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Freeze-drying of drug-free and drug-loaded solid lipid nanoparticles (SLN)

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Abstract

Solid lipid nanoparticles (SLN) of a quality acceptable for i.v. administration were freeze-dried. Dynasan 112 and Compritol ATO 888 were used as lipid matrices for the SLN, stabilisers were Lipoid S 75 and poloxamer 188, respectively. To study the protective effect of various types and concentrations of cryoprotectants (e.g. carbohydrates), freeze-thaw cycles were carried out as a pre-test. The sugar trehalose proved to be most effective in preventing particle growth during freezing and thawing and also in the freeze-drying process. Changes in particle size distribution during lyophilisation could be minimised by optimising the parameters of the lyophilisation process, i.e. freezing velocity and redispersion method. Lyophilised drug-free SLN could be reconstituted in a quality considered suitable for i.v. injection with regard to the size distribution. Loading with model drugs (tetracaine, etomidate) impairs the quality of reconstituted SLN. However, the lyophilisate quality is sufficient for formulations less critical to limited particle growth, e.g. freeze-dried SLN for oral administration. © 1997 Elsevier Science B.V.

Keywords: Solid lipid nanoparticles; Lyophilisation; Cryoprotectants; Trehalose; Intravenous colloidal drug carrier

1. Introduction

Solid lipid nanoparticles (SLN) (Müller and Lucks, 1991, 1992) are an alternative colloidal carrier system to polymeric nanoparticles, liposomes and emulsions. They attracted increasing attention during the last few years as indicated by the increasing number of research groups active in this field (Schwarz et al., 1994; Müller et al., 1995; Gasco, 1991; Domb, 1993; Boltri et al., 1993; Speiser, 1990). Production of lipid nanoparticles, physicochemical characterization and drug incorporation has been intensively studied. A crucial point for the use of the SLN as a colloidal drug carrier is their physical and chemical long-term stability. It has been shown that optimized SLN are physically stable as an aqueous dispersion for

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12 months (Freitas et al., 1995; Siekmann and Westesen, 1992) and recently, 24 months (Schwarz, 1995). However, some SLN formulations may be highly attractive with regard to toxicological considerations (e.g. well tolerated surfactant) but are not physically stable as aqueous dispersions. Another problem is chemical stability in the case of drugs susceptible to hydrolysis. For such cases, it is highly desirable to have a freeze-dried SLN formulation available. A prerequisite is a good reconstitution performance, whereas the required quality of the reconstituted SLN will depend on the method of administration, e.g. oral administration or intravenous injection. Therefore, the lyophilisation of SLN was investigated and optimized and the effect of drug incorporation on the lyophilisation process was studied.

2. Materials and methods

2.1. Materials

Dynasan 112 (glycerol trilaurate) was provided by Hüls AG (Witten, Germany) and Compritol 888 ATO (glycerol behenate) by Gattefossé (Weil AR, Germany). Pluronic F 68 (poloxamer 188) was a gift from BASF AG (Ludwigshafen, Germany) via the distributor Tensidchemie (Düren, Germany). Lipoid S 75 (soy lecithin) was provided by Lipoid KG (Ludwigshafen, Germany). Intralipid 10 and 20% were obtained from Kabi Pharmacia GmbH (Erlangen, Germany). All other chemicals (cryoprotectants) were purchased from Sigma (Deisenhofen, Germany).

2.2. Methods

Solid lipid nanoparticles were produced by high pressure homogenization (Schwarz et al., 1994) at increased temperature (5–10% above the melting point of the lipid, respectively). The dispersions contained 10% lipid (w/w), the Dynasan–SLN were stabilized with 5% Lipoid S 75 and the Compritol–SLN with 5% poloxamer 188. Particle size distribution was determined by photon correlation spectroscopy (PCS) (Malvern Zetasizer IV, Malvern Instruments, UK) and laser diffraction particle size analysis (Mastersizer E, Malvern Instruments, UK). The PCS diameter is based on the intensity of the light scattering from the particles. Similar to parenteral fat emulsion micelles and—in the case of lipoid—liposomes are present but will contribute little to the mean diameter due to their very weak scattering intensity compared to the emulsion droplets. The number of large particles in the SLN dispersions was determined by means of a Coulter Counter Multisizer II (Coulter Electronics, Krefeld, Germany) using a 30 μm capillary ($n = 3$). The Coulter counter signals were used directly for calculation of particle number/volume unit, i.e. the number of particles greater than 1, 2, 5, 7 and 10 μm. From the PCS data, the mean particle diameter of the bulk population and the polydispersity index (PI) of the SLN dispersions could be calculated. From the laser diffractometry data, the diameters 10, 50, 90, 95 and 99% (D (10%), D (50%), D (90%), D (95%) and D (99%)) were used to characterise the SLN dispersions. The diameters were calculated using the volume distribution.

The SLN were lyophilised using a Gamma 2-20 (Christ, Osterode a.H., Germany). The SLN dispersions were diluted (1:1) with the cryoprotectant solutions before freezing, yielding a lipid content of 5% (w/w). Of this diluted dispersion, 5 ml placed in 20 ml glass vials. Slow freezing was carried out on the shelves in the freeze-drier (shelf temperature -25°C). Rapid freezing of the sam-

Table 1
List of excipients and their concentrations used in the freeze thaw pre-tests of Dynasan and Compritol nanoparticles

Cryoprotectant	Concentrations (%)
Trehalose	2.5, 5, 10, 12.5, 15
Glucose	2.5, 5, 10, 12.5, 15
Maltose	2.5, 5, 10, 12.5, 15
Mannose	2.5, 5, 10, 12.5, 15
Lactose	2.5, 5, 10, 12.5, 15
Mannitol	2.5, 5, 10, 12.5, 15
Sorbitol	2.5, 5, 10, 12.5, 15
Glycine	0.5, 2.5, 5, 10, 15
Polyvinylpyrrolidone (PVP)	0.5, 0.75, 1.5
Polyvinylalcohol (PVA)	0.5, 1.25, 2.5
Gelatine	0.05, 0.125, 0.25, 0.5

ples was performed either by dipping the whole vial containing the SLN preparations into liquid nitrogen, or alternatively, by adding the SLN dispersion dropwise to liquid nitrogen.

The samples were lyophilised for 24 h at a temperature of −25°C and a vacuum of 0.370 mbar, followed by a secondary drying phase of one day at 20°C and maximum vacuum. Reconstitution of the lyophilised products was performed either by manual shaking, shaking and subsequent sonication (2 min, 160 W, Bandelin Sonorex, Bandelin, Berlin, Germany) or by using a Decapeptyl-Depot® dispergator (Ferring Arzneimittel GmbH, Kiel, Germany).

3. Results and discussion

3.1. Freeze-thaw tests for the screening of suitable cryoprotectants

Freeze-thaw experiments were conducted to select the excipients with the highest potential for cryoprotection. The freeze-thaw studies were conducted as pre-tests to avoid too many time consuming freeze-drying processes. If an excipient cannot protect the nanoparticles during the first step of lyophilisation, i.e. freezing, it is not likely to be an effective cryoprotectant. To assess the most suitable concentrations, the excipients were added in increasing amounts (Table 1). Glucose, mannose, maltose and trehalose were identified as being the most suitable excipients from those listed in Table 1. Concentrations between 10 and 15% proved to be most efficient. The increases in the mean particle size and the polydispersity index were negligible when thawing the frozen Compritol–SLN (Fig. 1). In the same concentrations, these excipients also proved to be the most efficient for Dynasan–SLN (data not shown) and were therefore chosen for the lyophilisation studies.

3.2. Effect of cryoprotectants during lyophilisation

The excipients which proved most effective in the freeze-thaw pre-tests were employed in a standard lyophilisation process, i.e. freezing on the pre-cooled shelves of the freeze drier (−25°C) as well as primary and secondary drying for 24 h, respectively. In general, the increase in particle size and polydispersity index was distinctly larger for Compritol–SLN than for Dynasan–SLN (Fig. 2). This may be attributed to different behaviour of the lipids and/or the stabilising surfactants (poloxamer 188 and Lipoid S 75,

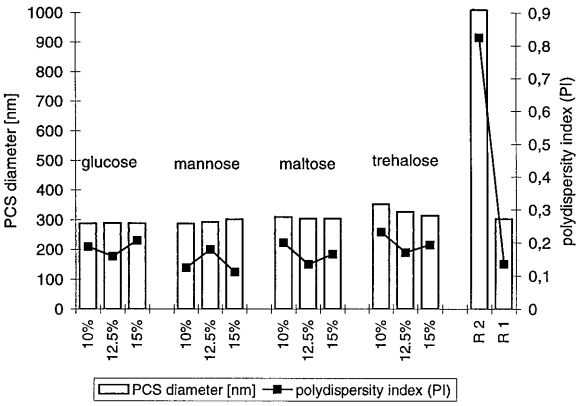

Fig. 1. Freeze-thaw pre-test of Compritol–SLN: Mean particle size (nm) and polydispersity index (PI) after thawing of the frozen SLN in the presence of different cryoprotectants, compared to the references (R1, SLN before freezing; R2, frozen and thawed SLN without cryprotectant). Cryoprotectant concentrations employed: 10, 12.5 and 15%; nanoparticle concentration, 5%.

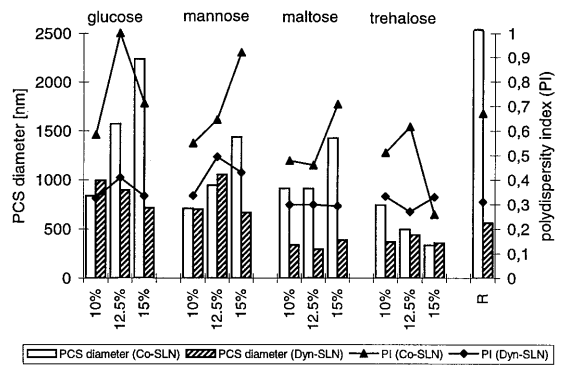

Fig. 2. PCS diameter and polydispersity index (PI) of reconstituted Compritol–SLN (Co–SLN) and Dynasan–SLN (Dyn–SLN) as a function of type and concentration of cryoprotectant. R, reference of lyophilised and reconstituted SLN without cryoprotectant; redispersion by sonic treatment for 2 min.

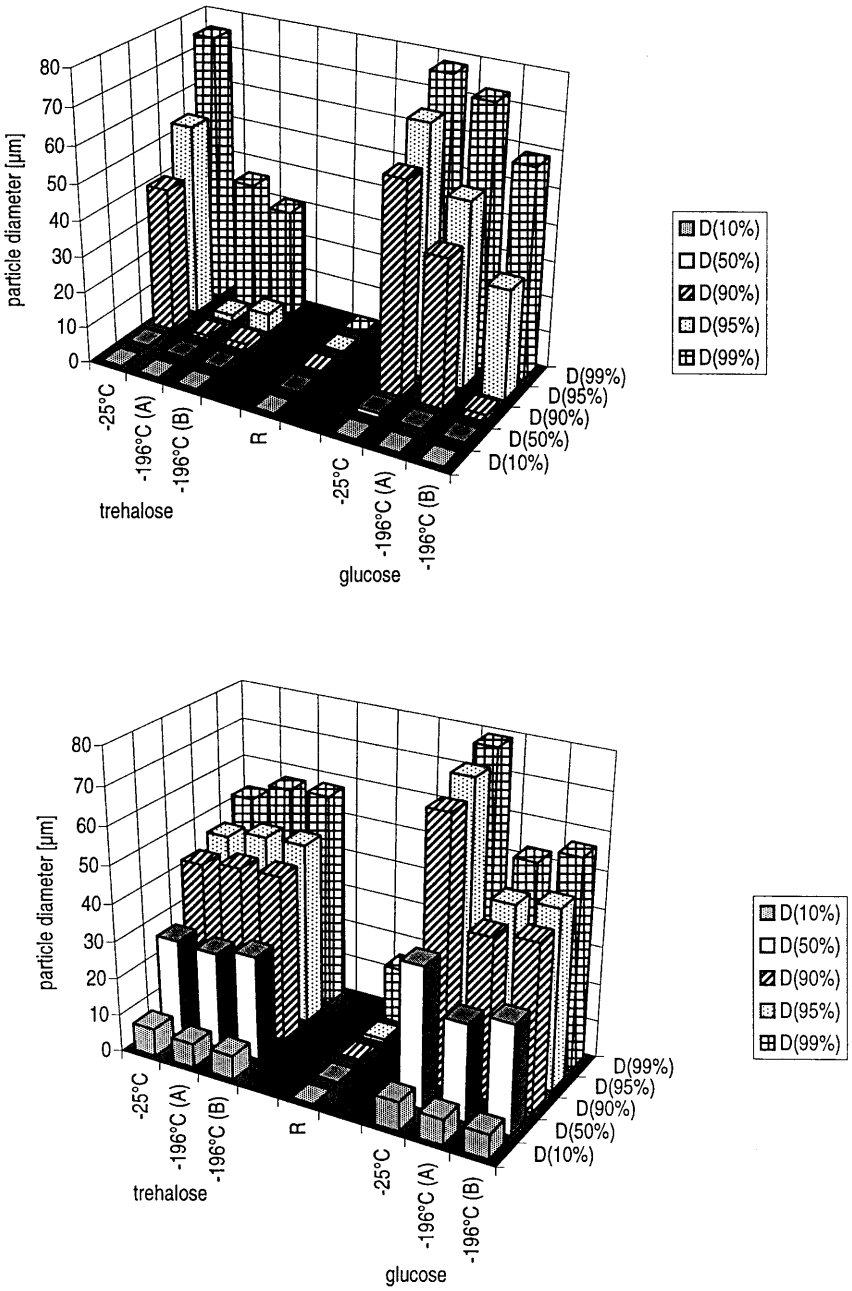

Fig. 3. Diameters 10, 50, 90, 95 and 99% of reconstituted lyophilised Dynasan–SLN (upper) and Compritol–SLN (lower). Cryoprotectants were trehalose 15% (left) and glucose 15% (right). Freezing methods: slow freezing on the shelves (− 25°C), rapid freezing by addition of SLN dropwise to liquid nitrogen (− 196°C (A)) or by dipping the whole vial into liquid nitrogen (− 196°C (B)). Redispersion by manual shaking.

respectively). Interestingly, the opposite was found in the freeze-thaw pre-tests. Unfortunately it was not possible to perform studies using Compritol–SLN stabilised with Lipoid S 75 to assess whether or not the difference is mainly due to the role of the surfactant. Homogenising Compritol

with Lipoid S 75 failed to produce a good product.

The lyophilisation process, or simple drying in air generally increased the degree of crystallinity in the case of partially crystallized SLN particles. Particles still in the liquid state after production—Dynasan 112–SLN (Schwarz et al., 1994)—were transferred to the crystalline state (degree of crystallinity of drug-loaded SLN: 87–98% compared to drug-free particles (Schwarz, 1995)).

Looking at different concentrations of the cryoprotectant used, in general, high concentrations proved to be most effective for Dynasan–SLN. Dynasan–SLN gave good results even without cryoprotectant, although cryoprotectant addition lead to slightly smaller PCS diameters in the case of maltose and trehalose. Apart from trehalose, high concentrations were distinctly less effective for Compritol–SLN (Fig. 2).

Trehalose proved to be most effective for both Compritol–SLN and Dynasan–SLN; the optimum concentration was 15% (Fig. 2). At 15% trehalose, the mean particle size of Dynasan–SLN increased from 103 nm before lyophilisation to 360 nm after reconstitution and for Compritol–SLN from 160 to 330 nm. Trehalose proved to be a very effective cryoprotectant for SLN as previously reported for liposomes, for example (Ausborn et al., 1992; Strauss et al., 1986; Grit and Crommelin, 1992). It was therefore chosen for further investigations to assess whether the modification of the freezing velocity could further improve the quality of the product. In addition, glucose was used as a second, less effective cryoprotectant to investigate if freezing parameters could improve its performance. Apart from the concentration, the nature of the cryoprotectant (e.g. mono- and disaccharide) is also of importance (Doebbler, 1966; Madden et al., 1985; Crowe and Crowe, 1991). Trehalose and glucose are therefore ideal model compounds to study this contribution.

3.3. Influence of freezing velocity on quality of reconstituted SLN

To study the effect of freezing velocity, the SLN dispersion was added dropwise to liquid nitrogen (method A) or the whole vial of SLN was dipped into liquid nitrogen (method B in Fig. 3). An important criterion regarding i.v. injectability is the formation of aggregates in the micrometer range. The content of these particles was therefore used as quality measure, i.e. determination of diameter 10, 90 and 99% by laser diffractometry. Fast freezing proved to be most efficient for trehalose and glucose protected Dynasan–SLN, whereas dipping of the whole vial (method B) was slightly more effective than method A (Fig. 3, upper). Again, trehalose was more effective than glucose. Generally, Compritol–SLN was less protected than Dynasan–SLN as indicated by the larger diameters (Fig. 3, lower).

3.4. Influence of redispersion method

Redispersion by sonication is a less convenient method for application in the clinic than, for example, manual shaking or the use of a manual redispersion system (e.g. Decapeptyl-Depot® dispergator). Therefore, an investigation into whether good redispersion can be achieved using these methods was carried out. Both manual shaking and the dispergator were slightly less effective for Dynasan–SLN than sonication (Fig. 4). They were inappropriate for reconstitution of Compritol–SLN as indicated by the strong increase in PCS diameter. Large aggregates were macroscopically

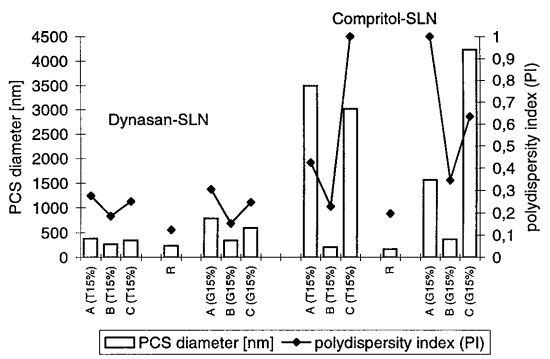

Fig. 4. PCS diameter of lyophilised and reconstituted Dynasan–SLN and Compritol–SLN as a function of redispersion method: A, redispersion by manual shaking; B, by sonication in a bath (160 W, 2 min); and C, by means of a Decapeptyl-Depot® dispergator. Cryoprotectants used: G 15%, glucose 15%; T 15%, trehalose 15%. References: R, non-lyophilised SLN.

Table 2
Number of particles greater than 1, 2, 5, 7 and 10 μm of Dynasan– and Compritol–SLN (Dyn-SLN, Co-SLN) before and after lyophilisation and reconstitution (Dyn–LYO, Co–LYO), respectively

System	Absolute number of particles/μl, dispersion $\pm$ S.D.				
	>1 μm	>2 μm	>5 μm	>7 μm	>10 μm
Dyn–SLN	308 172 $\pm$ 78 252	22 762 $\pm$ 5631	688 $\pm$ 237	209 $\pm$ 65	46 $\pm$ 14
Dyn–LYO	1 770 066 $\pm$ 58 386	48 552 $\pm$ 4267	943 $\pm$ 125	235 $\pm$ 68	85 $\pm$ 46
Co–SLN	97 479 $\pm$ 4303	8682 $\pm$ 467	666 $\pm$ 31	251 $\pm$ 11	72 $\pm$ 9
Co–LYO	7 125 963 $\pm$ 434 335	243 998 $\pm$ 99 453	62 483 $\pm$ 25 553	25 720 $\pm$ 6351	1736 $\pm$ 534
Intralipid 10%	598 067 $\pm$ 65 659	25 478 $\pm$ 4702	1473 $\pm$ 909	821 $\pm$ 626	475 $\pm$ 537
Intralipid 20%	603 748 $\pm$ 33 490	19 581 $\pm$ 5874	1559 $\pm$ 578	777 $\pm$ 450	459 $\pm$ 278

References are fat emulsions for parenteral nutrition (Intralipid 10 and 20%). The cryoprotectant is trehalose 15%; redispersion is by sonication; S.D., standard deviation (normalization for number of particles, calculated assuming 10% SLN dispersions and emulsions).

visible. Considering the relatively smaller increase in PCS diameter and the macroscopically good appearance of the Dynasan systems when dispersing them by shaking, it appears possible to obtain a manually redispersable product after further optimization.

3.5. Suitability of reconstituted drug-free SLN for i.v. application

The limiting factor for i.v. administration is the number of particles >5 μm which can potentially block capillaries. There are specifications in the pharmacopoeia regarding the limits of contamination by particulates from the packing material, e.g. in infusions. There are no definite limits specified for the number of microparticles in dispersions for i.v. administration, such as emulsions for parenteral nutrition. The German pharmacopoeia states only that the particle size should be measured, but gives no further specifications. To assess i.v. injectability, the SLN are therefore compared with emulsions for parenteral nutrition, with regard to the particles in the micrometer range (Müller et al., 1995). Non-lyophilised aqueous Compritol– and Dynasan–SLN are similar to parenteral emulsions regarding the proportion of particles greater than 5, 7 and 10 μm (Table 2). Especially when considering the difference in administration volume (e.g. 500 ml for emulsion and approximately 10 ml for SLN), the SLN dispersions can be administered intravenously. The required maximum injection volume of SLN dispersion can be estimated by considering the necessary single dose of the drug, the typical loading capacity of the lipid matrix (up to 30%) and the maximum lipid content of the SLN dispersion (30%).

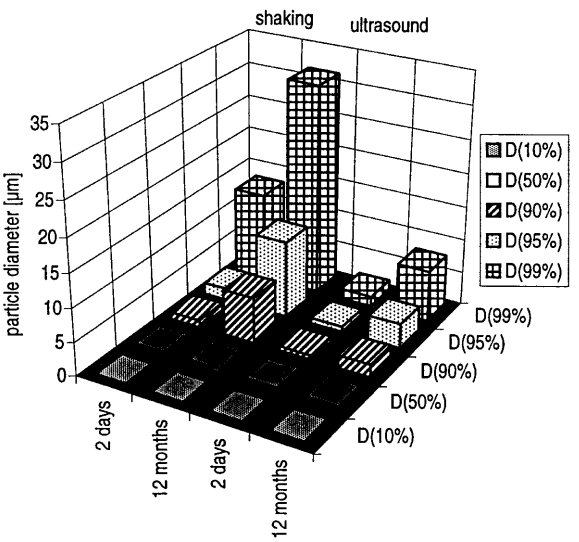

Fig. 5. Diameters 10, 50, 90, 95 and 99% (volume distribution) of reconstituted Dynasan–SLN immediately after lyophilisation and after 12 months of storage. Redispersion by manual shaking and by ultrasound, respectively. Cryoprotectant: trehalose 15%.

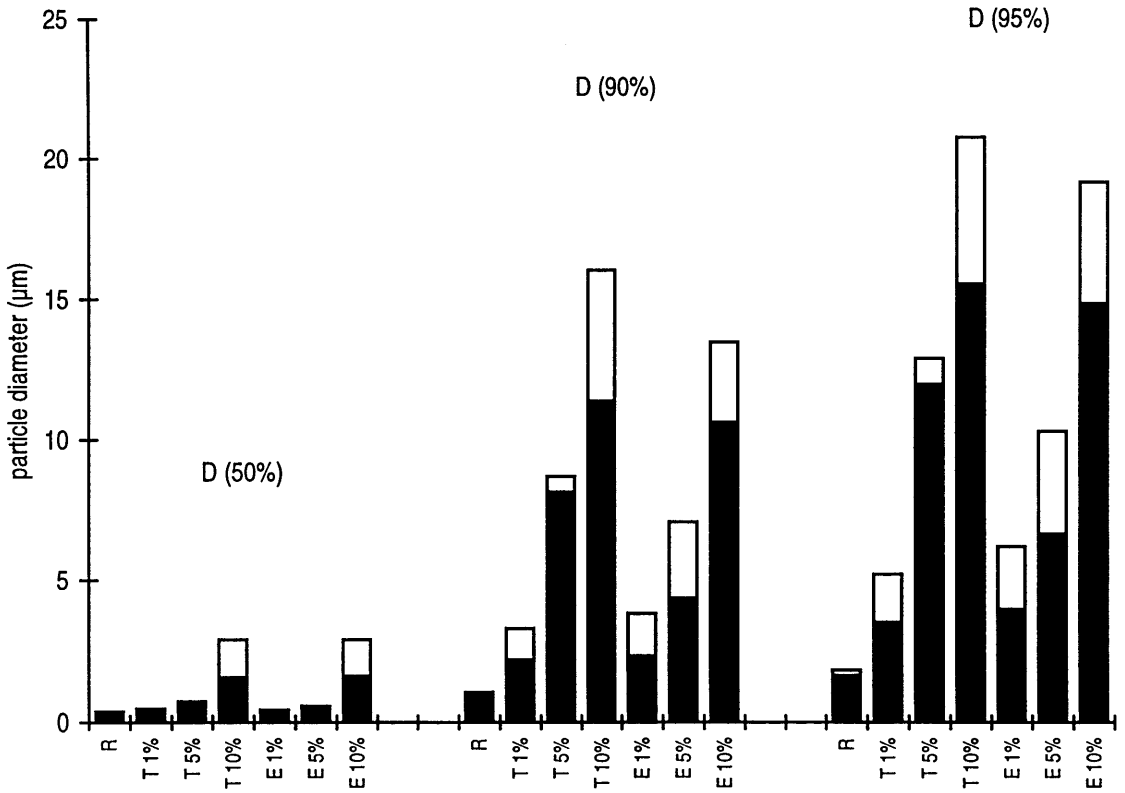

Fig. 6. Diameters 50, 90 and 95% (volume distribution) of reconstituted drug-loaded Dynasan–SLN (white bar: S.D.). Drugs: tetracaine (T) and etomidate (E) in concentrations of 1, 5 and 10% (calculated as percentages of the lipid matrix). Reference (R), lyophilised and reconstituted drug-free Dynasan–SLN; cryoprotectant for drug-loaded and drug-free SLN, trehalose 15%.

Lyophilisation and reconstitution of Compritol–SLN leads to distinct formation of large particles prohibiting i.v. administration. Dynasan–SLN shows very limited particle growth. Principally, the number of particles in the range of 1–2 μm increases, while little change occurs for the relevant particle fractions > 5 μm. Consequently, the Dynasan–SLN are of injectable quality with regard to size distribution.

3.6. Long-term stability of lyophilised SLN

Important for product quality, is the reconstitution performance of the lyophilisate after storage. Reconstitution behaviour can be worsened, for example, due to a sinter process accompanied by irreversible particle aggregation. For SLN lyophilisates, it has been reported that particle growth occurs when reconstituting the lyophi lised SLN after 12 months of storage (Siekmann and Westesen, 1994). However, the optimized Dynasan–SLN showed only a minor increase when redispersing them with ultrasound (Fig. 5). At present, further optimization is being performed.

3.7. Lyophilisation of drug-loaded SLN

Tetracaine and etomidate were incorporated in the Dynasan–SLN formulation, because it showed the best redispersion performance after lyophilisation. Lyophilisation of the drug-loaded nanoparticles should provide information on the extent to which the drug interferes with the

lyophilisation process. Trehalose was used as cryoprotectant because it was most effective for drug-free Dynasan–SLN. Both drugs, tetracaine and etomidate, impaired the quality of the reconstituted product. The number and size of large aggregates increased with increasing drug concentration in the SLN dispersion, as shown by the increase in the diameters 50, 90 and 95% (Fig. 6). Even concentrations as low as 1% led to a distinct increase in the diameters 90 and 95%, which is a sensitive parameter for the formation of aggregates. These instabilities were mainly attributed to free drug in the dispersion medium. During the freezing process, water will crystallize—at the same time the concentration of the dissolved drug in the water will increase until reaching the eutecticum. Presence of electrolytes (e.g. protonated drug) in the water reduces the zeta potential with increasing concentration, i.e. with progression of the freezing process. The reduction in zeta potential is considered to be one—most likely the major—cause of aggregation. Taking these factors into consideration, it may be advantageous to remove the free drug before lyophilisation, in cases where dispersions do not lead to a lyophilized product with sufficient redispersion properties. There may also be a change in the properties of the lipid matrix compared to drug-free SLN. To sum up, in contrast to drug-free SLN, the quality of the lyophilised drug-loaded SLN is not yet sufficient for i.v. injection of the reconstituted product. However, the quality is already sufficiently high for the production of lyophilised SLN for oral administration, e.g. in capsule form. Quality criteria are the relatively low content of microparticles and the uniform appearance of the reconstituted product, i.e. absence of macroscopically visible aggregates.

4. Conclusions

Freeze-drying of drug-free SLN under optimized conditions leads to a lyophilisate with good reconstitution properties. As optimized SLN formulation, the reconstituted SLNs are suitable for i.v. administration with regard to the size distribution. Drug loading of the particles impairs the reconstitution quality. However, the lyophilisates of drug-loaded SLN can be used for formulations less critical with regard to the presence of microparticles than i.v. injectables. Further optimization of the lyophilisation parameters to obtain an i.v. injectable product appears feasible.

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