1 Solid Lipid Nanoparticles — Concepts, Procedures, and Physicochemical Aspects

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1.1 SUMMARY

Solid lipid nanoparticles (SLN) have attracted increasing scientific and commercial attention during the last few years. This chapter highlights the main features of SLN, including the concept of SLN, different methods of production, and their applications. Special attention is paid to the relation among drug incorporation, the heterogeneity of the lipid particle, and the presence of other colloidal species. Strategies of SLN stabilization to avoid particle growth or gelation are discussed. The biological fate of the particles and the suitability of SLN for drug targeting are reviewed.

1.2 INTRODUCTION TO COLLOIDAL DRUG CARRIERS

Many existing drugs and drug candidates have poor solubility in biological fluids, which results in low and highly variable bioavailability and a high food dependency after oral administration. Intravenous injection of these drugs is not possible because of their low solubility. Appropriate formulation technologies might solve these problems. Colloidal drug carriers (CDC) have gained the most attention. The main therapeutic and commercial aims of CDC include:

- Enhancement of oral bioavailability
- Decrease in variability and food dependency
- Development of intravenous injectable formulations
- Drug targeting to specific tissues (with reduction of general toxicity)
- Life cycle management (protection by propriety formulation techniques)

CDC are defined only by their size (most scientists agree on sizes below 1 μm; others set 0.5 μm as the upper limit). CDC are very heterogeneous in all other aspects (e.g., thermodynamic stability, chemical composition, and the physical state, including solid, liquid, or liquid-crystalline dispersions) [1]. The most prominent examples are nanoparticles, nanoemulsions, nanocapsules, liposomes, nanosuspensions, (mixed) micelles, microemulsions, and cubosomes. Some CDC have reached the commercial market. Probably the best known example is the microemulsion preconcentrate of cyclosporine (Sandimmun-Neoral), which minimized the high variability of pharmacokinetics of the Sandimmun formulation. In addition, intravenous injectable CDC have been on the commercial market for many years. Examples include nanoemulsions of etomidate (Etomidat-Lipuro) and diazepam (Diazepam-Lipuro) [2–4], mixed micelles (Valium-MM, Konakion), and liposomes (AmBisome) [5].

However, overall only a very limited number of CDC has reached the marketplace. It is expected that this number will increase as a result of requirements for drug safety and of the increasing number of poorly soluble compounds in the pipeline. More and more molecules have to be formulated with a sophisticated drug delivery system to achieve predictable pharmacokinetics. Patents of other molecules (e.g., cyclosporine) have expired, and the first generics of cyclosporine microemulsion preconcentrates have entered the market. Companies develop strategies to protect or to get market shares based on formulation technology using CDC. Therefore, the modification of existing CDC (to circumvent existing patents) or the development

of new CDC (preferably with new advantages and patent protection) is considered crucial by many companies.

The main efforts to improve current CDC are related to:

- Increasing drug load
- Possibility of controlled release
- Possibility of drug targeting
- Increasing feasibility of large-scale production
- Increasing physical and chemical storage stability
- Minimizing overall costs

1.3 FROM NANOEMULSIONS TO NANOPELLETS: SLN — HISTORY AND SCOPE

Advantages of nanoemulsions include toxicological safety and a high content of the lipid phase as well as the possibility of large-scale production using high-pressure homogenization (HPH). However, controlled drug release from nanoemulsions is very unlikely because of the small size and the liquid state of the carrier. It has been estimated that retarded release requires very lipophilic drugs: Their octanol/water partition coefficient should be larger than 1,000,000:1 [6]. The use of solid lipids instead of liquid oils is a very attractive idea for achieving controlled drug release because drug mobility in a solid lipid should be considerably lower compared with a liquid oil. In addition, the stability of certain drugs might be higher in a solid matrix compared with in a liquid lipid. Therefore, solid lipid micro- and nanoparticles were developed as "frozen nanoemulsions" to realize controlled drug delivery and to increase the chemical stability of incorporated drugs.

The pioneer in this field was Speiser, who developed nanopellets for peroral administration [7]. These nanopellets were produced by dispersing melted lipids with high-speed mixers or with ultrasound. A relatively large amount of microparticles was present in these formulations, which might not be a serious problem for peroral administration, but they exclude an intravenous injection. Lipospheres, produced by high-shear mixing or ultrasound, were developed by Domb and represent similar systems [8–10]. They also contain large amounts of microparticles.

It was soon found that HPH is more effective for the production of submicron-sized dispersions of solid lipids than is high-shear mixing or ultrasound [11–13]. Dispersions obtained in this way are called SLN. Most SLN dispersions produced by HPH are characterized by an average particle size of around 100 to 200 nm and a low microparticle content. Alternative production procedures were also investigated, including the combination of organic solvents and HPH (HPH/solvent evaporation) [14], the dilution of microemulsions [15,16], and solvent injection [17].

It has been claimed that SLN combine the advantages of other colloidal carriers and avoid their disadvantages [18]. Proposed advantages include:

- Possibility of controlled drug release and drug targeting
- Increased drug stability
- High drug payload

- Feasible incorporation of lipophilic and hydrophilic drugs
- Lack of biotoxicity of the carrier
- Avoidance of organic solvents
- No difficulties with large-scale production and sterilization

1.4 SLN INGREDIENTS AND FORMULATION PROCESSES

1.4.1 Ingredients

General ingredients include the drug, solid lipids, emulsifiers, and water. Depending on the application, other ingredients might be present (osmotic agents, matrices for lyophilization, buffers, etc.).

The danger of acute and chronic toxicity resulting from the SLN lipids is rather low because, in general, physiological lipids are used (there are few exceptions, such as amphiphilic calixarenes [19]). The term "lipid" is generally used in a very broad sense and includes triglycerides (e.g., tristearin), partial glycerides (e.g., monostearate), fatty acids (e.g., stearic acid), steroids (e.g., cholesterol), and waxes (e.g., cetyl palmitate). More attention should be given to the physicochemical properties of the lipid and to classifying them in relation to their interactions with water, according to Small [20].

The choice of emulsifier depends on the administration route and is more limited for parenteral administrations. A large variety of ionic and nonionic emulsifiers of different molecular weight has been used to stabilize the lipid dispersion. The most frequently used compounds include different kinds of poloxamer, polysorbates, lecithin, and bile acids. In many cases, the combination of emulsifiers was more efficient at preventing particle agglomeration than was the use of a single surfactant.

1.4.2 Production Processes for SLN

1.4.2.1 High-Shear Homogenization and Ultrasound

High-shear homogenization and ultrasound were initially used for the production of solid lipid nanodispersions [7,8]. Both methods are widespread and easy to handle. However, in many cases, bimodal size distributions are obtained with one population in the micrometer range. In addition, metal contamination has to be considered if ultrasound is used.

Ahlin et al. used a rotor-stator homogenizer to produce SLN by melt-emulsification [21]. They investigated the influence of different process parameters — including emulsification time, stirring rate, and cooling conditions — on the particle size and the zeta potential. In most cases, average particle sizes in the range of 100 to 200 nm were obtained using stirring rates of 20,000 to 25,000 rpm for 8 to 10 min and controlled cooling with a stirring rate of 5,000 rpm.

1.4.2.2 High-Pressure Homogenization

HPH has emerged as a reliable and powerful technique for the preparation of SLN. HPH has been used for years for the production of nanoemulsions for parenteral nutrition. In contrast to other techniques, scaling up represents no or minor problems in most cases. High-pressure homogenizers push a liquid with high pressure (10 to

200 MPa) through a narrow gap (in the range of few microns). The fluid accelerates on a very short distance to very high velocity (over 1000 km/h). Very high-shear forces disrupt the particles down to the submicron range. Typical lipid contents range between 5 to 10% of the fluid and represent no problem to the homogenizer. Even lipid concentrations up to 40% have been homogenized to lipid nanodispersions [22].

Two general approaches of the homogenization step, the hot and the cold homogenization techniques, can be used for the production of SLN [13,23,24]. In both cases, a preparatory step involves incorporating the drug into the bulk lipid by dissolving or dispersing the drug in the lipid melt.

1.4.2.2.1 Hot Homogenization

Hot homogenization is carried out at temperatures above the melting point of the lipid and can therefore be regarded as the homogenization of an emulsion. A preemulsion of the drug-loaded lipid melt and the aqueous emulsifier phase (same temperature) is obtained by a high-shear mixing device (Ultra-Turrax). The quality of the preemulsion affects the quality of the final product to a large extent, and obtaining droplets in the size range of a few micrometers is desirable. HPH of the preemulsion is carried out at temperatures above the melting point of the lipid. In general, higher temperatures result in lower particle sizes because of the decreased viscosity of the inner phase [25]. However, high temperatures may also increase the degradation rate of the drug and the carrier. Furthermore, many surfactants have decreased solubilities and HLB values at a higher temperature, which might have a negative impact on homogenization efficacy. The homogenization step can be repeated several times. It should always be kept in mind that HPH increases the temperature of the sample (approximately 10˚C for 500 bar [26]). In most cases, 3 to 5 homogenization cycles at 500 to 1500 bar are sufficient. Increasing the homogenization pressure or the number of cycles often results in an increase of the particle size because of particle coalescence, which occurs as a result of the high kinetic energy of the particles [27].

The primary product of the hot homogenization is a nanoemulsion resulting from the liquid state of the lipid. Solid particles are expected to be formed by the cooling of the sample to room temperature or below. Because of the small particle size and the presence of the emulsifiers, lipid crystallization may be highly retarded, and the sample may remain as a supercooled melt (nanoemulsion) for several months [28]. Westesen and Bunjes found that purported "SLN" data published by another group were, in fact, measurements from supercooled melts [29].

1.4.2.2.2 Cold Homogenization

Cold homogenization is carried out with the solid lipid and can therefore by regarded as a high-pressure milling of a lipid suspension. Effective temperature control and regulation is needed to ensure the unmolten state of the lipid because of the increase in temperature during homogenization [26]. Cold homogenization has been developed to overcome the following three problems of the hot homogenization technique:

- Temperature-induced drug degradation
- Drug distribution into the aqueous phase during homogenization
- Complexity of the crystallization step of the nanoemulsion leading to several modifications or supercooled melts

The first preparatory step is the same as in the hot homogenization procedure and includes the solubilization or dispersing of the drug in the melt of the bulk lipid. However, different steps follow. The drug-containing melt is cooled very rapidly (e.g., by means of dry ice or liquid nitrogen). The high cooling rate favors a homogenous distribution of the drug within the lipid matrix. The solid, drug-containing lipid is milled by means of ball or mortar milling in the range of 50 to 100 μm. Low temperatures increase the fragility of the lipid and, therefore, favor particle disruption. The solid lipid microparticles are dispersed in a chilled emulsifier solution. The presuspension is subjected to HPH at or below room temperature. In general, compared with hot homogenization, larger particle sizes and a broader size distribution are observed in cold-homogenized samples [30]. The method of cold homogenization minimizes the thermal exposure of the sample, but it does not avoid it because of the melting of the lipid/drug mixture in the initial step. Most investigators use the hot homogenization process because of its higher efficacy and the avoidance of the cold milling process. It must be also mentioned that the rapid cooling of the lipid melt in the first step favors metastable lipid modifications (with higher drug loading capacity), which might transform with time into more stable polymorphs (with the expulsion of the incorporated drug).

1.4.2.3 SLN Prepared by Solvent Emulsification/Evaporation

Sjöström and Bergenståhl used a solvent emulsification/evaporation method to prepare solid lipid nanodispersions [14]. The lipophilic material is dissolved in a water-immiscible organic solvent (e.g., cyclohexane) that is emulsified in an aqueous phase to give an oil/water (o/w) emulsion. On evaporation of the solvent by reduced pressure, a solid lipid nanoparticle dispersion is formed. The mean diameter of the obtained particles was 25 nm, with cholesterol acetate as the model drug and using a lecithin/sodium glycocholate blend as the emulsifier. The reproducibility of these results was confirmed by Siekmann and Westesen [31], who also prepared nanoparticles of tripalmitin by dissolving triglyceride in chloroform. Mean particle sizes of the final particles ranged from 30 to 100 nm, depending on the lecithin/cosurfactant blend. The smallest particle diameters were obtained by using bile salts as cosurfactants. Comparable small-particle-size distributions are not achievable by melt emulsification of a similar composition. The mean particle size depends on the concentration of the lipid in the organic phase. Very small particles could only be obtained with low fat loads (5 w%) related to the organic solvent. With increasing lipid content, the efficiency of the homogenization declines because of the higher viscosity of the dispersed phase. The advantage of this procedure over the cold homogenization process described before is the avoidance of any thermal stress. A clear disadvantage is the use of organic solvents.

1.4.2.4 Solvent Injection Method

The production of polymeric nanoparticles by dilution of polymer solutions in water has been described by De Labouret et al. [32]. The particle size is critically determined by the velocity of the distribution processes. Nanoparticles were produced only with polar solvents, which distribute very rapidly into the aqueous phase (e.g.,

acetone, ethanol, isopropanol, methanol), whereas larger particle sizes were obtained with more lipophilic solvents. The process also can be easily used for the production of lipid nanodispersions [17]. A requirement is the solubility of the lipid in the polar organic solvent, which limits the application range of this procedure. A further disadvantage is the low concentration of the lipid nanoparticles (typically 1% or less). Higher amounts of the organic solvent increase the solubility of the lipid in the aqueous phase and lead to an increase in particle size resulting from Ostwald ripening. The main advantage of the method is the avoidance of thermal stress.

1.4.2.5 Microemulsion-Based SLN Preparations

SLN preparation techniques that are based on the dilution of microemulsions have been developed by Gasco [16]. It should be mentioned that there are different definitions and opinions about the structure and dynamics of microemulsion in the scientific community. An extended review has recently been published by Moulik and Paul [33].

Gasco and other scientists describe microemulsions as two-phase systems composed of an inner and outer phase (e.g., o/w microemulsions). Microemulsions are made by stirring at 65 to 70°C an optically transparent mixture that is typically composed of a low-melting fatty acid (e.g., stearic acid), an emulsifier (e.g., polysorbate 20, polysorbate 60, soy phosphatidylcholine, taurodeoxycholic acid sodium salt), coemulsifiers (e.g., butanol, sodium monooctylphosphate), and water. The hot microemulsion is dispersed in cold water (2 to 3°C) under stirring. Typical volume ratios of the hot microemulsion to cold water are in the range of 1:25 to 1:50. The dilution process is critically determined by the composition of the microemulsion. According to the literature [34,35], the droplet structure is already contained in the microemulsion, and, therefore, no energy is required to achieve submicron particle sizes.

In addition to the composition, the temperature gradient and the pH value are key parameters for the quality of the final lipid nanosuspension. High-temperature gradients facilitate rapid lipid crystallization and prevent aggregation [36,37]. Because of the dilution step, achievable lipid contents are considerably lower compared with the HPH-based formulations.

1.4.2.6 Comparison of Different Formulation Procedures

A reliable comparison between different formulation procedures can be made only by the same investigator, who has used the same batches of ingredients, the same storage conditions, and the same equipment for particle sizing. Otherwise, impurities in the ingredients and differences in particle-sizing technologies might lead to misleading results.

Siekmann and Westesen investigated the influence of the formulation procedure on the quality of tyloxapol- (1.5 w%) and soy lecithin– (1 w%) stabilized tripalmitin (3 w%) nanoparticles [27]. They demonstrated the principal possibility of obtaining size distributions in the range of 30 to 180 nm by ultrasonification. However, these small particle sizes required long sonication times (>15 min), which raises concerns about metal contamination of the product. Moreover, it is difficult to disperse higher

fat concentrations homogeneously by probe sonication, and, therefore, ultrasound is only of limited use. However, HPH proved to be a very effective dispersing technique in this study. A reduction of the average particle size from 474 to 155 nm was obtained after just the first homogenization cycle (800 bar). The maximum dispersing grade was observed after five homogenization cycles. Results reported by other investigators show similar dependences of the particle size from the homogenization pressure and the number of cycles [13,38].

The solvent emulsification/evaporation process was compared by Siekmann and Westesen to the melt-homogenization method [31]. In the case of lecithin/sodium glycocholate-stabilized tripalmitin dispersions, solvent emulsification yielded significantly smaller particles than melt-homogenization of similarly composed systems under the same production conditions. The mean particle size of the melt-homogenized tripalmitin nanoparticles was 124 nm, and that of the evaporated solvent only 28 nm. This result might be partly explained by the lower homogenization efficiency of the lecithin/sodium glycocholate blend in the emulsified tripalmitin melt compared to the organic solvent-in-water emulsion, as the mobility of phospholipid molecules is lower in the lipid melt than in the solvent. Moreover, the melt of the lipid/emulsifier blend is much more viscous than the solvent, so the homogenization requires more energy input. However, solvent emulsification is not always superior to melt homogenization with respect to the dispersing degree. In contrast, for systems stabilized by phospholipids and nonionic surfactants, melt homogenization produced smaller particles than the solvent emulsification procedure. These results show that the particle size heavily depends on the composition of the emulsifiers. Solvent emulsification is a suitable alternative method to prepare small, homogeneously sized lipid nanoparticle dispersions. An important advantage of that technique is the avoidance of any heat. However, solvent-emulsified suspensions are relatively diluted (0.5 to 2.5 w% tripalmitin) because of the limited solubility of the tripalmitin in organic solvents. Furthermore, it has to be considered that solvent emulsification may cause regulatory and toxicological problems arising from the solvent residues.

The dispersing grade depends on the power density and the power distribution in the dispersion volume. High-power densities result in more effective particle disruption, and high-pressure homogenizers reach by far the highest power densities (10^{12} to 10^{13} W/m^3). A homogeneous distribution of the power density is necessary to obtain narrow size distributions. Otherwise, particles localized in different volumes of the sample will experience different dispersing forces, and, therefore, the degree of particle disruption will vary within the sample volume. Inhomogeneous power distributions are observed in high-shear homogenizers and ultrasonifiers. High-pressure homogenizers are characterized by a homogenous power distribution because of the small size of the homogenizing gap (25 to 30 μm).

1.4.2.7 Influence of Ingredient Composition on Product Quality

1.4.2.7.1 Influence of the Lipid

Unfortunately, only a few investigators pay sufficient attention to detailed consideration of the lipid characteristics. A monoglyceride and a triglyceride will behave differently in an aqueous environment. The used lipids should be classified, according

to Small [20], into nonpolar and different classes of polar lipids. This classification is very helpful for understanding the interplay among drug, lipid emulsifier, and water. The general lipid composition (mixed chain lengths or triglycerides made from one fatty acid) will have different crystallinities and capacities for accommodating foreign molecules. In addition, pH levels may change the behavior of lipids considerably. Fatty acids are, in the protonated form (e.g., myristic acid), insoluble, nonswelling lipids, and they behave similarly to triglycerides. Unprotonated fatty acids (e.g., sodium myristate) can form micelles and are soluble amphiphiles with lyotropic mesomorphism. Around the pK_a (which varies in strong dependency of the environment), fatty acid–fatty acid salt complexes form lyotropic liquid crystalline lamellar phases and represent insoluble swelling lipids. The interaction between the fatty acid and the drug will be very different for each species.

Furthermore, even triglycerides with the same fatty acid composition will behave differently, depending on the localization of the fatty acid on the glycerol. For example, cacao butter has a rather sharp melting point because of the defined localization of the oleic (2 position) and palmitic and stearic acids (1 and 3 positions). Random localization of the fatty acids leads to a broadening of the melting point to a melting range, which means that a certain amount of liquid lipids will be present over a large temperature range.

Jenning and Gohla found that high crystallinity of lipid matrices was linked with good physical stability but a low degree drug incorporation, whereas lipid matrices with low crystallinity were able to accommodate higher amounts of drug and showed poor physical stability [39]. However, further parameters for nanoparticle formation will be different for different lipids. Examples include the melting point, the velocity of lipid crystallization, and the shape of the lipid crystals (and therefore the surface area). Higher-melting lipids led to an increase in particle size [11,30]. These results are in agreement with the general theory of HPH [26] and can be explained by the higher viscosity of the dispersed phase.

It is also noteworthy that most of the lipids used represent a mixture of several chemical compounds. The composition might, therefore, vary among different suppliers and might even vary for different batches from the same supplier. Small differences in the lipid composition (e.g., impurities) might have a significant effect on the quality of SLN dispersion (e.g., by changing the zeta potential or retarding crystallization processes). For example, lipid nanodispersions made with cetyl palmitate from different suppliers had different particle sizes and storage stabilities (A. Lippacher, personal communication).

The influence of lipid composition on particle size was also confirmed for SLN produced via high-shear homogenization [21]. The average particle size of Witepsol W35 SLN was found to be significantly smaller (117.0 ± 1.8 nm) than the average particle size of Dynasan 118 SLN (175.1 ± 3.5 nm). Witepsol W35 contains shorter fatty acid chains and considerable amounts of mono- and diglycerides, which possess surface-active properties.

Increasing the lipid content over 10% leads to larger particles (including microparticles) and broader particle size distributions [27,30]. Both a decrease of the homogenization efficiency and an increase in particle agglomeration cause this phenomenon.

1.4.2.7.2 Influence of the Emulsifier

The choice of the emulsifiers and their concentration has a great impact on the quality of the SLN dispersion [13,30]. Siekmann and Westesen determined that 2 w% tyloxapol was insufficient to stabilize a 10 w% tripalmitin dispersion. Increasing the tyloxapol concentration to 10 w% resulted in 85-nm particles with unimodal size distribution [27].

High concentrations of the emulsifier reduce the surface tension and facilitate the particle partition during homogenization. The decrease in particle size is connected with a tremendous increase in surface area. The increase of the surface area during HPH occurs very rapidly. Therefore, kinetic aspects have to be considered. The process of a primary coverage of the new surfaces competes with the agglomeration of uncovered lipid surfaces. The primary dispersion must contain excessive emulsifier molecules, which should rapidly cover the new surfaces. The excessive emulsifier molecules might be present in different forms, for example, molecularly solubilized or in the form of micelles or liposomes. The timescale of the redistribution processes of emulsifier molecules between particle surfaces, water-solubilized monomers, and micelles or liposomes is different. In general, SDS and other micelle-forming, low-molecular weight surfactants will rapidly achieve the new equilibrium. Redistribution processes will take a longer time for high-molecular weight surfactants (poloxamer) or stabilizers with poor solubility in water (lecithin).

However, it is not recommended that one use rapidly distributing surfactants exclusively because surfactant mixtures (e.g., Lipoid S75/poloxamer 188 [30] or tyloxapol/lecithin [27]) might lead to lower particle sizes and higher storage stability compared with formulations with only one surfactant. The addition of sodium glycocholate to the aqueous phase as coemulsifying agent decreases the particle size, too [27].

Different emulsifier compositions might require different homogenization parameters. For example, the maximal degree of dispersing was obtained with 500 bar and 3 cycles for poloxamer 188–stabilized systems [38]. Homogenization with pressures of 1000 or 1500 bar did not result in further reduction of the particle size. In contrast, pressures of 1500 bar proved to be the best for lecithin- (Lipoid S75) stabilized systems. A possible explanation for this observation is the different velocity of the coverage of the new lipid surfaces.

The choice of emulsifiers might also influence the crystallization behavior. For example, glycocholate was able to preserve the alpha polymorph of the lipid tripalmitin for long times, whereas transitions to more stable polymorphs occurred more rapidly for the other surfactants [40].

1.4.3 Sterilization

Parenteral administration requires sterile products. Aseptic production, filtration, γ-irradiation, and heating are normally used to achieve sterility. Filtration sterilization of dispersed systems requires high pressure and is not applicable to particles larger than 0.2 μm.

The impact of different sterilization techniques (steam sterilization at 121˚C [15 min] and 110˚C [15 min], γ-sterilization) on SLN characteristics has been

investigated [38,41,42]. Particle aggregation might occur as a result of the treatment. Critical parameters include sterilization temperature and SLN composition, and increased temperatures might significantly affect the mobility and the hydrophilicity of the emulsifier. Schwarz found that lecithin is a suitable surfactant for steam sterilization because there was only a minor increase in particle size and number of microparticles [38]. In contrast, steam sterilization induced a significant increase in particle size for poloxamer 188–stabilized Compritol SLN. For emulsifiers of this type, it is well known that increased temperatures lead to dehydration of the PEG-units and a decrease of the HLB value, which finally causes a decrease in the thickness of the protecting layer. It has been demonstrated by ^{1}H-NMR (nuclear magnetic resonance) spectroscopy on poloxamer-stabilized lipid nanoparticles that even a moderate temperature increase from room temperature to 37˚C decreases the mobility of the ethylene glycol chains on the particle surface [43].

Drug loading might have a large effect on sterilization-induced destabilization. Steam sterilization of 5% tetracaine–loaded poloxamer 188/Compritol SLN induced a broader size distribution and an increase in the mean particle size (from 160 to 260 nm) [41]. Even a larger increase in particle size (>500 nm) was observed for a higher tetracaine loading of 10%. These results indicate that drug-related phenomena contribute to destabilization processes in addition to changes of the emulsifier film. The destabilizing effect of tetracaine and etomidate has also been observed in steam sterilization of fat emulsions and is probably caused by the distortion of the mechanical properties of the surfactant film [44].

Experiments conducted by Freitas indicated that lowering the lipid content (to 2%), surface modification of the glass vials, and purging with nitrogen prevent the particle increase to a large extent and prohibit gelation [45]. In addition, it was observed that purging the sample with nitrogen showed a protective effect during sterilization. Cavalli et al. studied the influence of steam sterilization on particle size and zeta potential of SLN produced via microemulsions [46]. The lipid phase was made of stearic acid, behenic acid, or Acidan N12 (monostearate monocitrate diglyceride); and Epikuron 200 (soy phosphatidylcholine 95%) and taurodeoxycholate were used as stabilizers. SLN were dispersed in aqueous trehalose (2%) or poloxamer 188 solution (2%). Steam sterilization (121˚C, 15 min) did not change the average particle size of Acidan N12 SLN, but increased particle sizes were observed for SLN composed of behenic acid (from 70 to 135 nm) and of stearic acid (from 55 to 110 nm). After 1 year, increased particle sizes were observed for all systems (Acidan N12 SLN, 350 nm; behenic acid SLN, 120 nm; stearic acid SLN, 450 nm).

Particle sizes of diazepam-loaded SLN showed similar changes after sterilization as did drug-free systems. Steam sterilization (121˚C, 20 min) did not cause changes in particle size and zeta potential of azidothymidine palmitate–loaded SLN (trilaurin, phospholipid stabilized) [47].

An alternative method to steam sterilization for temperature-sensitive samples could be γ-irradiation. Comparative studies on SLN sterilization by steam and γ-rays were conducted by Schwarz and others [38,41,42]. Lecithin-stabilized SLN proved to be superior to poloxamer-stabilized SLN for the process of steam sterilization. However, this difference was not observed for γ-sterilized samples, which indicates the importance of heat-induced changes. Compared to steam sterilization at 121˚C,

the increase in particle size after γ-irradiation was lower, but was comparable to steam sterilization at 110°C.

Unfortunately, most investigators did not search for steam sterilization– or irradiation-induced chemical degradation. It should be kept in mind that degradation does not always cause increased particle sizes. In contrast, the formation of species like lysophosphatides or free fatty acids might even preserve small particle sizes, but might also cause toxicological problems (e.g., hemolytic activity). Detailed studies that involve the aspects of chemical stability are clearly necessary to permit valid statements of the possibilities of SLN sterilization.

1.4.4 Stabilization by Drying

It has been shown that particle sizes of aqueous SLN dispersions might be stable over 12 to 36 months [30]. However, this stability is not a general feature of SLN dispersions, and, in most cases, physical (increases of the particle size) or chemical instability (degradation reactions, hydrolysis) will be observed in a shorter period of time.

The particles must have a very narrow size distribution to avoid crystal growth by Ostwald ripening. The SLN formulation should be resistant to temperature changes that will occur during shipping. Furthermore, mechanical stress resulting from transport might lead to sudden gelation of the SLN dispersions. In addition, microbiological aspects also have to be kept in mind. A strategy to solve these problems is to remove the water and produce SLN powders, which can be stored for longer times. The addition of water just before administration should lead to the particle sizes of the SLN dispersion.

Lyophilization is a widely used process for water removal from sensitive samples. However, two additional transformations between the formulations are necessary and may be the source of additional stability problems. The first transformation, from aqueous dispersion to powder, involves the freezing of the sample and the evaporation of water under vacuum. Freezing of the sample might cause stability problems because of the freezing-out effect, which results in changes of the osmolarity and the pH. The second transformation, resolubilization, involves, at least in its initial stages, situations that favor particle aggregation (low water and high particle content and high osmotic pressure).

The protective effect of the surfactant can be compromised by lyophilization [48]. It has been found that, to prevent an increase in particle size, the lipid content of the SLN dispersion should not exceed 5%. Direct contact of lipid particles is decreased in diluted samples. Furthermore, diluted SLN dispersions will also have higher sublimation velocities and a higher specific surface area [49]. The addition of cryoprotectors will be necessary to decrease SLN aggregation and to obtain a better redispersion of the dry product. Typical cryoprotective agents are sorbitol, mannose, trehalose, glucose, and polyvinylpyrrolidone.

The best results occurred when the lyophilization of SLN was obtained with the cryoprotectors glucose, mannose, maltose, and trehalose in concentrations of 10 to 15% [50]. The observations come into line with the results of studies on liposome lyophilization, which indicated that trehalose was the most sufficient substance to prevent liposome fusion and leakage of the incorporated drug [51].

Mean particle sizes and size distributions of Compritol and Dynasan 112 SLN were only slightly increased after freeze-thaw experiments [50].

The results of unloaded SLN do not predict the quality of drug-loaded lyophilizates. Even low concentrations of 1% tetracaine or etomidate caused a significant increase in particle size, which excludes an intravenous administration [50].

Siekmann and Westesen investigated the lyophilization of tripalmitin SLN (surfactants: 4.5% tyloxapol and 3% soybean lecithin [Lipoid S100]) [52]. Glucose, sucrose, maltose, and trehalose were used as cryoprotective agents in concentrations of 5, 10, and 20%. Handshaking of redispersed samples was an insufficient method, whereas bath sonification produced better results. Average particle sizes of all lyophilized samples with cryoprotective agents were 1.5 to 2.4 times higher than the original dispersions. Cryoprotector-free samples showed very high particle aggregation. Samples with a lipid content below 10% showed less aggregation than more highly concentrated samples. The efficiency of the cryoprotectors decreases in the following order: trehalose > sucrose > glucose and maltose. Surprisingly, it was found that the time at which the cryoprotector is added influences the quality of the final formulation. Best results were obtained when the cryoprotector was added to the sample before homogenization. Under these circumstances, average particle size remained almost unchanged, though storage over 1 year caused significant increases in particle sizes. Average particle sizes were 4 to 6.5 times larger than in the original dispersion. In contrast to the lyophilizates, the aqueous dispersions of tyloxapol/phospholipid-stabilized tripalmitin SLN exhibited remarkable storage stability. The average particle size increased only very slightly, from 56 to 65 nm, over 1 year. The instability of the SLN lyophilizates can be explained by the sintering of the particles.

Cavalli et al. also observed increased particle sizes (2.1 to 4.9 times) after lyophilization [46]. A trehalose concentration of 2% was insufficient to prevent lyophilization-induced particle aggregation. Increasing the concentration of trehalose to 15% resulted in average particle sizes around 100 nm and in polydispersity indices of 0.25 after reconstitution.

Heiati compared the influence of four cryoprotectors (trehalose, glucose, lactose, and mannitol) on the particle size of azidothymidine palmitate–loaded SLN lyophilizates [47]. Trehalose was found to be the most effective cryoprotector for preventing aggregation during lyophilization and subsequent reconstitution of SLN. A sugar/lipid weight ratio of 2.6 to 3.9 was recommended.

The freezing process has an effect on the product quality. Rapid freezing in liquid nitrogen was suggested by Schwarz and Mehnert [50]. In contrast, other researchers observed the best results after a slow freezing process. Zimmermann et al. found that optimization of the lyophilization parameters results in formulations that are intravenously injectable, with regard to particle size [53]. Again, best results were obtained with samples of low lipid content and with the cryoprotector trehalose. In contrast to the results of Schwarz, slow freezing in a deep freeze (–70˚C) was superior to rapid cooling in liquid nitrogen. Furthermore, introduction of an additional thermal treatment to the frozen SLN dispersion (2 h at –22˚C followed by a 2-h temperature decrease to –40˚C) was found to improve the quality of the product.

Recent studies of Gasco's group indicate that drying with a nitrogen stream at low temperatures (3 to 10˚C) might be superior to lyophilization [54]. Compared to lyophilization, the advantages of this process are the avoidance of freezing and the energy efficiency resulting from the higher vapor pressure of water.

Spray drying might be an alternative procedure to lyophilization to transform an aqueous SLN dispersion into a dry product. This method has been used scarcely for SLN formulation, although spray drying is cheaper than lyophilization. By spray drying, Freitas and Müller obtained a redispersable powder that complies with the general requirements regarding particle size and selection of ingredients for intravenous injections [55]. Spray drying might potentially cause particle aggregation as a result of high temperatures, shear forces, and partial melting of the particles. Freitas and Müller recommend the use of lipids with melting points greater than 70˚C for spray drying. Furthermore, the addition of carbohydrates and low lipid contents favor the preservation of the colloidal particle size in spray drying. The melting of the lipid can be minimized by using ethanol/water mixtures as a dispersion medium instead of pure water because of the lower inlet temperatures. The best result was obtained with SLN concentrations of 1% in solutions of 30% trehalose in water or 20% trehalose in ethanol/water mixtures (10/90 v/v).

1.5 CHARACTERIZATION OF SLN

Appropriate characterization of the solid lipid nanodispersion is a necessary and very difficult task because of the submicron size of the particles and the complexity of the system, which also includes dynamic phenomena. The following statement of Laggner about lipids should always be kept in mind [56]:

> Lipids and fats, as soft condensed material in general, are very complex systems, not only in their static structures but also with respect to their kinetics of supramolecular formation. Hysteresis phenomena or supercooling can gravely complicate the task of defining the underlying structures and boundaries in a phase diagram (p. 334).

This is especially true for lipids in the colloidal size range. Many analytical tools do not permit direct measurement in the undiluted SLN dispersion. Therefore, sample preparation might cause artifacts (e.g., removal of emulsifier from particle surface by dilution, induction of crystallization processes, changes of lipid modifications). Pushing SLN dispersions though a syringe needle might result in the immediate transformation of the low, viscous SLN dispersion into a viscous gel. In this case, the artifact caused by sample preparation is clearly visible, although in many other cases it will not be.

The most important parameters include:

- Particle size
- Degree of crystallinity and lipid modification
- Coexistence of additional colloidal structures (micelles, liposomes, supercooled melts, drug nanoparticles) and timescale of distribution processes
- Zeta potential

Photon correlation spectroscopy (PCS) and laser diffraction (LD) are the most frequently applied techniques for measurements of particle size. PCS (also known as dynamic light scattering) measures the fluctuation of the intensity of the scattered light caused by particle movement and covers a size range from a few nanometers to about 3 μm. New developments (back scattering, cross polarization) permit measurement in undiluted or less diluted samples. Microparticles are not detected by PCS, but they can be visualized by means of LD measurements. This method is based on the dependence of the diffraction angle on the particle radius. Smaller particles cause more intense scattering at high angles than do larger ones. New developments of LD expanded the lower limit of measurable particle sizes from 40 to 100 nm.

However, despite this progress, it is highly recommended that both PCS and LD be used simultaneously. It should be kept in mind that both methods are not "measuring" particle sizes. Rather, they detect light-scattering effects that are used to calculate particle size. For example, uncertainties may result from nonspherical particle shapes and from the assumption of certain parameters that are used to calculate the particle size. Platelet structures commonly occur during lipid crystallization [57] and have also been observed for SLN [11,40,58]. The presence of several populations and other colloidal structures adds further difficulties.

The use of additional techniques is recommended. Light microscopy is not sensitive to the nanometer size range but gives a fast indication of the presence of microparticles. Electron microscopy provides, in contrast to PCS and LD, direct information on the particle shape. However, the investigator should pay special attention to possible artifacts that may be caused by the sample preparation. For example, solvent removal may cause modification changes that will influence the particle shape [57].

Atomic force microscopy (AFM) has also been applied to image the morphological structure of SLN [59]. The size of the visualized particles was of the same magnitude as the results of PCS measurements. The AFM investigations revealed the disklike structure of the particles. Dingler and others investigated cetyl palmitate SLN (stabilized by polyglycerol methylglucose distearate, Tegocare 450) by electron microscopy and AFM and observed spherical forms of the particles [60,61]. Different SLN shapes were reported by Westesen and others for SLN made of well-defined lipids of high purity (e.g., pure triglycerides) [11,62]. A disadvantage of AFM is the required fixation of the particles (by removal of water), which changes the status of the emulsifier and might also cause polymorphic transitions of the lipid.

The particle sizing by field flow fractionation (FFF) is based on the different effect of a perpendicular applied field on particles in a laminar flow [63–66]. The separation principle corresponds to the nature of the perpendicular field and may, for example, be based on different mass (sedimentation FFF), size (cross-flow FFF), or charge (electric-field FFF). Cross-flow FFF has been applied recently to investigate nanoemulsions, SLN, and nanostructured lipid carriers (NLC, particles composed of liquid and solid lipids) [58]. Although all samples had comparable particle sizes in PCS, their retention in the FFF was very different. Compared to the spherical droplets of the nanoemulsion, SLN and NLC were pushed more efficiently to the bottom of the channel because of their anisotropic shape. Their very different shapes have been confirmed by electron microscopy.

Special attention must be paid to the characterization of the degree of lipid crystallinity and the modification of the lipid, because these parameters are strongly related to drug incorporation and release rates. Both thermodynamic stability and lipid packing density increase, while drug incorporation rates decrease in the following order:

supercooled melt > alpha modification > beta′ modification > beta modification

Because of the small size of the particles and the presence of emulsifiers, lipid crystallization and modification changes might be highly retarded. For example, it has been observed that polymorphic transitions might occur very slowly and that Dynasan 112 SLN, if crystallization is not artificially induced, may remain a supercooled melt over several months [29,62]. Q_{10} nanodispersions remain also stable as supercooled melts over several months [1].

Differential scanning calorimetry and x-ray scattering are widely used to investigate the status of the lipid. Differential scanning calorimetry is based on the fact that different lipid modifications possess different melting points and melting enthalpies. By means of x-ray scattering, it is possible to assess the length of the long and short spacings of the lipid lattice. Measuring the SLN dispersions themselves is highly recommended because solvent removal will lead to modification changes. Sensitivity problems and long measurement times of conventional x-ray sources might be overcome by synchrotron irradiation [62]. In addition, this method allows for conducting time-resolved experiments and allows the detection of intermediate states of colloidal systems that will be undetectable by conventional x-ray methods [56]. Unfortunately, this source has limited accessibility for most investigators.

NMR is a very useful tool for investigating colloidal systems. NMR active nuclei of interest are ^{1}H, ^{13}C, ^{19}F, and ^{31}P. Because of the different chemical shifts, it is possible to attribute the NMR signals to particular molecules or their segments. Simple ^{1}H-NMR spectroscopy permits an easy and very rapid detection of supercooled melts caused by the low line widths of the lipid protons [67]. This method is based on the different proton relaxation times in the liquid and semisolid/solid states. Protons in the liquid state give sharp signals with high signal amplitudes, whereas semisolid/solid protons give weak and broad NMR signals under these circumstances. Simple ^{1}H-NMR spectroscopy also allows the characterization of lipid particles composed of solid and liquid lipids (NLC) [68,69]. The great potential of NMR, with its variety of different approaches (solid-state NMR, determination of self-diffusion coefficients, etc.), has scarcely been used in the SLN field, although it will provide unique insights into the structure and dynamics of SLN dispersions.

Electron spin resonance (ESR) is, as is NMR, a noninvasive method that does not require dilution of the sample. Paramagnetic spin probes are used as model drugs to investigate SLN dispersions. A large variety of spin probes is commercially available. The corresponding ESR spectra give information about the microviscosity and micropolarity. ESR permits the direct, repeatable, and noninvasive characterization of the distribution of the spin probe between the aqueous and the lipid phases. Experimental results demonstrate that storage-induced crystallization of SLN leads to an expulsion of the probe out of the lipid into the aqueous phase [43]. Furthermore,

by using an ascorbic acid reduction assay, it is possible to monitor the timescale of the exchange between the aqueous and the lipid phase. It recently has been shown that, by this method, lipophilic model drugs are not protected from the aqueous environment in NLC and SLN [69].

Similar results to the ESR experiments can be obtained with fluorescence measurements [69]. Further analytical methods include infrared and raman spectroscopy (to study the arrangement of the lipids chains) [70], rheometry (to investigate gel formation [22]), and measurement of the zeta potential (to predict storage stability of colloidal dispersion [71]).

1.6 ADVANTAGES AND DISADVANTAGES OF SLN AND NLC

Clear advantages of SLN include the ease of scaling up, the avoidance of organic solvents, and the high content of nanoparticles. Critical points relate to high pressure–induced drug degradation, the coexistence of different lipid modifications and different colloidal species, the low drug loading capacity, and the kinetics of distribution processes.

HPH might decrease the molecular weight of polymers [25]. High-molecular weight compounds and long-chain molecules are more sensitive than low-molecular weight drugs or molecules with a spherical shape. For example, it was found that HPH causes degradation of DNA and albumin [72].

Lipid crystallization is an important point for the performance of SLN carriers. Lipid crystallization might be delayed, and supercooled melts (nanoemulsions) remain. The crystallization will involve several polymorphic forms with different melting behaviors, different capacities to incorporate foreign molecules (drugs), and different particle shapes. In most cases, these polymorphic transitions have been observed to last over long periods of time and to continue during the storage of the sample. The handling of the sample (changes of temperature, mechanical vibration, or pushing it through a syringe) might catalyze polymorphic transitions and induce gelation of the sample.

The degree of drug incorporation is, in general, low as a result of the crystalline nature of the lipid, with even triglycerides with minor differences in chain length forming separated lipid crystals. In most cases, the investigators did not differentiate between drug incorporation or drug association with the lipid. Lukowski and Pflegel observed by electron diffraction that acyclovir is not molecularly dissolved in the lipid matrix [73]. Bunjes used NMR techniques to characterize the physicochemical state of diazepam, the lipid and the emulsifier [74]. Cryo transmission electron microscopy studies of SLN give clear evidence that PCS sizes of 130 nm correspond to only one to five lipid layers [58]. Therefore, a much higher amount of the drug will be localized directly on the surface of the particles, which is in conflict with the general aim of the SLN systems (drug protection and controlled release caused by the incorporation of the drug in the solid lipid).

NLC have been proposed as the SLN of a new generation, with higher drug loadings and controlled release properties [75,76]. Several structures were proposed, including the presence of oil droplets in a solid lipid matrix (which should combine

high drug loading caused by the liquid lipid and controlled release caused by the solid lipid). Unfortunately, the proposed structures were not backed up by experimental data. Recent studies demonstrate that NLC possess no advantages over nanoemulsions. It has been shown that the liquid lipid forms a half drop on the solid platelet [58,69].

The presence of several colloidal species is an important point that has been overlooked by many scientists. Stabilizing agents are not localized exclusively on the lipid surface but also in the aqueous phase in different forms, which might serve as an alternative location to host the drug molecules. Sometimes the amount of stabilizers exceeds the amount of the lipid phase. For example, stearic acid (as lipid phase), Epikuron 200 (lecithin), and taurocholate have been formulated in the ratio of 3:4:6 [54].

Published NMR spectra of diazepam indicate a high mobility of the drug, which indicates a localization of the drug in other colloidal species of high mobility [74] (an association with the solid lipid would cause extensive line broadening [69]).

Therefore, micelle-forming surfactant molecules (e.g., SDS) will be present in three different forms, namely, on the lipid surface, as micelles, and as monomeric surfactant molecules in solution. Lecithin will form liposomes, which have also been detected in nanoemulsions for parenteral nutrition [77]. Mixed micelles have to be considered in glycocholate/lecithin-stabilized and -related systems. Micelles, mixed micelles, and liposomes are known to solubilize drugs, and are therefore attractive alternative drug-incorporation sites (especially with respect to the low incorporation capacity of lipid crystals).

A more detailed investigation of the SLN and NLC — including the appropriate characterization of drug incorporation, the presence of other colloids, and their *in vivo* fate — is necessary to understand and judge the real potential of these colloids.

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