Production and Characterization of a Budesonide Nanosuspension for Pulmonary Administration

Claudia Jacobs[1] and Rainer Helmut Müller[1,2]

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Purpose. This study describes the production of a budesonide nanosuspension by high-pressure homogenization for pulmonary delivery from 40 mL up to 300 mL. The aim was to obtain a nanosuspension that can be nebulized and is also long-term stable.

Methods. The nanosuspension was produced by high-pressure homogenization. Particle size analysis was performed by laser diffraction and photon correlation spectroscopy. For further particle characterization, zeta potential was determined. To investigate the aerosolization properties, the nanosuspension was nebulized and afterward analyzed on particle size.

Results. It was possible to obtain a long-term stable budesonide nanosuspension. Mean particle size of this nanosuspension was about 500–600nm, analyzed by photon correlation spectroscopy. Analysis by laser diffraction showed that the diameters 95% and 99% were below 3 μm. Budesonide nanosuspension showed a long-term stability; no aggregates and particle growth occurred over the examined period of 1 year. The PCS diameter before and after aerosolization did not change, and the LD diameters increased negligibly, showing the suitability for pulmonary delivery. The scale-up from 40 mL up to 300 mL was performed successfully.

Conclusions. High-pressure homogenization is a production method to obtain nanosuspensions with budesonide for pulmonary application.

KEY WORDS: budesonide nanosuspension; high-pressure homogenization; aerosolization; pulmonary delivery.

INTRODUCTION

Budesonide is a corticosteroid used in asthma therapy of moderate to severe asthma (1). It represents, along with other corticosteroids, one of the most valuable therapeutic agents for the prophylactic treatment of asthma. Corticosteroids reach their application site, the receptor, by diffusing into cells. Therefore, a fast dissolution of the drug in the airways is desired.

Budesonide is a poorly soluble drug, *e.g.,* the solubility in water is < 1 mg/100 mL (2). The drug is applied through dry powder inhalers or metered dose inhalers. The drug powder for these systems is jet milled; the mean particle size achieved with this technology is in the lower micrometer range of ~3–25 μm (3). To improve the properties of budesonide formulation, a nanosuspension was developed.

The general advantages of nanosuspensions compared with microparticulate systems are their increased dissolution velocity and saturation solubility, especially below 1–2 μm. A steep increase in intrinsic dissolution rate is reported for particles smaller than 1 μm (4,5). For the poorly soluble drug budesonide, it would mean that larger concentration of drug would occur more rapidly in the lung, leading to higher local drug levels at the absorption site. This formulation would deliver drug more efficiently than particles produced by traditional approaches.

The higher saturation solubility can be explained by the Kelvin and Ostwald-Freundlich equations (6,7). The Kelvin equation describes the transition of molecules from a liquid phase to a gas phase, which can also be applied to the transition of molecules from a solid phase (drug particle) to a liquid phase. The smaller the droplets or particles, the stronger the curvature and as a result an increased vapor pressure (or in case of particles increased dissolution pressure) occurs. The dependency of the saturation solubility on the particle size is also explained in the Ostwald-Freundlich equation. The increased dissolution velocity can be explained by the Noyes-Whitney and the Prandtl equations (8,9). According to these principles, the bioavailability of drug nanosuspensions is increased (10,11).

Based on these generally known principles, the advantages of nanosuspensions can be used for new or improved drug formulations, especially for drugs with poor solubility in aqueous and simultaneously organic media (12,13) such as budesonide.

An additional advantage is the adhesivness of small particles onto surfaces. It is well known from powder technology that fine powders or particles generally possess an increased adhesiveness to other particles or surfaces compared with larger particles. The tendency of the particles to stick to mucosal surfaces at the absorption site over an extended period of time achieves an enhanced absorption rate. Although the dissolution time of nanosuspensions compared to microparticulate suspensions is increased, a prolonged residence time at the site of absorption would be still beneficial for the uptake of budesonide, because microparticles will be transported out of the lungs by cilia movement (14), whereas nanoparticles can adhere a longer time onto the mucosal surface (15) and in that way increase the absorption of drug. Nanosuspensions generally possess a very low fraction of microparticles (16), which reduces unwanted deposition of particles in mouth and pharynx and in this way decreases local and systemic side effects of budesonide.

Nanoparticles can be obtained by different production methods. If the drug is soluble in an organic solvent, precipitation (17) would be a possible method. Another way to obtain nanoparticles is pearl milling (18). The drug suspension is filled into a pearl mill and ground to nanoparticles for several days. In this study, the nanoparticles are obtained by high-pressure homogenization, with a piston gap homogenizer (19,20). The drug suspension is pressed through a small gap at high pressure, and the cavitation forces are high enough to disrupt the microparticles into nanoparticles. For drug suspensions, pressures of about 1500 bar are usually applied.

Pulmonary delivery of budesonide drug nanoparticles would be realized by nebulizing the aqueous drug nanoparticle suspension (nanosuspension) by using a commercially available system (*e.g.,* Pariboy, medic aid). Compared to the same single dose in the form of microparticles, transfer to

[1] Free University of Berlin, D-12169 Berlin, Germany
Department of Pharmaceutics, Biopharmaceutics and Biotechnology, D-12169 Berlin, Germany.
[2] To whom correspondence should be addressed. (e-mail: mpharma@zedat.fu-berlin.de)

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nanoparticles increases the number of particles to the extent that statistically in each aerosol droplet drug particle can be found. This leads to more efficient and even more delivery to the lungs (Fig. 1).

The objective of this study was to develop a budesonide nanosuspension by high-pressure homogenization and to investigate the aerosolization properties of this nanosuspension to use them for pulmonary delivery. In addition, scale-up and long-term stability properties were examined.

MATERIALS AND METHODS

Materials and Chemicals

The drug budesonide, used as a model drug for pulmonary administration, was provided by SkyePharma AG (Muttenz, Switzerland) (jet-milled quality, LD diameter 50% about 2 μm and 99% about 11 μm). Soya lecithin (Lipoid S 75) was supplied by Lipoid GmbH (Ludwigshafen, Germany). Tyloxapol, Span 85, cetyl alcohol, and Thiomersal were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Lecithin and Span 85 are approved for delivery to the lungs by regulatory agencies. Cetyl alcohol is a substance with GRAS status, and tyloxapol is the active ingredient of Exosurf®Neonatal and SupraVent,™ which are used for cystic fibrosis and chronic bronchitis. Phase 1 clinical trial showed safety and tolerability of inhaled tyloxapol.

Preparation of Nanosuspensions

The surfactants were dissolved or dispersed in warm (~40°C) bidistilled water by using an Ultra Turrax (Jahnke und Kunkel GmbH, Staufen, Germany) until the surfactants had completely dissolved or were finely dispersed. The drug powder was dispersed in the aqueous surfactant solution by using again an Ultra Turrax for 1 min at 9500 rpm. The obtained premix was homogenized by using an APV Gaulin Micron LAB 40 homogenizer (APV Deutschland GmbH, Lübeck, Germany). At first, two cycles at 150 bar and two cycles at 500 bar as a kind of premilling were applied and then 20 homogenization cycles at 1500 bar were run to obtain the final product. This production method is a discontinous production method, because the homogenizer has to be refilled with the prior homogenized suspension. To produce this suspension in a continuous production way, the LAB 40 was modified by the company APV in a way that a continuous production is possible. Production of batches up to 0.5 L is possible with this technical variation.

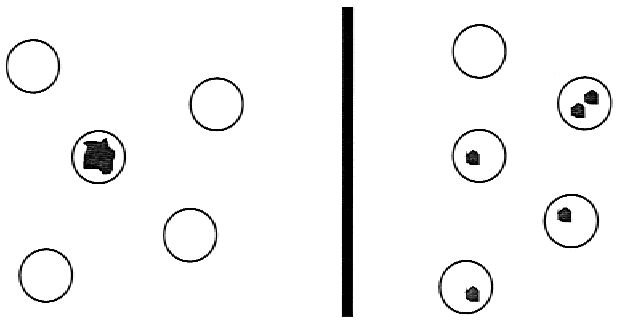

Fig. 1. Left drug as a 3-μm particle, right as 500-nm particles distributed in more droplets.

All nanosuspensions were preserved with 0.001% Thiomersal.

Particle Size Analysis

The particle size analysis was performed by laser diffraction using a LS 230 from Coulter Electronics (Krefeld, Germany). The nanosuspension was diluted before the measurement with deionized water to achieve the required dilution for LD analysis. The diameters were calculated by using the volume distribution. Diameters 50%, 90%, and 99% mean that 50% (respectively, 90% and 99%) of the particles are below the given size. In addition, photon correlation spectroscopy (PCS) using a Malvern Zetasizer 4 (Malvern Instruments, UK) was performed to determine the mean diameter of the bulk particle population and the polydispersity index (PI). A sample dilution is required for PCS analysis also; it was performed with deionized water. The PI ranges from 0 for a perfectly monodispersed particle population to 1.0 for a very broad size distribution. The laser diffraction data are volume based, and the PCS mean diameter is light intensity weighted size; therefore, the PCS mean diameter and the diameter 50% from the LD are not necessarily identical (LD data are generally higher).

Zeta Potential Measurements

Zeta potential measurements were performed in distilled water with conductivity adjusted to 50 $\mu S/cm^2$ by addition of sodium chloride, to determine the surface charge. To estimate the long-term stability properties, zeta potential was also measured in the original dispersion medium. The analysis was performed by using the Malvern Zetasizer 4 (Malvern Instruments, UK), large bore capillary cell, field strength 20 V/cm. The electrophoretic mobility was converted to the zeta potential via the Helmholtz-Smoluchowski equation.

All measurements were performed in triplicate.

Aerosolization

The nanosuspension was nebulized by a nebulizer (Pari Inhalierboy, Starnberg, Germany). The suspension is nebulized through a nozzle by air pressure. To determine the aerosolization properties of the nanosuspension particles, the nebulized nanosuspension droplets were collected in a beaker. The collected droplets were diluted with deionized water and immediately afterward analyzed by laser diffraction and PCS. In addition, the collected suspension was diluted as described above to determine the zeta potential.

RESULTS AND DISCUSSION

Screening for the Optimal Formulation Composition

A screening of formulations was designed with different types and concentrations of surfactants. The surfactants were chosen from a selection of excipients regarded as suitable for inhalation (14,21,22) or are proved to be safe for human use; they are listed in Table I. Based on this screening, the most successful surfactant combination for stabilizing budesonide as nanosuspension turned out to be formulation B4. The LD 50% of B1 and B2 was at 5 and 2 μm, and the LD 99% was at 25 μm and 75 μm; this is more in the particle size range for

Table I. Composition of Budesonide Formulation B1–B4

Formulation	Budesonide (% w/w)	Lecithin (% w/w)	Span 85 (% w/w)	Tyloxapol (% w/w)	Cetyl alcohol (% w/w)
B1	1.0	0.5	0.5		
B2	1.0			0.2	0.1
B3	1.0			0.5	
B4	1.0	0.5		0.2	

microsuspensions than for nanosuspensions. The LD 99% of B3 and B4 was below 3 μm and the LD 50% was 1 μm and less. These two formulations were also analyzed by PCS. The bulk population of B3 was 599 nm with a PI of 0.278; the formulation B4 reached a mean particle size of 500 nm and a PI of 0.397.

The minimum size that can be achieved mainly depends on the hardness of the drug and the homogenization parameters applied (number of cycles and pressure). However, the surfactant mixture is the determining factor for possible aggregation of the ultrafine drug nanoparticles. B4, a combination of lecithin 0.5% and tyloxapol 0.2% proved to be most suitable to stabilize budesonide as nanosuspension. B3 seemed to be able to stabilize this nanosuspension too; the results were similar to B4. Judging from the results shown in Fig. 2, it is obvious that the surfactant mixture B1 and B2 were not efficient in stabilizing budesonide nanoparticles. During the homogenization procedure, aggregates can form because of insufficient coverage of surfaces by surfactants within the milling process (newly generated surfaces need to be covered; diffusion of the surfactant to these surfaces requires time). Therefore, formed aggregates need to be deaggregated again in the next homogenization cycle. Depending on the properties of the surfactants (functional groups responsible for hydrophobic/hydrophilic interactions of surfactant and drug, zeta potential, *etc.*), stabilizing is successful or not. In fomulation B1 and B2, the deaggregation of the particles could not be achieved, and the surfactant was not efficient to stabilize the particles in principle. Deaggregation of nanoparticles is important because as aggregates they do not have the special features of nanosuspensions (4).

Similar results were found for nanosuspensions of paclitaxel (23). Growth of paclitaxel crystals was not observed, only aggregation of nanoparticles when the surfactant mixture was not optimized. For the amount of microparticles, the nanosuspension with formulation B3 and B4 have a good quality, *i.e.,* the number of microparticles is low. This is shown by the LD diameter 99%, which was for both formulations <4 μm.

To investigate the homogenization process in more detail, formulation B4 was taken, and particle size reductions as a function of applied homogenization cycles were determined. Depending on the hardness of the drug powder and the required fineness of the particle material, the homogenization process can take from 3 up to 20 cycles (20). For each drug and application, dependent on the requirements of the application route [*e.g.,* i.v. formulations must not have a high number of particles above 5 μm (24)], the number of cycles has to be optimized. Figure 3 shows the particle size reduction of budesonide nanosuspension 1.0% drug content as a function of homogenization cycles (applied pressure per cycle: 1500 bar). Little change in the diameter 50% was observed after 10 cycles; LD 50% 0.959 μm decreased to 0.802 μm after 20 cycles. By applying higher cycle numbers, one can reach a more uniform product with only a small amount of microparticles (further decrease in diameter 95% and 99%). To minimize the microparticulate fraction of formulation B4, 10 more cycles were run. The LD 99% decreased from 2.75 μm (after 10 cycles) to 2.06 μm (after 20 cycles), and the LD 95% decreased from 2.23 μm to 1.806 μm. In further studies, every nanosuspension was produced by applying 20 homogenization cycles with 1500 bar.

Zeta Potential

Zeta potential analysis was performed to get information about the surface properties of the nanoparticles. It is an indication for the long-term stability of particulate systems. For a physically stable suspension stabilized by electrostatic repulsion, a zeta potential of approximately ± 30 mV is re-

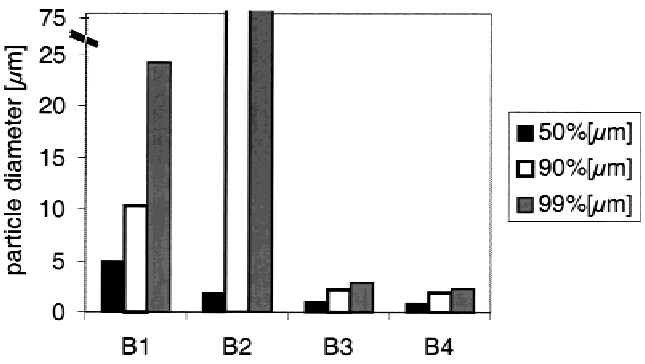

Fig. 2. LD diameters 50%, 95% and 99% (volume based) after 10 homogenization cycles of the screening formulations B1, B2, B3 and B4.

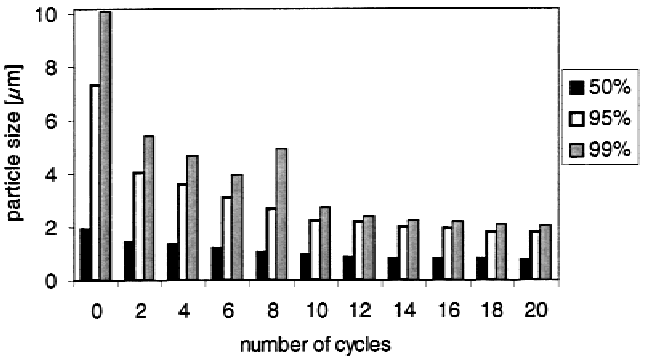

Fig. 3. LD diameters 50%, 95%, and 99% (volume based) as a function of homogenisation cycles containing budesonide 1.0%, lecithin 0.5%, and tyloxapol 0.2%.

quired as minimum (25,26). In a combined electrostatic and steric stabilization, as a rough guide line ±20 mV are sufficient. The investigated nanosuspensions were stabilized through a combination of an electrostatic stabilizer, soya lecithin, and a steric stabilizer, tyloxapol. First, the analysis was performed in bidistilled water (conductivity 50 μS/cm). Measurements in bidistilled water gives information about the covering of the particle surface. The zeta potential of formulation B4 (three batches were produced; batch size was 40 mL) was −41.1 mV (±4.9 mV); the zeta potential for formulation B3 (stabilized with tyloxapol and cetyl alcohol) is −12 mV.

The second step was to determine the zeta potential in dispersion medium, which is the storage medium. These data will be an indicator for the long-term stability. For formulation B4, −49.8 mV (± 3.0 mV) was measured. Formulation B3 had a zeta potential in original medium of about −5 mV; this low value indicates a physically instable suspension. The dispersion medium of formulation B4 itself had a potential of −51.8 mV (±0.6 mV). This can be explained because of the presence of soya lecithin, which is able to form liposomes spontaneously.

The zeta potential of formulation B4 turned out to be independent from the production modus (continuous or discontinuous). The batches produced in a continuous modus (three batches of 300 mL were produced) had practically the same zeta potentail in bidistilled water (−45.3 ± 1.5 mV) and in dispersion medium (−51.2 ± 2.8 mV) as the batches produced in the discontinuous modus (data see above).

Scale-Up

Because formulation B4 achieved the best results for budesonide as nanosuspension, this combination of surfactants was taken to examine the possibility to produce this suspension in a continuous production way with the modified LAB 40, the production of to 0.5-L suspensions is possible.

Three batches of 300 mL (formulation B4, 1% drug content) were produced and compared with three 40-mL batches of the same composition for particle size. In Fig. 4 left, the results are shown. The scale-up of budesonide from 40 to 300 mL was successful, and the same particle sizes were achieved. Diameter 50% were 0.73 ± 0.01 μm (40-mL batches) and 0.81 ± 0.04 μm (300-mL batches), characterizing the bulk population. Diameter 99%, sensitive parameters for the microparticulate content, were for the 40-mL batches 2.20 ± 0.28 μm and for the 300 ml batches 2.25 ± 0.19 μm showing the unproblematic scaling-up. The obtained mean PCS diameters (Fig. 5 triangles) of 500 nm, polydispersity index (PI) of 0.373 for the 40-mL batches and 460 nm, and PI of 0.377 for the 300-mL batches are in good agreement with the data obtained by laser diffraction analysis. The zeta potentials of the 40-mL batches were about −43 mV (±2.7 mV) and of the 300-mL batches were −45 mV (±1.5 mV), which indicates that no other adsorption or desorption phenomenon occurred during the continuous production of batch B6.

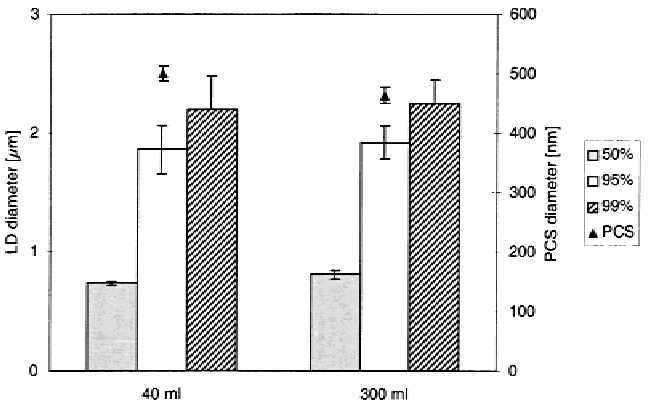

Fig. 4. LD diameter 50%, 95%, and 99% (volume based) and PCS mean diameter (triangle) of budesonide nanosuspension, processed on the LAB 40 discontinuous mode (40 mL) and LAB 40 continuous mode (300 mL).

Increase of Drug Content

To be flexible in variations with the drug content of a formulation, it was investigated if it is possible to stabilize an even higher drug content with the same amount of surfactants. Depending on the further processing of the nanosuspension or to get a suitable low volume for application purposes, a distinct concentration of drug is desired. A nanosuspension with a drug content of 10% (discontinuous production) was produced. Figure 5 compares the LD diameters 50%, 90%, and 99% of a 1% and a 10% batch; both batches contain the same amount of surfactants from formulation B4. The LD and PCS data of the 10% batch are slightly better. PCS diameter of the 1% batch was 500 nm and of the 10% batch was 435 nm. This can be explained by higher shear forces and collision in the homogenization gap when a higher amount of drug powder is present. The 10% batch was also stable over the examination period of 1 year (data until now).

Long-Term Stability

As indicated by the zeta potential of about −44 mV for formulation B4, the nanosuspensions were stable during 1 year stored at room temperature. The exemplary LD distribution of one batch (formulation B4, continuous production modus) on the day of production and 1 year later is shown in Fig. 6. The two distribution curves are practically identical. The particle diameters 50%, characteristic for the bulk population, were 0.84 μm on the day of production and 0.85 μm 1 year later. The absence of particle growth due to Ostwald ripening or aggregation effects can be shown by the particle

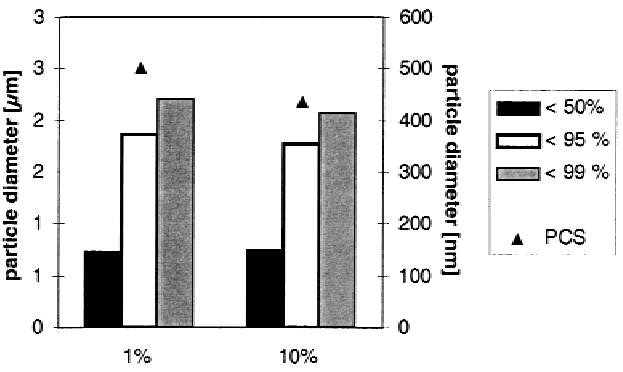

Fig. 5. Comparison of budesonide nanosuspension containing 1% and 10% drug (w/w), LD diameter 50%, 95%, and 99% (volume based), and PCS mean diameter (triangle).

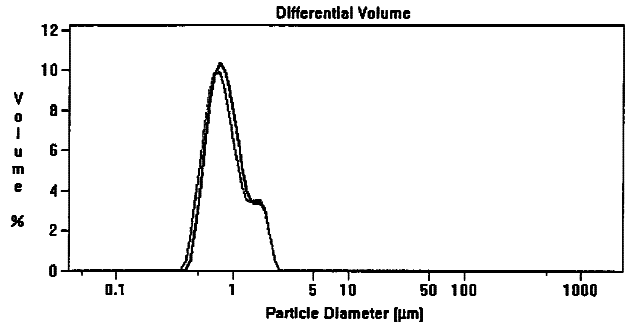

Fig. 6. LD-distribution of a budesonide nanosuspension formulation B4 on the day of production and after 1 year of storage at room temperature.

diameters 99%. On the day of production they were at 2.04 µm and after 1 year storage at 2.12 µm.

In Fig. 7 LD diameters 50%, 90%, and 99% and PCS diameters of 1% budesonide nanosuspension (formulation B4, 40 mL, three batches and 300 mL, three batches) are displayed as a function of storage time at room temperature. There was no difference between the 40-mL batches and the 300-mL batches over the examined period of 1 year. The bulk population of the 40-mL batches, characterized by the LD diameter 50%, increased from 0.73 to 0.80 µm, whereas the 50% diameters of the 300-mL batches remained the same. The LD diameter 99% of the batches did not increase over the examined period of time; it remained <2.5 µm. The additional PCS measurements underline the results of the LD measurement. The PCS values of both the 40-mL and the 300-mL batches were around 460 nm and did not increase over the storage period.

Influence of Nebulization on Suspension Particle Size

To examine the aerosolization properties of nanosuspensions, they were nebulized by a commercial nebulizer. The results can be seen in Fig. 8. The PCS diameter remained the same, 491 nm before and 496 nm after aerosolization. The particle diameters analyzed by LD increased only slightly (~0.2 µm). One factor could be that water of the dispersion media evaporated during the nebulization process from very fine droplets, which might cause aggregates, especially when a

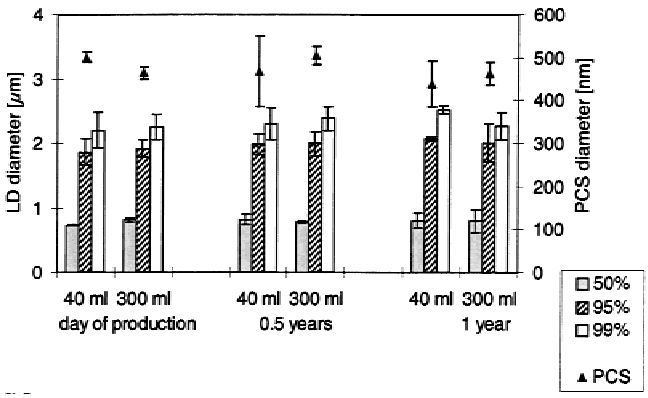

Fig. 7. LD diameter 50%, 95%, and 99% (volume based) and PCS mean diameter (triangles) of budesonide nanosuspension processed as 40 mL (three batches) and 300 mL (three batches) batches, as a function of storage time (stored at room temperature).

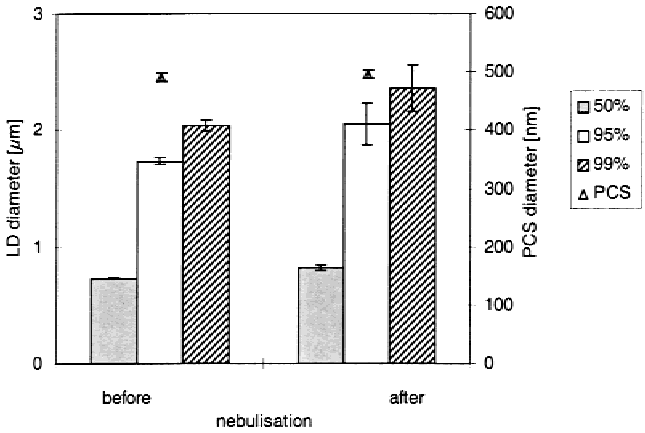

Fig. 8. LD diameter 50%, 95%, and 99% (volume based) and PCS mean diameter (triangles) of budesonide nanosuspension before and after nebulization (six batches).

droplet completely evaporates and solid particles remain. However, this has no practical relevance, because the diameter 99% is still 2.4 µm after nebulization.

CONCLUSION

The possibility of creating a long-term stable aqueous budesonide nanosuspension with 1% and 10% drug content was examined. Furthermore, the successful scaling up of this formulation from 40 to 300 mL was evaluated. In addition, the aerosolization of budesonide nanosuspension was successful. This result opens the opportunity to formulate budesonide as a pulmonary formulation being long-term stable as aqueous suspension (no Ostwald ripening, no aggregation) and to administer it by using a conventional nebulizer or, alternatively, a portable inhaler.

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