

3.3. Large-scale production with the APV Gaulin 5.5

The Gaulin 5.5 production line available was equipped with containers for 60 kg product. The batch size produced was 20 kg, because there are no basic differences when moving up in the batch size. The pumping velocity was reduced from 160 to 60 L/h, for reasons of comparability with the LAB 60. In the continuous mode, the sample taken at 2 min homogenization time showed a mean particle size of 67.5 nm with a low polydispersity index of 0.162, and LD diameter 99% of 0.254 μm (Fig. 5a). This is practically identical with the sizes of the products obtained on lab and medium scale. However, the particle size increased with increasing circulation time. The product analyzed at 30 min showed a PCS particle size to 115.3 nm with a polydispersity index of 0.132. In contrast to the LAB 60, a 30 min circulation time with the increased batch volume from 10 to 20 kg was not sufficient, to yield a product with the specification of a PCS diameter $\ll$ 100 nm. The continuous mode with the Gaulin 5.5 is not suitable with batch size of 20 kg and above.

Interestingly, in the discontinuous mode a steady decrease in particle size was obtained from cycle 1 to cycle 5 (Fig. 5b). This is in contrast to the LAB 60, where an increase in size was observed. The PCS diameter decreased from 90.6 nm (cycle 1) to 63.3 nm after 5 cycles, the LD diameter 50% dropped from 0.135 to 0.111 μm. After 5 cycles a narrow polydispersity index of 0.158 was achieved. Similar sizes as on lab scale were obtained, when applying 5 cycles in the discontinuous mode of the Gaulin 5.5. The homogenization time for 20 kg is 20 min with a pumping velocity of 60 L/h and only 8 min with a velocity of 160 L/h. This is shorter than the 30 min in the continuous mode, which even yielded a product of insufficient quality (i.e. size not $\ll$100 nm).

3.4. Large-scale production with the Avestin C50

In further extension of this study, the large-scale production unit with the Gaulin 5.5 was compared to the Avestin C50. Both homogenizers are based on the piston-gap principle, but made from different manufacturers, have consequently differences in the design of the homogenization chamber. The design of the chambers affects very much the efficacy, but up to now there are no mathematical models around to predict the optimal design of a homogenization chamber for a certain dispersion task. Therefore it is still necessary to find out empirically which chamber design is most suitable for a certain product. Form these considerations it made sense to compare the machines. The pneumatic Avestin C50 model was selected which is able to produce larger quantities, because it is distinctly cheaper than the hydraulic version C55. The hydraulic driven version has the advantage that the capacity 55 L/h is not decreasing with increasing pressure.

The batch size was again 10 kg. The Avestin C50 was able to generate the desired particle size range, i.e. 61.3 nm PCS diameter in the continuous mode at 2 min circulation time, low polydispersity index of 0.169. Further increase in homogenization time resulted in a subsequently pronounced increase in particle size. The size of the product in the container measured after 30 min homogenization was 106.8 nm (Fig. 5c). This is practically identical to the Gaulin 5.5 in the continuous mode (115.0 nm). The Avestin C50 is also not suitable in the continuous mode.

In discontinuous mode, the Avestin C50 generated a particle size of 35.8 nm with a polydispersity index of 0.150 after 1 cycle (Fig. 5d, Table 1). Further cycles lead to a slight increase in PCS and LD diameters, e.g. PCS from 35.8 to 48.5 nm.

3.5. Concluding comparison of the different homogenizers

The LAB 40 generated a PCS size of about 53 nm after 1 cycle at 800 bar, being a pressure suitable for large-scale production. Employing the other homogenizers for medium- and large-scale enabled to obtain a similar size of the bulk population (36/54 to 70 nm) and similar low values for the LD diameters, e.g. diameter 99% (range 0.203–0.238 μm, compared to 0.238 with the LAB 40)

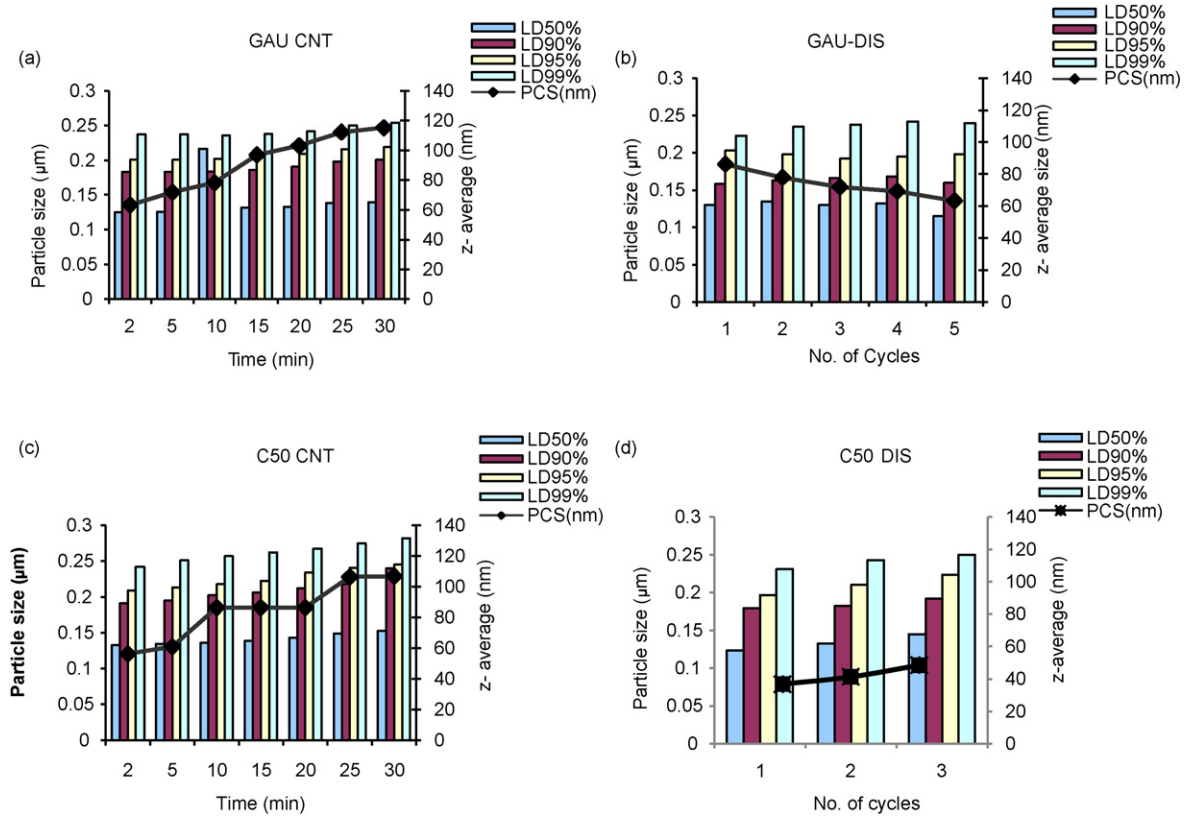

Fig. 5. PCS size and LD diameters 50%, 90%, 95% and 99% of SLN as a function of cycle number (discontinuous production mode (DIS)) or as a function of circulation time (continuous mode (CNT)): (a) the Gaulin 5.5 in continuous (GAU CNT) and (b) in discontinuous mode (GAU DIS), (c) the Avestin C50 in continuous (C50 CNT) and (d) in discontinuous mode (C50 DIS).

(Table 1 and Fig. 6). However, the production conditions to reach this size differ for the respective machines, an overview is given in Table 1 and Fig. 6.

Interestingly, with all four homogenizers LAB 40, LAB 60, Gaulin 5.5 and Avestin C50 the smallest size was obtained at identical pressure of 800 bar. In the discontinuous mode, the LAB 60 and the Avestin C50 yielded the smallest particle size after just 1 homogenization cycle, increase of cycles had no or little effect (Figs. 4c and 5d). A very small size of about 36 nm was obtained with the C50, which cannot be explained straight away, because this size is even below the size in the continuous mode of the Avestin C50. In case a larger size is required, the simple solution would be to run the C50 with a slightly pressure lower than 800 bar.

In contrast, the Gaulin 5.5 in the discontinuous mode yielded the smallest particle size after 5 homogenization cycles. However, it should be noted, that after 1 cycle, the PCS diameter was already very small with 87.6 nm. To reduce the number of cycles to just one, increasing slightly the pressure is an option. The continuous (circulation) mode was only suitable when using the LAB 60 (Fig. 4b), the Gaulin 5.5 and the Avestin C50 yielded particles above 100 nm after 30 min circulation time. Increasing the circulation time is not meaningful, the production takes too long, the product is exposed

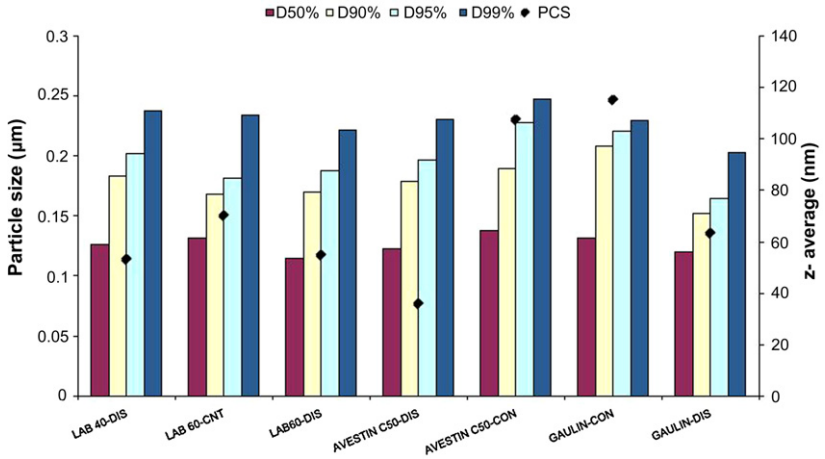

Fig. 6. Comparative effect on final mean PCS particle size (nm) and LD diameters (µm) applying the optimal production parameters listed in Table 1 for the different homogenizers (DIS, discontinuous mode; CNT, continuous production mode).

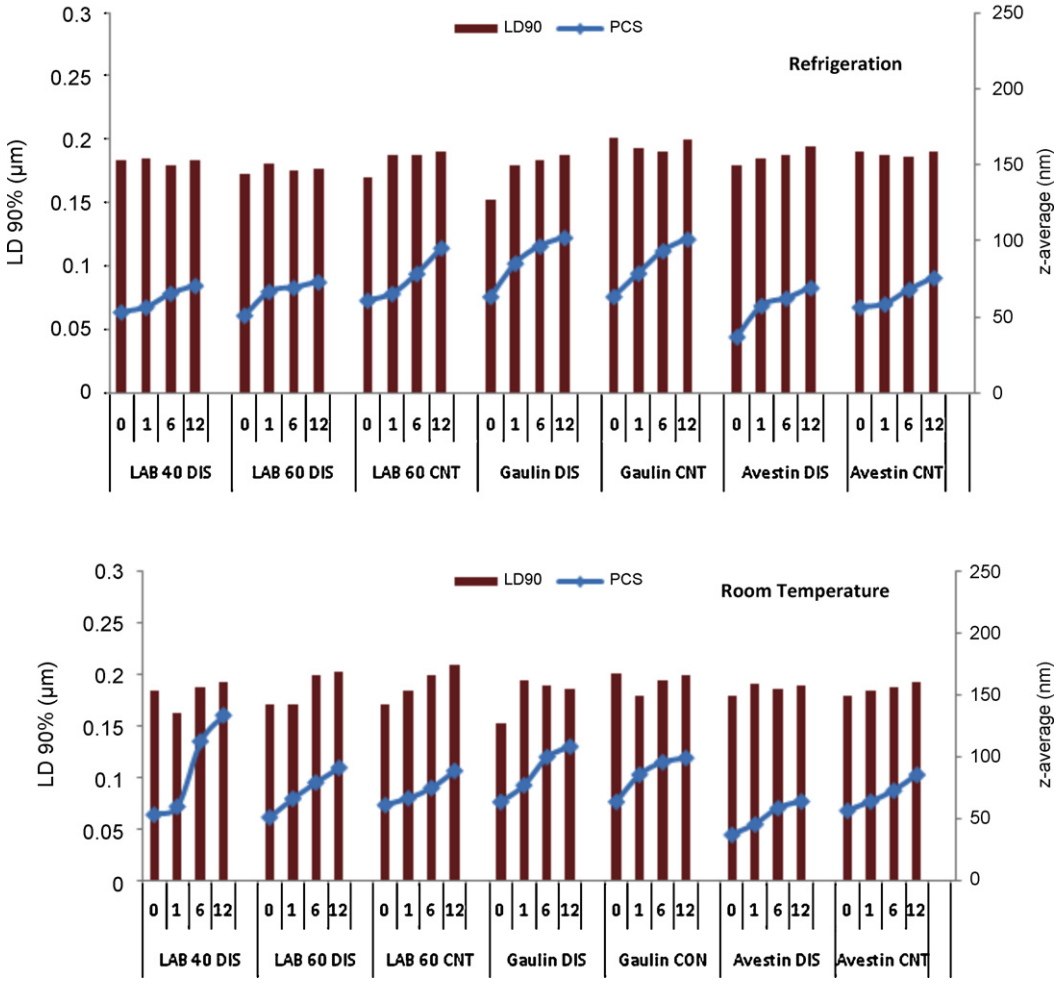

Fig. 7. Long-term stability as function of months (1, 6 and 12) of stavudine lipid nanoparticles produced using: (a) APV LAB 40 (DIS), (b) APV LAB 60 (CNT/DIS), (c) Gaulin C 5.5 (CNT/DIS), and (d) Avestin (CNT/DIS) at refrigeration (above) and room temperature (below).

too long to heat. As a summary, the continuous mode – even when using the LAB 60 – should rather be employed only for a batch size distinctly below 10 kg, e.g. 2 kg coming closer to the circulation times in the equation by Jarchau.

The minor differences in mean diameter and polydispersity index do not impair the quality of the particle dispersion and its performance in the final product. Scaling up to larger production units creates differences in the heat transfer between product and surfaces/metal parts of the production unit, as well as in the cooling rate of the final product. Obviously, these heat transfer effects had little impact on the mean size and size distribution of the lipid particle dispersion. In summary, increasing the production scale from 40 g to 20 kg, i.e. by a factor of 500, could be performed without changing the production parameters of pressure and temperature. If desired, a fine tuning can be performed by modifying slightly the production pressure. It should be noted, that sizes of SLN can be different when producing a drug-free formulation (blank formulation, SLN with lipid only) and the corresponding SLN suspension with additionally drug incorporated in the lipid particle phase. If the drug is lipophilic and incorporated mainly throughout the bulk of the lipid, this will have little effect on the interfacial stabilizer layer and consequently the sizes will be similar. The situation change, when the drug is amphiphilic in character, localizes in the interface and becomes part of the stabilizing interfacial layer (e.g. Amphotericin, which localizes in parenteral emulsions in the lecithin layer). To be on the safe side, scale up should be performed with the final formulation desired, not with blank SLN.

Differences in drug loading as a function of batch size were not investigated within this study, but can be excluded as minimal due to physics. During production, each drug will partition according to its partitioning coefficient $K_{o/w}$ between the molten and later solidified lipid phase and the aqueous surfactant phase (2 phase partitioning). The constant $K_{o/w}$ is independent on size of the lipid phase, and differs only slightly for amphiphilic drugs localizing in the interface (localization is function of interface size, 3 phase partitioning).

3.6. Long-term stability

Despite having the same or similar size, the long-term stability might differ. There are differences in the stability depending on the minor content of larger droplets. In general homogenous populations are more stable, therefore one tries in emulsion production to minimize large droplets by additional homogenization cycles despite the diameter of the bulk population does not change. Differences in the homogenization process might lead to differences in the coverage of the interface with surfactants (spatial arrangement), not detectable by, e.g. ZP measurements. Therefore all samples were put on a long-term stability test for 1 year at 3 different temperatures. Used for the study were the SLN suspensions from the last production cycle in the discontinuous production mode or after 2 min circulation time in the continuous mode. The latter still had a size in the targeted range, the lipid nanoparticle suspensions after 30 nm circulation time were >100 nm. Aim was

to compare the stability of SLN similar in size, to exclude effects on stability due to size differences.

From the ZP measurements in original dispersion medium of about −17 mV, they were not fully electrostatically stabilized. This would require at least about 30 mV. However, the surfactant mixture contains also sterically stabilizing Tween and Poloxamer, which could compensate the missing charge. ZP measurements in water are actually measurements of the Stern potential, being related to the surface potential (Nernst potential). The ZP values were about −20 to −24 mV, i.e. only slightly higher than in the original dispersion medium (Table 1). This supports the presence of non-ionic, non-charged adsorbed stabilizers. They hardly desorb upon dilution (e.g. multipoint attachment of Poloxamer), covering surface charges and can lead to low measured ZP values compared to non-covered surfaces.

Long-term stability studies at refrigeration and room temperature showed only a slight increase in particle size and polydispersity index over the period of 12 months. The PCS diameter increased by about 20–30 nm in the SLN suspensions produced with the larger scale homogenizers, by about 50 nm in the suspension on lab scale from the LAB 40 stored at room temperature. There was a tendency that the larger scale increased stability at room temperature. There was little change in the LD diameter 50% for all batches (Fig. 7, please note scale of axis). Light microscopy revealed the absence of aggregates (data not shown), which might not be detectable by LD. The diameter 99% stayed below 0.25 μm. In contrast, the samples stored at 40 °C degree showed a marked increase in particle size after the 14th day, resulting subsequently in formation of thick gel for all prepared batches. To summarize, the stavudine SLN suspension from all homogenizers show moderate size increase, still being injectable after 1 year of storage. Prerequisite is, however, storage at room temperature or below, avoiding higher temperatures which promote aggregation and finally gelling.

Of course, it has to be pointed out that the physical stability has to be supplement by chemical stability data, i.e. of the drug itself and – often forgotten – the lipid excipients! It is a highly dispersed system, large interface, and the question of, e.g. lipid oxidation or lipid degradation occurs. Long-term stability of various lipids in aqueous SLN suspensions was shown previously (Radomska et al., 1999) attributed to the solid state of the lipid in the particles—in contrast to the liquid droplets of o/w emulsions.

4. Conclusions

Scaling up of the stavudine production was possible using medium- and large-scale homogenizers, based on the same homogenization principle piston-gap (APV Gaulin machines). It was also possible to use a differently designed homogenizer (homogenization chamber) from a different manufacturer (Avestin C50). At least for the investigated formulation, the homogenization system of the piston-gap arrangement seems to be rather robust, yielding practically identical or very similar sizes when using the discontinuous production mode, or the continuous mode with the LAB 60. The robustness is supported by the fact, that exactly the same production pressure of 800 bar was used for all machines, no adaptation of the pressure was necessary. However, the continuous mode with the batch sizes used is not recommended with the Gaulin 5.5 and the Avestin C50.

For nanoemulsions with a size typically above 200 nm and a higher content of lipid (10% or 20%) a few cycles (2–4) are typically required to achieve the smallest size (maximum dispersitivity). Surprisingly with the stavudine formulation 1 cycle yielded the smallest size (except Gaulin 5.5), additional homogenization cycles rather promoted particle growth. Obviously formulations with very low lipid content (2%, w/w) and a very high surfactant to lipid ratio behave differently to the conventional nanoemulsions.

Obviously, the type of homogenizers does not affect the long-term stability of similar sized systems. Large-scale homogenizers seem rather to yield tendentially more stable suspensions. The observed instability at 40 °C (gelling) is attributed to the SLN formulation itself.

Based on the obtained data, homogenization scale up – compared to scale up in other processes – proved to be relatively easy without the need of intensive screening for optimized production parameters in each scale up step. If desired a certain specific target size can be obtained by variation of the production pressure. In contrast to the parameter pressure, screening is obviously recommended for the cycle number, as shown by the different behavior regarding size increase and decrease. The production parameters established for the large-scale machines in the discontinuous mode can be used for even larger quantities, just by increasing the capacity of the supply containers in the production line.

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