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Influence of different parameters on reconstitution of lyophilized SLN

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Abstract

Drug-loaded solid lipid nanoparticles (SLN) suitable for parenteral administration were freeze-dried. The lipid matrix Imwitor 900 (concentration, 2.5%) was stabilized with Lipoid E 80 and sodium glycocholate. The influence of different parameters of lyophilization like the protective effect of cryoprotectants, freezing velocity, and thermal treatment was investigated. The results of this study demonstrate that, by optimizing critical process parameters, i.v.-injectable SLN-dispersions can be freeze-dried, preserving their small particle size.

Keywords: Solid lipid nanoparticles; Lyophilization; Colloidal drug carrier

One major challenge of drug delivery is the development of i.v.-injectable drug carriers for poorly water-soluble substances. Solid lipid nanoparticles (SLN) (Müller and Lucks, 1996) are of increasing interest as alternatives to liposomes, nanoemulsions, and polymeric nanoparticles (Gasco et al., 1992; Siekmann and Westesen, 1992; Schwarz et al., 1994; Müller et al., 1995). Physical and chemical long-term stability is still an important problem of certain SLN formulations. The intravenous use of SLN, especially, has strong demands with respect to microparticle contamination and general toxicological considerations. Average diameters above 5 μm might cause death due to embolism. For that reason, aggregation and particle growth have to be avoided during storage. The chemical stability of hydrolizable drugs is another problem. One possible solution for both stability problems is lyophilization. The freeze-dried formulations must have a good reconstitution and fulfill the requirements of intravenous injectable drug carriers. Therefore, the critical process parameters have to be determined and optimized.

It was possible to develop a SLN formulation for the poorly water-soluble drug RMEZ98 (Novartis) which meets the requirements of i.v. administration. However, this formulation is only stable for 2 days, making it necessary to develop a freeze-dried product to obtain a long-term stability with required quality after reconstitution.

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The aim of this study was to determine the critical process parameters and to optimize the lyophilization of the RMEZ98-loaded SLN dispersion with regards to an i.v. application.

Polyvinyl alcohol (PVA), av. molecular weight 30 000–70 000, polyvinyl pyrrolidone (PVP), av. molecular weight 40 000, mannite, fructose, lactose, glucose, sorbitol, trehalose, maltose, and mannose were purchased by Sigma–Aldrich (Steinheim, Germany). Lipoid E 80 was a gift from Lipoid KG (Ludwigshafen, Germany). Imwitor 900 (glyceryl monostearate 40–50%) was provided by Condea (Witten, Germany). Sodium glycocholate was used from Fluka (Neu-Ulm, Germany). Solid lipid nanoparticles were produced by high-pressure homogenization using a piston-gap homogenizer LAB 40 (APV Homogenizer GmbH, Germany). The melted lipid (Imwitor 900) with the dissolved drug was poured into the hot surfactant solution (Lipoid E 80 and sodium glycocholate) at 80°C and dispersed using a high-speed rotor-stator stirrer. Afterwards, the pre-emulsion was homogenized at 500 bar for three cycles. The final SLN-dispersion contained 2.5% lipid.

Particle-size analysis was performed by photon correlation spectroscopy (PCS) (Malvern Zetasizer IV, Malvern Instruments, UK). To characterize the content of micrometer particles, laser diffractometry (LD) was employed using an LS 230 (Coulter Electronics, Germany). Characterization parameters were the diameters 50, 90 and 99% of the volume distribution. Especially the LD (90%) and LD (95%) are very sensitive towards the presence of even few microparticles or aggregates.

The SLN were lyophilized using a Gamma 2-20 apparatus (Christ, Osterode a. H., Germany). Differential scanning calorimetry (DSC) was performed using a Mettler DSC 821e (Mettler Toledo, Gießen, Germany).

By using the hot homogenization, it was possible to produce drug-loaded SLN suitable for i.v. administration. The mean particle size was about 150 nm and LD (99%) 500 nm, which means 99% of the particles were smaller than 500 nm. Unfortunately, this aqueous dispersion was only stable for 2 days, making it necessary to increase stability by means of lyophilization.

The protective effect of cryoprotectants has been widely investigated (Doebbler, 1966; Madden et al., 1985; Crowe et al., 1986; Crowe and Crowe, 1991). In this study, eight different carbohydrates and two polymers were tested in different concentrations, making a pre-choice after freeze–thaw experiments. Trehalose, a disaccharide, was one of the most effective cyroprotectants. In contrast to the optical impression (collapsed SLN-cake), fructose, a monosaccharide, showed the best results after reconstitution. The polymers PVA and PVP had no sufficient protective effect.

The concentration of the cryoprotector was maximized to reach the highest dilution of the nanoparticles in the cryoprotectant matrix with respect to the osmolarity of the reconstituted system (reconstitution with double distilled water).

Other parameters with a positive effect towards better reconstitution and prevention of the formation of aggregates were the time point of lyophilization (immediately after production) and dilution of the SLN dispersion before freezing. A longer drying period (40 h instead of 21 h) gave no better results.

An important parameter is the freezing velocity. Samples were frozen under two different conditions: (A) −196°C by adding the SLN dispersion dropwise to liquid nitrogen; (B) freezing at −70°C in a deep freeze. In general, quick freezing favors the formation of small ice crystals and a heterogeneous structure or amorphous glasses (Rupprecht 1992). During the following drying, the watersteam escapes at first with high velocity, but then, because of the fine porosity of the already dried parts, more and more slowly (Rupprecht 1992). Slow freezing leads to a more crystalline structure with large pores and a quick sublimation during the drying process. Disadvantageous is the affection of freeze-concentration.

In contrast to other studies (Schwarz and Mehnert, 1997), a slower freezing proved to be the better method for this formulation, with freezing at −196°C leading to greater formation of aggregates after redispersion.

Due to the presence of particles larger than 5 μm, the size distribution still was not acceptable for i.v. injection.

Therefore, the influence of thermal treatment was investigated, combining the advantages of slow and fast freezing. After freezing to a sufficient low temperature, the temperature was raised right under collapse-temperature and kept constant for a certain time. Afterwards, the sample was cooled down again to an appropriate temperature for the drying step. This treatment leads to bigger crystals with a more homogenous structure and a transformation of metastable, amorphous areas into stable crystalline ones. To prevent thawing during lyophilization, DSC was used as a means to determine an optimal temperature regulation. By this, we calculated the parameters recorded in Fig. 1.

The vials were closed under vacuum in the freeze-drier. Reconstitution of the lyophilized products was performed by manual shaking. With the previously described parameters, we obtained freeze-dried SLN dispersions with a submicron particle size distribution after reconstitution (Fig. 2). They are considered suitable for parenteral administration. But, with regards to scaling up for industrial purposes, further optimization of the process will be necessary.

Optimized lyophilization process:

- dilution of the aqueous SLN-dispersion with a solution of trehalose or fructose
- freezing right after production at –70°C in a deep-freeze,
- thermal treatment at -22°C for 2 hours
- cooling down to –40°C for another 2 hours
- primary drying at 1,03 mbar (-30°C 7h, -10°C 2h, 20°C 12h)
- secondary drying for 3h 30°C at 0,001mbar

Fig. 1. Parameters of the optimized lyophilization process.

In conclusion, this study demonstrates that by optimizing different critical parameters of the lyophilization process, drug-loaded SLN dispersions can be freeze-dried to i.v.-acceptable formulations with regard to particle-size distribution after reconstitution.

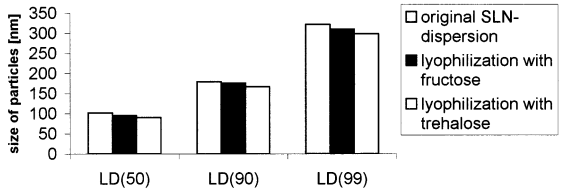

Fig. 2. Particle-size distribution by means of laser diffractometry before and after lyophilization.

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