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SOLID LIPID NANOPARTICLES: COLLOIDAL CARRIER SYSTEMS FOR DRUG DELIVERY

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ABSTRACT

Solid lipid nanoparticles (SLN) are aqueous colloidal dispersions, the matrix of which comprises of solid biodegradable lipids. They are introduced in 1990 as an alternative carrier system to traditional colloidal carriers, such as emulsions, liposomes and polymeric micro- and nanoparticles. SLN combine advantages of the traditional systems but avoid some of their major disadvantages. They exhibit major advantages such as modulated release, improved bioavailability, protection of chemically labile molecules like retinol, peptides from degradation, cost effective excipients, improved drug incorporation and wide application spectrum. This paper presents an overview about the selection of the ingredients, different ways of SLN production, drug incorporation and release, characterization of SLN quality and structure, Sterilization, storage and stability of SLN dispersions and SLN applications.

INTRODUCTION: In recent years it has become more and more evident that the development of new drugs alone is not sufficient to ensure progress in drug therapy. Development of suitable drug carrier is very important. Because the in vivo fate of the drug is no longer mainly determined by the properties of the drug, but by the carrier system, which should permit a controlled and localized release of the active drug according to the specific needs of the therapy [1]. Solid lipid nanoparticles (SLN) are colloidal carriers developed at the beginning of the 1990s as an alternative particulate carrier system to emulsions, liposomes and polymeric nanoparticles.

In the case of polymeric microparticles the degradation of the polymer might possibly cause systemic toxic effects by impairment of the reticuloendothelial system [2] or by accumulation at the injection site; cytotoxic effects have been indeed observed in vitro after phagocytosis of particles by human macrophages and granulocytes [3]. In addition, organic solvent residues derived from the preparation procedures such as the solvent evaporation technique often used for liposome and polyester microparticles can be present in the delivery system and could result in severe acceptability and toxicity problems. SLN's combine the advantages and avoid the disadvantages of other colloidal carriers.

Similar to emulsions and liposomes they are composed of physiologically well tolerated excipients and can be produced on large industrial scale by high pressure homogenization. Identical to polymeric nanoparticles their solid matrix protects incorporated active ingredients against chemical degradation and provides the highest flexibilities in the modulation of the drug release profiles [4]. SLN formulations for various application routes (parenteral, oral, dermal, ocular, pulmonary, and rectal) have been developed and thoroughly characterized *in vitro* and *in vivo*. SLN are produced by replacing the liquid lipid (oil) of an o/w emulsion by a solid lipid or a blend of solid lipids, i.e. the lipid particle matrix being solid at both room and body temperature. SLN are composed of 0.1% (w/w) to 30% (w/w) solid lipid dispersed in an aqueous medium and if necessary stabilized with preferably 0.5% (w/w) to 5% (w/w) surfactant. The incorporation of cosmetic and pharmaceutical actives is also feasible. The mean particle size of SLN is in the submicron range, ranging from about 40 to 1000nm [5].

Advantages of SLN:

- Use of biodegradable physiological lipids.
- Avoidance of organic solvents related to the production method or methods.
- Wide application spectrum (oral, i.v., dermal).
- Improved bioavailability of poorly water-soluble molecules.
- Site specific delivery of drugs via i.v. injection route.
- Enhanced drug penetration into the skin, localization in certain skin layers, via dermal application.
- Possibility of scaling up to industrial production level, by high-pressure homogenization, at low cost and in a relatively simple way.
- Protection of chemically labile agents from degradation in the gut and sensitive molecules from outer environment.

SLNs have a high specific surface due to their small diameters, a spherical shape and favorable zeta potential. Some other peculiarities of SLN may also be mentioned such as the drug pseudo-zero order kinetics, the prolonged release obtained in vitro for drugs incorporated in SLN, their rapid uptake (internalization) by cell lines (5-10 min), the possibility to prepare stealth SLN so as to avoid the Reticuloendothelial system (RES) [6]. Moreover, the

possibility of loading drugs with differing physico-chemical and pharmacological properties makes SLNs a highly versatile delivery system [7].

Disadvantages of SLN:

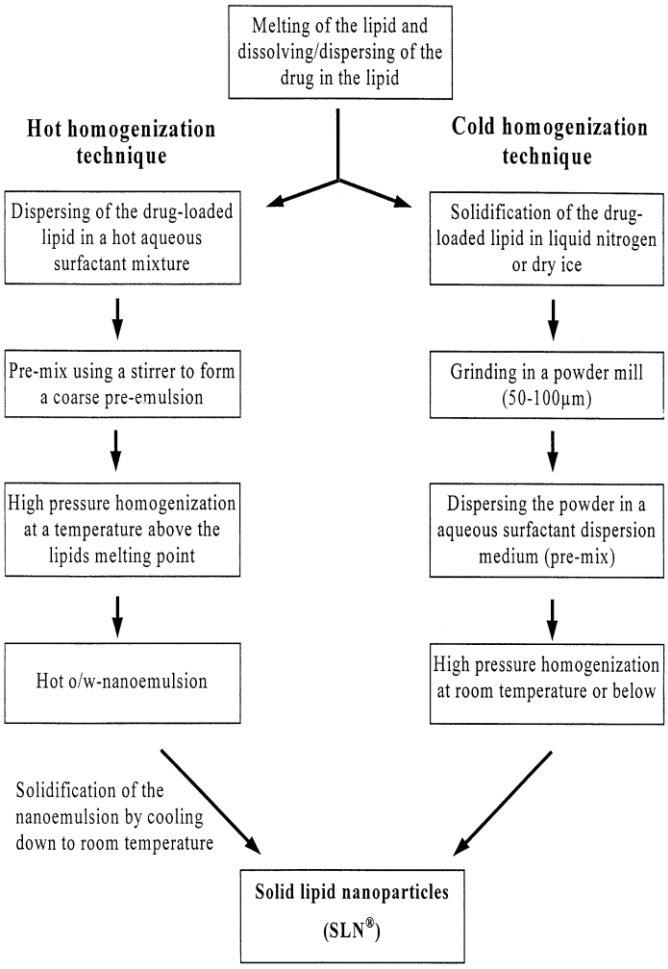
- Limited drug loading capacity due to crystalline structure of solid lipid.
- Adjustment of drug release profile.
- Drug expulsion during storage due to the formation of a perfect crystal.
- Particle growing.
- Unpredictable gelation tendency.
- Unexpected dynamics of polymorphic transitions.
- High water content of SLN dispersions.

General Ingredients of SLN's: Lipids and emulsifiers are generally used for preparation of solid lipid nanoparticles. The matrixes of SLN are the natural or the synthetic lipids which can be degraded, including triglyceride (Tricaprin, Trilaurin, Trimyristin, Tripalmitin, Tristearin), Hydrogenated coco-glycerides (SoftisanÒ 142), Hard fat types (WitepsolÒ W 35, WitepsolÒ H 35, WitepsolÒ H 42, WitepsolÒ E 85), Glyceryl monostearate (ImwitorÒ 900), Glyceryl behenate (CompritolÒ 888 ATO). Glyceryl palmitostearate (PrecirolÒ ATO 5), Cetyl palmitate, Fatty acids (e.g., Stearic acid, Palmitic acid, Decanoic acid, Behenic acid), steroid (e.g., cholesterin) waxes (e.g., microcrystal paraffin wax, whale ester wax).

Emulsifiers include the phospholipids [Soybean lecithin (LipoidÒ S 75, LipoidÒ S 100), Egg lecithin (LipoidÒ E 80)], Phosphatidylcholine (lecithin, EpikuronÒ 170, Epikuron 200), Nonionic wetting agent (e.g., poloxamer 188, 182, 407, 908), cholate (e.g., sodium cholate, sodium glycocholate, sodium taurocholate, deoxy-sodium taurocholate) short-chain spirits (e.g., butanol, butanoic acid), Polysorbate 20, Polysorbate 60, Polysorbate 80, Dioctyl sodium sulfosuccinate, Mono-octylphosphoric acid sodium. Amphipathic materials (e.g., ionic and nonionic type) can stabilize the dispersion of SLN, on the surface of SLN, hydrophobic parts stretch to the core, hydrophilic parts stretch to the disperse medium, so drug with low water-solubility can be entrapped in the SLN to form the colloidal drug system [8].

METHODS OF SLN PREPARATION:

1. **High pressure homogenization (HPH):** HPH is a suitable method for the preparation of SLN, and can be performed at elevated (hot HPH technique) or at or below room temperature (cold HPH technique) [9]. The schematic procedure was depicted in **Figure 1**.



2. **Hot homogenization:** In the hot homogenization method the lipid melt (heated 5 or 10°C above the melting point of the lipid) containing the active compound is dispersed in a hot surfactant solution of the same temperature (5-10°C above the melting point of the solid lipid or lipid blend) by high speed stirring [10]. The obtained emulsion (generally called pre-emulsion) is then passed through a high pressure homogenizer(piston gap homogenizer, MICRON LAB 40) adjusted to the same temperature generally applying three cycles at 500 bar or two cycles at 800 bar. The produced hot O/W nanoemulsion is cooled down to room temperature; the lipid recrystallizes and leads to solid lipid nanoparticles. Usually, lower particle sizes are obtained at higher processing temperatures because of lowered viscosity of the lipid phase.

3. **Cold homogenization:** The cold HPH is a suitable technique for processing temperature labile drugs and also hydrophilic drugs, because they would partition between the melted lipid and the water phase during the hot homogenization process. In the cold homogenization method, the active containing lipid melt is cooled down, the solid lipid ground to lipid microparticles (approximately 50-100 mm) and these lipid microparticles are then dispersed in a cold surfactant solution yielding a cold pre-suspension of micronized lipid particles.

   This suspension is passed through a high pressure homogenizer at room temperature applying typically 5-10 cycles at 1500 bar [11]. This process avoids, or minimizes, the melting of the lipid and therefore minimizing loss of hydrophilic drugs to the water phase. To further minimize the loss of hydrophilic compounds to the aqueous phase of the SLN dispersion, water can be replaced by liquids with low solubility for the drug, e.g. oils or PEG 600. In general, compared to hot homogenization, larger particle sizes and a broader size distribution are observed in cold homogenized samples. The influence of homogenizer type, applied pressure, homogenization cycles and temperature on particle size distribution has been studied extensively [12]. Both HPH techniques are suitable for processing lipid concentrations of up to 40% and generally yield very narrow particle size distributions (polydispersity index < 0.2).

4. **SLN produced by microemulsion technique:** Gasco and co-workers developed SLN preparation techniques which are based on the dilution of microemulsions [13]. Microemulsions are two-phase systems composed of an inner and outer phase (e.g. o/w microemulsions). They are made by stirring an optically transparent mixture at 65-78^{o}C which is typically composed of a low melting fatty acid (e.g. stearic acid), an emulsifier (e.g. polysorbate 20, polysorbate 60, soy phospha tidylcholine, and taurodeoxycholic acid sodium salt), co-emulsifiers (e.g. butanol, sodium monooctylphosphate) and water.

   The hot microemulsion is dispersed in cold water (2-38^{o}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. Considering microemulsions, the temperature gradient and the pH value fix the product quality in addition to the composition of the microemulsion. High-temperature gradients facilitate rapid lipid crystallization and prevent aggregation [14]. Due to the dilution step, achievable lipid contents are considerably lower compared with HPH based formulations.

5. **SLN prepared by solvent emulsification /evaporation:** The lipophilic materials are dissolved in a water-immiscible organic solvent (e. g., cyclohexane, toluene, chloroform, and dichloromethane) that is emulsified in an aqueous phase. Upon evaporation of the solvent,

nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium. This solution was emulsified in an aqueous phase by HPH. The organic solvent was removed from the emulsion by evaporation under reduced pressure (40-60mbar) [15]. However, from the technique, it is impossible to remove the additional organic solvent. 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.

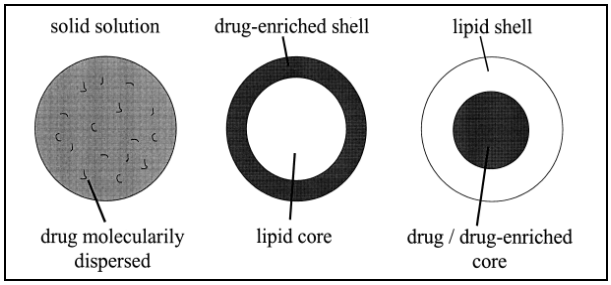
6. **Preparation by high speed stirring and/or ultrasonication:** The SLN were developed from lipid microparticles produced by spray congealing followed by lipid nanopellets produced by high speed stirring or sonication [16]. A great advantage of this method is the fact that the equipment is common in every lab and the production can easily be done. The problem of high speed stirring was a broader particle size distribution ranging into the micrometer range. This leads to physical instabilities such as particle growth upon storage. This could be improved by higher surfactant concentrations, which in order might be correlated with toxicological problems after parenteral administration. A further disadvantage is potential metal contamination due to ultra sonication.

7. **Preparation by w/o/w double emulsion method:** This is a novel method based on solvent emulsification-evaporation for the preparation of SLN loaded with hydrophilic drugs [17]. The hydrophilic drug is encapsulated along with a stabilizer to prevent drug partitioning to the external water phase during solvent evaporation in the internal water phase of a w/o/w double emulsion. This technique has been used for the preparation of sodium cromoglycate containing SLN.

Drug Incorporation and Loading Capacity: Many different drugs like AZT-P and derivatives, Camptothecin, Clobetasol propionate, Cortisone, Sodium cromoglycate, Cyclosporin A, Diazepam, Diminazenediaceturate, Doxorubicin, Etomidate, Hydrocortisone, Idarubicin, Magnetite, Mifepristone, Paclitaxel, Pilocarpine, Piribedil, Prednisolone, Progesterone, Retinoic acid, Tetracaine, Thymopentin, Tobramycin have been incorporated in SLN [18]. A very important point to judge the suitability of a drug carrier system is its loading capacity. The loading capacity is generally expressed in percent related to the lipid phase (matrix lipid + drug). Different loading capacities for different drugs were reported. For Ubidecarenone loading capacity of up to 50%, for Tetracaine and etomidate capacities of 10±20%, for retinol up to 5%, for coenzyme Q10 20% and for cyclosporin 20±25% was reported. Factors determining the loading capacity of drug in the Lipid [19] are;

- Solubility of drug in melted lipid;
- Miscibility of drug melt and lipid melt;
- Chemical and physical structure of solid lipid matrix;
- Polymorphic state of lipid material.

Drug Incorporation Models: Three models are widely used for describing the incorporation of active ingredients into SLN [20-22]. The structure obtained is a function of the formulation composition (lipid, active compound, and surfactant) and of the production conditions. The models are depicted in **Figure 2**.



Homogeneous Matrix Model: It is derived from solid solutions of lipid and active ingredient. A solid solution can be obtained when SLNs are produced by cold homogenization method or by the avoidance of potentially drug solubilizing surfactants. A lipid blend can be produced containing the active in a molecularly dispersed form. After solidification of this blend, it is ground in its solid state thus avoiding or minimizing of the enrichment of active molecules in different parts of the lipid nanoparticle. Etomidate SLN represents the homogeneous solid solution matrix model.

- **Drug enriched shell model:** It is produced by the hot homogenization technique. Tetracaine SLN's were prepared produced by hot HPH at a drug concentration well below saturation solubility in the lipid, i.e. during the production, the drug partitioned from the lipid phase to the water phase. Upon cooling, the lipid precipitates first due to phase separation. Simultaneously, the drug re-partitions into the liquid lipid phase and its concentration in the outer shell liquid lipid increases continuously. Finally, the drug enriched shell crystallizes. If the drug is located primarily in the shell of the particles, a burst release is highly likely. Other factors contributing to a fast release are large surface area, high diffusion coefficient (small molecular size), low matrix viscosity and short diffusion distance of the drug.

- **Drug enriched core model:** It is formed when the drug precipitates first before the lipid re-crystallizes. This is seen when the drug is dissolved in the lipid phase at or close to its saturation stability. Cooling of such a hot nanoemulsion prepared by HPH will lead to the super-saturation of the drug in the lipid melt and subsequently to drug crystallization prior to lipid crystallization. The drug-enriched core is surrounded by a practically drug-free lipid shell. Due to the increased diffusional distance and hindering effects by the surrounding solid lipid shell, the drug has a sustained release profile. SLN loaded with prednisolone released the drug in vitro (i.e., in absence of enzymes) over a period of more than 5 weeks.

Characterization of SLN Quality and Structure: An adequate characterization of the solid lipid nanodispersion is a necessity for the control of the quality of the product. Characterization of SLN is a serious challenge due to the small size of the particles and the complexity of the system, which includes also dynamic phenomena. Several parameters have to be considered which have direct impact on the stability and release kinetics:

- **Measurement of the particle size and zeta potential:** Photon correlation spectroscopy (PCS) and laser diffraction (LD) are the most powerful techniques for routine measurements of particle size. The Coulter Counter method is rarely used to measure SLN particle size because of difficulties in the assessment of small nanoparticles and the need of electrolytes which may destabilize colloidal dispersions. PCS (also known as dynamic light scattering) measures the fluctuation of the intensity of the scattered light which is caused by particle movement. This method covers a size range from a few nanometers to about 3 microns. This means PCS is a good tool to characterize nanoparticles, but it is not able for the detection of larger microparticles.

They can be visualized by means of LD measurements. This method is on the dependency of the diffraction angle on the particle radius (Fraunhofer spectra). Smaller particles cause more intense scattering at high angles compared to the larger ones. A clear advantage of LD is the coverage of a broad size range from the nanometer to the lower millimeter range. The development of phase-sensitive-intensity-difference- scattering (PIDS) technology greatly enhanced the sensitivity of LD

to smaller particles. However, despite this progress, it is highly recommended to use PCS and LD simultaneously. It should be kept in mind that both methods do not 'measure' particle size. Rather, they detect light scattering effects which are used to calculate particle size. For example, uncertainties may result from non-spherical particle shapes. Further, difficulties may arise both in PCS and LD measurements for samples which contain several populations of different size. Therefore, additional techniques might be useful. For example, light microscopy is recommended, although it is not sensitive to the nanometer size range. It gives a fast indication of the presence and character of microparticles (microparticles of unit form or microparticles consisting of aggregates of smaller particles).

Electron microscopy provides, in contrast to PCS and LD, direct information on the particle shape [23]. Atomic force microscopy (AFM) is attracting increasing attention. This technique utilizes the force acting between a surface and a probing tip resulting in a spatial resolution of up to 0.01 nm for imaging. Striking advantages of AFM are the simplicity of sample preparation, as no vacuum is needed during operation and that the sample does not need to be conductive.

Therefore, it has the potential for the direct analysis of the originally hydrated, solvent containing samples. Zeta potential is an important product characteristic of SLNs since its high value is expected to lead to de-aggregation of particles in the absence of other complicating factors such as steric stabilizers or hydrophilic surface appendages. It is usually measured by zeta meter. The measurement of the zeta potential allows predictions about the storage stability of colloidal dispersion [24]. In general, particle aggregation is less likely to occur for charged particles (high zeta potential) due to electric repulsion. However, this rule cannot be strictly applied for systems which contains steric stabilizers, because the adsorption of steric stabilizer will decrease the zeta potential due the shift in the shear plane of the particle.

- **Degree of crystallinity and Lipid Modification:** The degree of lipid crystallinity and the modification of the lipid are strongly correlated with drug incorporation and release rates. Thermodynamic stability and lipid packing density increase, and drug incorporation rates decrease in the following order: Super cooled melt < α-modification < β'-modification < β-modification. Due to the small size of the particles and the presence of emulsifiers, lipid crystallization and modification changes might be highly retarded. Differential scanning calorimetry (DSC) and X-ray scattering are widely used to investigate the status of the lipid.

DSC uses 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 spacing of the lipid lattice. It is highly recommended to measure the SLN dispersion themselves 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 [25]. However, this source has limited accessibility for most investigators. Infrared and Raman spectroscopy are useful tools to investigate structural properties of lipids [26]. However, their potential to characterize SLN dispersions remains to be investigated.

- **Coexistence of additional colloidal structures (micelles, liposomes, super cooled melts, drug nanoparticles) and time scale of distribution processes:** The magnetic resonance techniques, NMR and ESR (Nuclear Magnetic Resonance and Electron Spin Resonance) are powerful tools for

investigating dynamic phenomena and the characteristics of nano-compartments in colloidal lipid dispersions. NMR active nuclei of interest are ^{1}H, ^{13}C, ^{19}F and ^{35}P. Due to the different chemical shifts it is possible to attribute the NMR signals to particular molecules or their segments. Simple ^{1}H-spectroscopy permits an easy and rapid detection of super cooled melts [27]. It permits also the characterization of liquid nano-compartments in recently developed lipid particles, which are made of blends from solid and liquid lipids [28]. This method is based on the different proton relaxation times in the liquid and semisolid/solid state.

Protons in the liquid state give sharp signals with high signal amplitudes, while semisolid/solid protons give weak and broad NMR signals under these circumstances. ESR requires the addition of paramagnetic spin probes 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 non-invasive characterization of the distribution of the spin probe between the aqueous and the lipid phase. Experimental results demonstrate that storage induced crystallization of SLN leads to an expulsion of the probe out of the lipid into the aqueous phase [29]. ESR spectroscopy and imaging will give new insights about the fate of SLN in vivo.

Influence of Ingredients on SLN:

- **Influence of the lipid:** Using the hot homogenization, it has been found that the average particle size of SLN dispersions is increasing with higher melting lipids. The influence of lipid composition on particle size was also confirmed on SLN produced via high-shear homogenization [30]. The average particle size of WitepsolÒW35 SLN was found to be significantly smaller (117.061.8nm) than the size of DynasanÒ118 SLN (175.163.5nm). WitepsolÒW35 contains shorter fatty acid chains and considerable amounts of mono- and diglycerides which possess surface active properties. Increasing the lipid content over 5-10% in most cases results in larger particles (including microparticles) and broader particle size distributions. Both a decrease of the homogenization efficiency and an increase in particle agglomeration cause this phenomenon.

Most importantly, the lipid composition will determine the type of crystal that is generated upon cooling, thereby influencing the stability of SLN and the release characteristics of the encapsulated bioactive from the SLN. Triacylglycerides (TAGs) are commonly used as carrier lipids. TAGs exhibit polymorphism upon cooling; that is, the individual chains of the lipid molecules may assume a variety of possible association configurations giving rise to longitudinal stacking of TAG molecules in lamellae that lead to the formation of α, β′, and β crystals with hexagonal, orthorhombic, and triclinic unit structures, respectively [30]. These crystals differ in their lattice spacing from 4.15Å for the thermodynamically least stable and lowest melting α-form to 4.6Å for the thermodynamically most stable and highest melting β-form.

Pure homogenous lipids tend to form predominantly perfect crystals with the typical plated-like pattern of β-modification. This can lead to aggregation and destabilization in the cooling process and higher oxidative instability due to increased surface area and must therefore be avoided if possible. Using heterogeneous lipids will favor formation of spherical particles due to higher presence of α-crystals in the lipid phase [31]. In summary, a solid understanding of the polymorphic features of

the carrier lipid in combination with the bioactive lipid is required to form SLN that are physically and chemically stable.

- **Influence of the emulsifier:** The choice of the emulsifiers and their concentration is of great impact on the quality of the SLN dispersion [32].

Surfactant Type: In conventional emulsions, the emulsifier predominantly influences the final particle size that can be achieved during the homogenization and influences the stability of the dispersions after manufacturing by providing sufficient repulsive interaction forces that prevent droplets from coming into close contact leading to flocculation and or coalescence. However, in SLN, the surfactant plays an additional very important role in controlling the crystallization process. Because of the small size of the parent nanoemulsion, the number of lipid molecules interacting with the hydrophobic emulsifier tail groups is large enough to modulate the crystallization process.

Moreover, the surfactant can subsequently improve the kinetic stability of the generated crystal structure even if that crystal structure is thermodynamically less stable than that of a corresponding alternative polymorphic form and thereby prevent re-crystallization during storage that may lead to destabilization of the dispersion during storage. To fulfill both the demand of particle stabilization and crystal modulation, mixtures of nonionic surfactants providing for the modulation and ionic surfactants providing for the repulsive interactions are commonly used.

Surfactant Concentration: Surfactant concentration influences the final size that can be achieved during homogenization and therefore the physical properties of the system. High concentration of surfactants will decrease the surface tension and stabilize newly formed surfaces during homogenization leading to smaller droplets. In addition, in SLN, insufficient amount of surfactant can lead to increased instability if recrystallization processes. For example, the thermodynamically more unfavorable α-crystal modification may transition into the β-form. This transition is associated with morphological changes, i.e., the β-crystals may undergo directional growth leading to formation of needle-like structures with increased surface areas. If these surfaces are not stabilized by surfactant molecules, hydrophobic interactions may lead to flocculation and destabilization of the SLN suspension.

Toxicity and Status of Excipients: One can anticipate that SLN are well tolerated in living systems because they are made from physiological compounds and therefore, metabolic pathways exist. The status of excipients for SLN has to be discussed as a function of the administration routes. Topical and oral administration of SLN is absolutely non-problematic regarding the excipients. For topical SLN, all excipients can be used which are currently employed for the formulation of pharmaceutical and cosmetic ointments and creams. For oral SLN, all the lipids and surfactants used in traditional dosage forms such as tablets, pellets and capsules can be exploited.

In addition all compounds of GRAS status or accepted GRAS status can be employed [33]. There is also the option to use lipids and surfactants from the food industry. For parenteral administration one can use glycerides composed of fatty acids which are contained in oils of parenteral fat emulsions. Therefore, no toxic effects are expected from the SLN degradation products. To formulate parenteral SLN, surfactants accepted for parenteral administration (surfactant of GRAS status) can be used, that means, e.g. lecithin, Tween 80, Poloxamer 188, PVP, sodium glycocholate, Span 85 etc. For the intravenous

route it is recommended to focus on the i.v. accepted surfactants (e.g. lecithin, Tween 80, Poloxamer 188, sodium glycocholate). The results of cytotoxicity studies (MTT test) indicated that SLN are less toxic than polymeric nanoparticles [34].

Sterilization of SLN: Sterilization of SLN is important in the case of pulmonary or parenteral administration. For lecithin-stabilized SLN it could be shown that autoclaving is possible [35]. The SLN melt during the autoclaving and recrystallize during the cooling down. However, autoclaving is not possible when a certain structure has been given to the SLN in a controlled way by adjusting the production parameters. This special structure leading to the desired modulated release profile would be lost when the particles melt again during the autoclaving and recrystallize in an uncontrolled way.

Autoclaving at 121°C cannot be performed when using sterically stabilizing polymers, e.g. poloxamer series [36]. The autoclaving temperature seems to be too close to the critical flocculation temperature (CFT) of the polymers, at least the polymer adsorption layer seems partially to collapse leading to insufficient stabilization and particle aggregation. This can be avoided by reducing the autoclaving temperature (e.g. 121° to 110°C, but simultaneously prolonging the autoclaving time).

The physical stability during autoclaving cannot be stated in a general manner, it depends very much on the composition of the SLN formulation. SLN dispersions can also be sterilized by filtration similar to emulsions for parenteral nutrition. It is highly important to filter them in the liquid state; this allows even particles with a size larger than the pores in the filter to be filtered[37]. This technology is well known from parenteral emulsions and easy to apply to SLN. Alternatively, the SLN can be produced aseptically, again identical to parenteral emulsions.

Storage and Stability of SLN Dispersions: The physical stability of SLN dispersions has been investigated intensively, e.g., by measurements of particle size (photon correlation spectroscopy, PCS; laser diffraction, LD), charge (ZP) and thermal analysis (differential scanning calorimetry, DSC). Physical stability of optimized aqueous SLN dispersions is generally more than 1 year [38] and Muller et al. could show stability for SLN made from glyceryl palmitostearate or tribehenate for up to 3 years by PCS [39]. The average diameter of the main population remained between 160 and 220 nm for the investigated period. Freitas and Muller investigated the effect of light and temperature on the physical stability of SLN dispersions composed of 10% tribehenate and 1.2% poloxamer 188 [40]. They found that particle growth could be induced by an input of kinetic energy (light, temperature) to the system.

Storage under artificial light lead to gelation of the system within 7 days of storage, under day light within 3 months and in darkness particle growth started after 4 months storage. The gelation was accompanied by a decrease in ZP from -24.7 to below -18 mV. The influence of the storage temperature on particle size has also been analyzed. They found that the particle size measured by LD increased rapidly at elevated temperatures and remained stable for more than 180 days when refrigerated. Again, particle growth could be correlated to a decrease in ZP from -24.7 to approximately -15 mV.

Freitas and Muller have also correlated the physical stability of the aforementioned SLN formulation with the polymorphic state of the lipid[41]. After hot HPH, the lipid is present in a mixture of β', α and sub α polymorphs. The input of kinetic energy causes a transformation to β' accompanied by gel formation. By inhibition of this transformation (refrigerated, dark storage), this transformation could be avoided. These studies

show that the development of optimal storage conditions can improve the physical stability of previously regarded unstable SLN formulations tremendously. For spray-drying, a melting point of the lipid matrix of >70°C is a pre-requisite. Typically, protectors such as trehalose are added to the dispersion in concentrations of about 20–25%. For best reconstitution effects, SLN concentration in the spraying medium should be approximately 1%.

The influence of lipid type and concentration, carbohydrate type and concentration, redispersion medium and spraying medium have been investigated by Freitas *et al*., [42]. Lyophilization can be employed as an alternative very sensitive drying method. It is a promising way to increase chemical and physical SLN stability over extended periods of time. Transformation into a solid form will prevent Ostwald ripening and avoid hydrolysis reactions. Lyophilization also offers principle possibilities for SLN incorporation into pellets, tablets or capsules. The process has been optimized with regard to operating conditions, lipid concentration, type and concentration of cryoprotectant and redispersing conditions [43]. The addition of cryoprotectors will be necessary to decrease SLN aggregation and to obtain a better redispersion of the dry product.

Heiati *et al*., have investigated the effect of cryoprotective sugars on the size of neutral and negatively charged SLN after lyophilization and reconstitution [44]. The PCS diameter and the polydispersity index increased upon redispering. No changes in ZP and in drug loading were observed. The efficiency of the cryoprotectors decreases in the following order: trehalose> sucrose»glucose and maltose. Trehalose was the most sufficient substance to prevent liposome fusion and leakage of the incorporated drug. The time of the addition of the cryoprotector influences the quality of the final formulation. Best results were obtained when the cryoprotector was added to the sample prior to homogenization. Under these circumstances, average particle size remained almost unchanged.

Administration Routes of Solid Lipid Nanoparticles (SLN):

- **Oral administration:** SLN granulates or powders can be put into capsules, compressed into tablets or incorporated into pellets. For the production of tablets the aqueous SLN dispersion can be used instead of a granulation fluid in the granulation process. Alternatively SLN can be transferred to a powder (e.g. by spray-drying) and added to the tabletting powder mixture. For the production of pellets the SLN dispersion can be used as wetting agent in the extrusion process [45].

SLN powders can be used for the filling of hard gelatin capsules; alternatively, the SLN can be produced directly in liquid PEG 600 and filled into soft gelatin capsules. Sachets are also possible using spray dried or lyophilized powders. The use of submicron-size particular systems in oral drug delivery, especially peptide drugs, has attracted considerable pharmaceutical interest. Therapeutically relevant peptides (e.g. calcitonin, cyclosporine A, insulin, LHRH, somatostatin), protein antigens (e.g. hepatitis B and malaria antigens) and model protein drugs (e.g. bovine serum albumin and lysozyme) have been incorporated in SLN [46].

Controlled release behavior of these systems is reported to enable the bypass of gastric and intestinal degradation of the encapsulated drug [47] and their possible uptake and transport through intestinal mucosa. The adhesive properties of nanoparticles are reported to increase bioavailability and reduce or minimize erratic absorption [48]. Absorption of nanoparticles occurs through mucosa of the

intestine by several mechanisms, namely through the Peyer's patches, by intracellular uptake or by the paracellular pathway. PEG coating of colloids leads to the increased attention in their oral use, especially in delivery of peptide drugs. Various companies are interested in solid lipid nanotechnology for oral drug delivery. Pharmatec (Italy) developed a cyclosporine SLN formulation for oral administration.

Avoidance of high plasma peak and low variability in plasma profile were provided in this case. AlphaRx have also rifampicin loaded SLN under preclinical phase (RifamsolinTM) (www.alpharx.com). Rifampicin is mainly used to treat tuberculosis, which requires long-term treatment due to poor cellular antibiotic penetration. AlphaRx aims to deliver this drug inside the human cell, to increase its efficacy and as a result to increase patient compliance.

- **Parenteral administration:** Basically SLN can be used for all parenteral applications suitable for polymeric nanoparticles. This ranges from intraarticular to intravenous administration. Unfortunately, the polymer particles proved to be cytotoxic in the concentrations necessary for the arthritis treatment [49]. As an alternative the lipophilic corticoids could be incorporated into SLN. SLN are very suitable for systemic delivery because they consist of physiologically well-tolerated ingredients and they have good storage capabilities after lyophilization and/or sterilization.

When injected intravenously, SLN are sufficiently small to circulate in the microvascular system and prevent macrophage uptake in case of hydrophilic coating. Therefore, SLN have been suggested for viral and non-viral gene delivery. Cationic SLN has been demonstrated to bind genes directly via electrostatic interactions, and to have potential benefits in targeted gene therapy in treatment of cancer. SLN formulations can be used for systemic body distribution with a minimized risk of blood clotting and aggregation leading to embolism. Also SLN provide a sustained release depot of the drug when administered subcutaneously or intramuscularly. Incorporated drug is gradually released on erosion (e.g. degradation by enzymes) or by diffusion from the particles. The rate of release may be controlled by the nature of the lipid material, particle size, and choice of surfactant and also by inner structure of the lipid particles. To facilitate drug targeting, for example in tumor tissue, reticuloendothelial system avoidance (stealth) facility may be incorporated.

This may be achieved using block polyoxyethylene polypropylene copolymers like Pluronic F188 in which the hydrophobic portion of the molecule forms the nanoparticle matrix while the water soluble polyoxyethylene block forms a hydrophilic coating on the particle. Stealth SLN increases the tumor accumulation, antibacterial activity of antiparasitic and antifungal drugs and allows brain delivery of anticancer drugs that are not capable of crossing the blood brain barrier (BBB). Treatment of central nervous system diseases such as brain tumors, AIDS, neurological and psychiatric disorders is often constrained by the inability of potent drugs to pass blood brain barrier (BBB), which is formed by the endothelium of the brain vessels, the basal membrane and neurological cells.

Hydrophilic coating of colloids improves the transport of these through BBB and tissue distribution [50]. In general, parenteral application of SLN reduces the possible side effects of drug incorporated with increased bioavailability. These systems are very suitable for drug targeting. Excellent properties of SLN make them attractive drug carrier systems even for

pharmaceutical companies. SLN products of several pharmaceutical companies can be given as follows: cationic solid lipid nanoparticles (SLN) for gene transfer namely TransoPlex® was produced by PharmaSol DDS (Germany) [51], AlphaRx (USA) is developing vancomycin and gentamicin products with VansolinTM and ZysolinTM trade names. They are very effective in treatment of life-threatening infectious disease such as pneumonia. The intention of incorporating them into SLN has been to increase their efficacy while reducing their side effects. SkyePharma (UK) also has formulations of nanoparticulate technology which includes nanosuspensions and solid lipid nanoparticles under preclinical development [52].

- **Pulmonary administration:** The potential of SLN in pulmonary drug delivery has not been sufficiently explored. Aqueous SLN dispersions were nebulized with a Pari-Boy, the aerosol droplets were collected and the size of SLN analyzed. It was observed that the particle size distributions of SLN before and after nebulization were almost identical, only very little aggregation could be detected which was of no significance for pulmonary administration. SLN powders might be used in dry powder inhalers. SLN could be spray-dried using, lactose.

Basic advantages of drug release from SLN in the lung are control of the release profile, achievement of a prolonged release and having a faster degradation compared to particles made from some polymeric materials. High tolerability of SLN can be exploited for drug targeting to lung macrophages [53]. Lymphatic drainage plays an important role in the uptake of particulates in the respiratory system. Assessment of inhaled radio-labeled SLN biodistribution has been described and the data showed an important and significant uptake of the radio-labeled SLN into the lymphatics after inhalation [54].

- **Topical Application:** Dermally applied lipid nanoparticles exhibit following features: Increased Hydration, Wrinkle Smoothing, Drug Release Modification, Modulation of Penetration, Protection of Chemically Labile Compounds, Pigment Effect, Adhesiveness, Occlusivity, Increased Efficiency of Molecular UV-Blockers and Reduced Side Effects [55]. They are well suited for use on damaged or inflamed skin because they are based on non-irritant and non-toxic lipids [56]. In most cases, the incorporation of the SLN dispersion in an ointment or gel is necessary in order to achieve a formulation which can be administered to the skin. During the last few years, SLN have been studied with active compounds such as vitamin E, tocopherol acetate, retinol, ascorbylpalmitate, clotrimazole, triptolide, phodphyllotoxin and a nonsteroidal anti androgen RU 58841 for topical application. Chemisches Laboratorium Dr. Kurt Richter (Germany) introduced a NCL formulation containing black currant seed oil for regenerative care of scaly and aged skin (NanoLipid RestoreTM) in the German market.

- **Ocular administration:** SLN showed an increased retention time in the eye. Biocompatibility and muco-adhesive properties of SLN improve their interaction with ocular mucosa and prolong corneal residence time of the drug, with the aim of ocular drug targeting. Cavalli et al studied pilocarpine delivery via SLN which is commonly used in glaucoma treatment, earlier [57]. They also evaluated SLN as carriers for ocular delivery of tobramycin in rabbit eyes [58]. In both cases SLN significantly enhanced the ocular bioavailability of drug. Another research group incorporated poorly water soluble drugs (hydrocortisone, estradiol hemihydrate and pilocarpine base) into SLN and performed in vitro drug permeation study through human organo typical cornea

construct [59]. They observed high loading capacity, because drugs were nearly completely incorporated within the nanoparticles due to their high lipophilic character. Consequently, permeation studies indicated prolonged drug release in all the formulations. In industrial fields, the incorporation of several antibiotics has been attempted in SLN, due to their broad antimicrobial spectrum. For an instance, OcusolinTM from AlphaRx is a gentamicin loaded-SLN product in the form of ophthalmic solution. It is still under preclinical development.

- **Rectal administration:** Plasma levels and therapeutic efficacy of rectally administered drugs were reported to be higher compared with those given orally or intramuscularly in the same dose Sznitowska *et al.,* incorporated diazepam into SLN for rectal administration in order to provide a rapid action [60]. They found that lipid matrix which is solid at body temperature is not an advantageous system for diazepam rectal delivery. They decided to employ lipids which melt around body temperature in their next experiments. This area seems very open to investigation, especially when the benefits of rectal route are taken into consideration. PEG coating seems to be a promising approach on rectal delivery and consequently, enhancement of bioavailability.

CONCLUSION: SLN constitute an attractive colloidal drug carrier system due to successful incorporation of active compounds and their related benefits. SLN offer an economical and patient-friendly device for administration of drugs by various routes. Coating of SLN with hydrophilic substances is very promising in the treatment of various diseases such as cancer and tuberculosis. They have good perspectives to be developed and marketed very successfully. Clear advantages of SLN include the composition (physiological compounds), the rapid and effective production process including the possibility of large scale production. The promising results of SLN prove their potential as versatile carrier systems for application in cosmetic and pharmaceutical formulations.

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