**Research Article**

# Solid Lipid Nanoparticles: A Critical Appraisal

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**ABSTRACT**

A new concept in nanotechnology is that of Solid lipid nanoparticles (SLNs), i.e. lipid nanocrystals in water possessing a solid core into which drugs are incorporated. The SLNs combine the virtues of more traditional drug carriers such as liposomes or polymeric nanoparticles while eliminating some of their disadvantages, e.g. the issues of burst release and long term stability in the case of liposomes as well as the problems of residual solvents and bulk production in the case of polymeric nanoparticles. The use of nanoparticles as drug-carrier system is a very attractive possibility to achieve controlled drug release. A clear advantage of SLNs over polymeric nanoparticles is the fact that the lipid matrix is made from physiological lipids, which decreases the danger of acute and chronic toxicity. There are various types of solid lipid nanoparticles and can be prepared using several methods. The present review covers all the aspects of solid lipid nanoparticles including their history, types, methods of preparations, evaluation parameters, advantages over conventional drug delivery systems and pharmaceutical applications which may be helpful in carrying out extensive research in their regards.

**Keywords:** Lipospheres, Micro emulsionsm novel drug delivery system, Nanospheres, Solid lipid nanoparticles.

## INTRODUCTION

Over the last twenty years, nanotechnology has affected practically quite all technical fields, as it has in pharmaceutics. Particular kinds of colloidal drug delivery systems that have been developed include Solid Lipid Nanoparticles (SLNs), as well as liposomes, dendrimers, and polymeric nanoparticles. SLNs were first prepared in the early 1990s by two different methods: the hot homogenisation technique and the warm microemulsions. The number of research groups working in the field slowly but continuously grew from that beginning and today they total some forty, mostly academics; during the same period the number of patents has also increased as preparation methods like the Solvent Emulsification Technique and the Solvent Diffusion Method have been filed.[1]

Main reasons for the therapy failure include:

1. Insufficient drug concentration due to poor absorption, rapid metabolism and elimination (e.g. peptides, proteins).

2. Drug distribution to other tissues combined with high drug toxicity (e.g. cancer drugs)

3. Poor drug solubility which excludes i.v. injection of aqueous drug solution

4. High fluctuation of plasma levels due to unpredictable bioavailability after peroral.

The Journal Paris Match described Professor Speiser in one of its articles as "the father of nanoparticles", in case of SLN we have "the parents", Prof. Maria Gasco and Prof. Rainer Müller. Credit should also be given to Professor Dr.

Kirsten Westesen, at this time with Braunschweig University, working also at the beginning of the 1990s on SLN production using high pressure homogenisation. The SLN generated worldwide a lot of interest, within the first years after the invention, and quite a number of research groups had started working with SLN as documented in the first SLN review from 1995.[2]

### Some peculiarities of SLNs

Excipients of SLNs are mostly lipids with relatively low melting points, some of which are present in foodstuffs and in the human body. For dermal and oral delivery, the excipients (lipids, surfactants) have already been accepted by the regulatory authorities for traditional dosage forms, such as creams, tablets, pellets and capsules. For parenteral administration, glycerides can be used, which are composed of fatty acids currently in use for parenteral nutrition. Solid lipid nanoparticles (SLNs), introduced in 1991, for combining the advantages but avoiding the disadvantages of other colloidal carriers have attracted increasing attention in recent years, and are regarded as an alternative carrier system to traditional colloidal systems, such as emulsions, liposomes and polymeric micro particles and nanoparticles. The predominant production technique till recently was the high-pressure homogenization (HPH) method. [3-11]The existence of different colloidal carrier systems raises the question as to which of them might be the most suitable for the desired purpose. Aspects to consider include:

1. Drug loading capacity

2. Possibility of drug targeting

3. *In vivo* fate of the carrier (interaction with the biological surrounding, degradation rate, accumulation in organs)

4. Acute and chronic toxicity

5. Scaling up of production

6. Physical and chemical storage stability

7. Overall costs

Figure 1 displays the most common morphologies that can be produced when forming SLNs. If the drug is miscible with the lipid, a solid solution can be formed, which is completely homogeneous. That is, more polar drugs would orient to the water phase to form a drug enriched shell; whereas more hydrophobic materials would move toward the core of the nanoparticle.
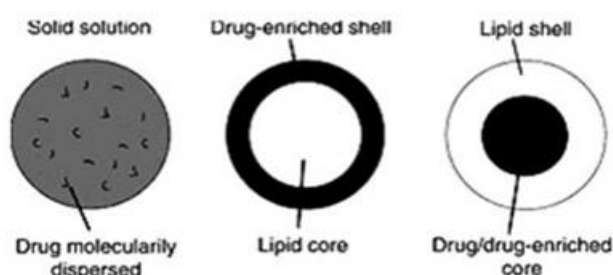


**Figure 1:** Drug incorporation models for solid lipid nanoparticles

### Historical Background and Production of Solid Lipid Nanoparticles

The first lipid particles were produced by the research group of Speiser, the father of the nanoparticles, in Zurich J. Paris Match). High-speed stirring was applied for the production of an o/w emulsion between a melted lipid phase and a hot aqueous surfactant solution. The obtained emulsion was then cooled and the inner lipid phase formed solid particles. The product developed by Speiser was called "lipid nanopellets" and was intended for oral delivery. Owing to the saturation of the organic solvent with water no solvent diffuses from the droplets into the external water phase from the o/w emulsion. Removal of solvent from the droplets and particle formation is achieved by adding additional water to the emulsion and extracting the solvent.[12-17]

### Solid Lipid Nanoparticle Size

To put the size of solid lipid nanoparticles in perspective, Table 2 sizes of various objects. Because of the comparable size of the components in the human cells, nanoparticles are of great interest in drug delivery.

### Solid Lipid Nanoparticle Surface

We can generally see and discern objects as small as 100,000 nm (100 mm). It is only in the past 300 years, with the invention of microscopes, that we can see smaller objects. Today, one can see objects as small as individual atoms (about 0.1 nm) using the scanning probe

microscope. Owing to their small size, nanoparticles exhibit interesting properties, making them suitable for a variety of drug delivery applications. The number of molecules present on a particle surface increases as the particle size decreases (Table 2). [18]

**Table 1:** Milestones in the field of Solid lipid nanoparticles manufacture

| Year | Milestone |
|---|---|
| **Nanoparticles formed by polymerization** | |
| 1976 | polyacylamide nanospheres |
| 1976 | polymethyl methacrylate nanospheres |
| 1979 | polyalkyl cyanoacrylate nanospheres |
| 1986 | polyalkyl cyanoacrylate nanocapsules |
| **Nanoparticles from performed polymers** | |
| 1972 | Albumin nanospheres by heat denaturation |
| 1978 | Gelatin nanospers by desolvation |
| 1979 | Albumin nanospheres by cross linking |
| 1981 | Poly Lactic acid nanospheres by |
| 1986 | emulsification solvent evaporation |
| 1988 | Poly Lactic acid nano capsules by interfacial Deposition |

**Table 2:** Typical size of various objects

| Object | Size (nm) |
|---|---|
| Carbon atom | 0.1 |
| DNA double helix (diameter) | 3 |
| Ribosome | 10 |
| Virus | 100 |
| Bacterium | 1,000 |
| RBC | 5,000 |
| Human hair(diameter) | 50,000 |
| Resolution of unaided human eyes | 100,000 |

**Table 3:** % Surface Molecules in particles

| Particle Size (nm) | Surface Molecule (%) |
|---|---|
| 1 | 100.00 |
| 10 | 27.10 |
| 100 | 2.97 |
| 1,000 | 0.30 |
| 10,000 | 0.03 |

### Morphology and Structure of Solid Lipid Nanoparticles

Different models have been described in the literature for how active molecules can be incorporated into SLNs. [103]

Therefore, three incorporation models have been proposed:

1. SLN Type I or homogeneous matrix model,

2. SLN Type II or drug-enriched shell model, and

3. SLN Type III or drug-enriched core model.

The SLN Type I or homogeneous matrix model is derived from a solid solution of lipid and active ingredient. [104]

The SLN Type II or drug-enriched shell model is achieved when SLNs are produced via the hot HPH technique, and the active ingredient concentration in the melted lipid is low.



**Figure 2:** Basic type of solid lipid nanoparticles Abbreviations: SLN, solid lipid nanoparticle

The SLN Type III or drug-enriched core model can take place when the active ingredient concentration in the lipid melt is high and at or relatively close to its saturation solubility.

### Aims of Solid Lipid Nanoparticles

It has been claimed that SLN combine the advantages and avoid the disadvantages of other colloidal carriers.[19] Proposed advantages include:

- Possibility of controlled drug release and drug targeting
- Increased drug stability
- High drug payload
- Incorporation of lipophilic and hydrophilic drugs feasible
- No bio toxicity of the carrier
- Avoidance of organic solvents
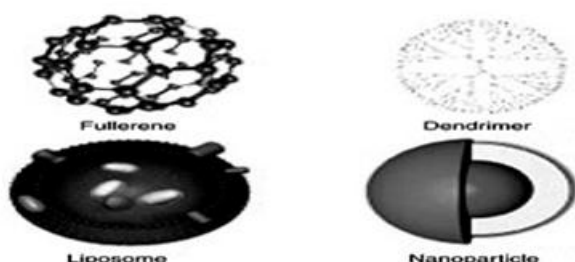- No problems with respect to large scale production and sterilization.



**Figure 3:** Different types of nano particulate structures

### Advantages of Solid Lipid Nano particulate Drug-Delivery Systems

The Solid lipid nanoparticles (SLNs) may offer some advantages such as protection of drugs against degradation, targeting the drugs to specific sites of action, organ or tissues, and delivery of biological molecules such as proteins, peptides, and oligonucleotides (Figure 3). A

number of different strategies have been proposed in order to modify the physicochemical characteristics of the NPs, and thus their interactions within the biological systems. [20-22]

### Physiology of Git with Relevance to Particulate Uptake

### Gastrointestinal Tract Physiology

The GIT serves to carry out the digestion of food and the absorption of water, nutrients, and electrolytes, and provides a selective barrier between the environment and the systemic circulation. Although the GIT is designed to prevent uptake of particulate matter (potentially toxic materials and pathogens) from the environ-ment, it is not a completely prohibitive barrier. Specialized mechanisms exist that allow internalization of macromolecules and particles. The mucus, built up from mucin molecules, covering the absorptive enterocytes in the intestines, acts as a barrier for oral absorption of foreign matter, including NPs. [23-24]

FAE, adapted for endocytosis/transcytosis of antigens and microorganisms to the organized lymphoid tissue within the mucosa and M cell basement membrane, appears to play an important role in facilitating antigen-to-cell and cell-to-cell interactions during an immune response. M cells lack fully developed microvilli in comparison to the neighbor- ing absorptive cells and deliver the particles taken up to the lymphatics from where they, in a size-dependent manner, are then released into the blood stream (Figure 3). [25-27]

### Channels of Uptake

To deliver their drug content in the blood, lymph, or target organs, SLNs have to cross the gastrointestinal barrier either by passive diffusion via transcellular or paracellular path- ways or by active processes mediated by membrane-bound carriers or membrane-derived vesicles (Figure 4). [28, 29]
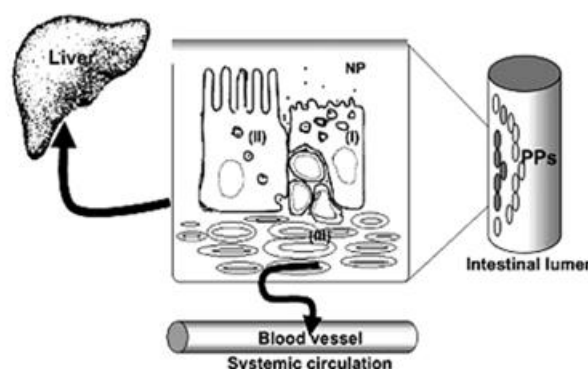


**Figure 4:** Uptake mechanism

### Lymphatic Uptake

Translocation of particulates via a transcellular mode of transport particularly across M cells was first shown in 1961 by Sanders and Ashworth who, using electron microscopy, reported the endocytosis of 200-nm latex particles in ordinary enterocytes that were transported to

the liver. Initially, only a small fraction of the total particle dose appears in the blood after oral administration, which may be due to entrapment of particles in Mcell pockets filled with lymphocytes and macrophages, non fenestrated capillary endothelium in PP and trapping in lymph nodes, which impedes direct access of particulates to circulation.

## SLN Production Procedures

### General ingredients

General ingredients include solid lipid(s), emulsifier (s) and water. The term lipid is used here in a broader sense and includes triglycerides (e.g. tri stearin), partial glycerides (e.g. Imwitor), fatty acids (e.g. stearic acid), Steroids (e.g. cholesterol) and waxes (e.g. cetyl palmitate). All classes of emulsifiers (with respect to charge and molecular weight) have been used to stabilize the lipid dispersion. It has been found that the combination of emulsifiers might prevent particle agglomeration more efficiently. An overview of ingredients which are commonly used is provided in Table 1. A clear advantage of SLN is the fact that the lipid matrix is made from physiological lipids which decreases the danger of acute and chronic toxicity. The choice of the emulsifiers depends on the administration route and is more limited for parenteral administrations.

### SLN Preparation

### High shear homogenization and ultrasound

High shear homogenization and ultrasound are dispersing techniques which were initially used for the production of solid lipid nano dispersions. Both methods are widespread and easy to handle. Furthermore, metal contamination has to be considered if ultrasound is used.

### SLN Preparation methods

The methods for preparing SLNs can be classified into seven categories:

A. High pressure homogenization

  a) Hot homogenization

  b) Cold homogenization

B. Solvent emulsification/evaporation

C. Microemulsion base

D. High shear homogenization and ultra sound

E. Membrane Emulsification Method

F. Multiple Emulsion Method

G. Solvent Injection Method

### High pressure homogenization

High pressure homogenization (HPH) has emerged as a reliable and powerful technique for the preparation of SLN. Homogenizers of different sizes are commercially available from several manufacturers at reasonable

prices. HPH has been used for years for the production of nano emulsions for parenteral nutrition.[72] Two general approaches of the homogenization step, the hot and the cold homogenization techniques, can be used for the production of SLN (Figure 1).[30-32]

### 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 pre-emulsion of the drug loaded lipid melt and the aqueous emulsifiers phase (same temperature) is obtained by high-shear mixing device (Ultra-Turrax)

### Schematic procedure for hot homogenization

### Problems/ Disadvantages of High Pressure Homogenization

1. Temperature induced drug degradation

2. Drug distribution into the aqueous phase during Homogenization.

3. Complexicity of the crystallization step of the nanoemulsion leading to several modifications and/or super cooled melts.

The primary product of the hot homogenization is a nanoemulsion due to the liquid state of the lipid. Solid particles are expected to be formed by the following cooling of the sample to room temperature or to temperatures below. Due to the small particle size and the presence of emulsifiers, lipid crystallization may be highly retarded and the sample may remain as a super cooled melt for several months.[33]

### Cold homogenization

Cold homogenization has been developed to overcome the following three problems of the hot homogenization technique:

1. Temperature-induced drug degradation

2. Drug distribution into the aqueous phase during homogenization

3. Complexity of the crystallization step of the nanoemulsion leading to Several modifications and/or super cooled melts

### SLN prepared by solvent emulsification/evaporation

Sjostrom and Bergenstahl described a production method to prepare nanoparticle dispersions by precipitation in o/w emulsions. The lipophilic material is dissolved in a water-immiscible organic solvent (e.g. cyclohexane) 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. The mean diameter of the obtained particles was 25 nm with cholesterol acetate as model drug and by using a lecithin/sodium glycocholate blend as emulsifier. The reproducibility of these results is confirmed by

Westesen.[131] The cholesterol acetate nanoparticles had a mean particle size of 29 nm (PCS number distribution) prepared according to Sjostrom with available equipment.[34]

## Microemulsion based SLN preparations

Gasco and co-workers developed SLN preparation techniques which are based on the dilution of microemulsions. It should be mentioned that there are different opinions in the scientific community about the

structure and dynamics of microemulsion. An extended review has recently been published by Moulik and Paul.[35,36]

## High shear homogenization and ultrasound

These are dispersing techniques which were initially used for solid lipid nano dispersion. Both methods are wide spread and easy to handle. Furthermore, metal contamination has to be considered if ultrasound is used.
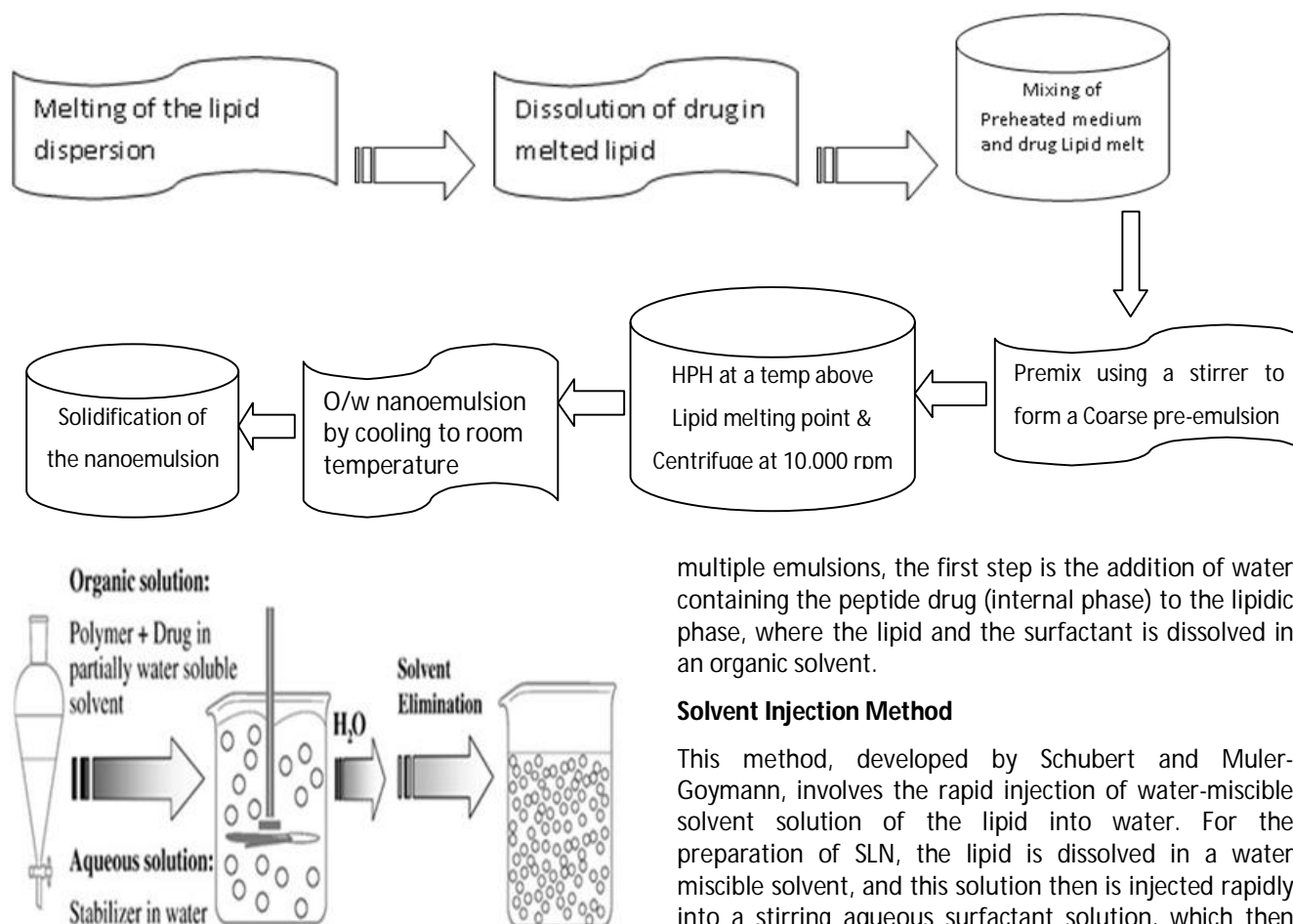




**Figure 6:** Schematic illustration of the solvent evaporation technique

## Membrane Emulsification Method

In this method, the drug substance, lipid and the lipophilic surfactant are dissolved in an organic solvent such as dicloromethane and concentrated to a constant weight under vaccum. A thin even lipid film is formed. Aqueous solution containing the hydrophilic surfactant is added to the lipid film, and the mixture is sonicated to produce the SLN system. Spherical SLN with high loading capacity can be obtained by this method.

## Multiple Emulsion Method

With the drawback of the high temperature that is required to disperse drugs to solid lipid particles, new technologies for thermolabile drug substances, for instance, proteins were developed. Among them is the multiple emulsion technique. For the preparation of

multiple emulsions, the first step is the addition of water containing the peptide drug (internal phase) to the lipidic phase, where the lipid and the surfactant is dissolved in an organic solvent.

## Solvent Injection Method

This method, developed by Schubert and Muler-Goymann, involves the rapid injection of water-miscible solvent solution of the lipid into water. For the preparation of SLN, the lipid is dissolved in a water miscible solvent, and this solution then is injected rapidly into a stirring aqueous surfactant solution, which then filtered. Almost all groups of lipids studied seem to be suitable for the preparation of SLN, and the addition of surfactant to the lipidic phase leads to the reduction in particle size.

## Comparison of different formulation procedures

Whenever possible, a direct comparison between the different formulation procedures should be made by the same investigator, using the same ingredients, the same storage conditions and the same equipment for particle sizing. Otherwise, impurities of the ingredients and differences in particle sizing technologies might lead to misleading results. Siekmann et al. 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.[37]

Ahlin and Kristl investigated the influence of the emulsifier concentration on the SLN particle size (production by rotor–stator homogenizer).[71] HPH and

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rotor–stator homogenizer can be attributed to different dispersing degrees of the liposomes which are obtained with both methods.

### Influence of ingredient composition on product quality

### 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. These results are in agreement to the general theory of HPH and can be explained by the higher viscosity of the dispersed phase. However, other critical parameters for nanoparticle formation will be different for different lipids. Examples include the velocity of lipid crystallization, the lipid hydrophilicity (influence on self-emulsifying properties and the shape of the lipid crystals (and therefore the surface area). For example, lipid nanodispersion made with cetyl palmitate from different suppliers had different particle sizes and storage stabilities (A. Lippacher, personal communication). Both a decrease of the homogenization efficiency and an increase in particle agglomeration cause this phenomenon which has been observed for lipid nanoemulsion, too.[38-40]

### Influence of the emulsifier

The choice of the emulsifiers and their concentration is of great impact on the quality of the SLN dispersion. Investigating the influence of the emulsifiers concentration on the particle size of ¨Compritol SLN dispersions, zur Muhlen obtained best results with 5 w% sodium cholate or poloxamer 188. 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 addition of sodium glycocholate to the aqueous phase as co-emulsifying agent decreases the particle size, too.[37, 38]

### Sterilization and secondary productions steps

### Sterilization

Parenteral administration requires sterile formulations. Aseptic production, filtration, g-irradiation and heating are normally used to achieve sterility. Filtration sterilization of dispersed systems requires high pressure and is not applicable to particles 0.2 mm. The sterilization should not change the properties of the formulation with respect to physical and chemical stability and the drug release kinetics.

Free radicals are formed during g-sterilization in all samples due to the high energy of the γ-rays. These radicals may recombine with no modifications of the sample or undergo secondary reactions which might lead to chemical modifications of the sample. It was also applied for liposomes. A possible concern is related to temperature-induced changes of the physical stability. Comparative studies on SLN sterilization by steam and γ-rays were conducted by Schwarz. Compared to lecithin stabilized systems, poloxamer stabilized SLN were found to be more unstable to steam sterilization. However, this difference was not detected for g-sterilized samples. Compared to steam sterilization at $121^0$C, the increase in particle size after γ-irradiation was lower, but comparable to steam sterilization at $110^0$C.[41-43]

### Lyophilization

Storage stability involves chemical and physical aspects and includes the prevention of degradation reactions (e.g. hydrolysis) and the preservation of the initial particle size. It requires that the SLN ingredients have sufficient chemical stability and that the particles have a very narrow size distribution to avoid crystal growth by Ostwald ripening. The SLN formulation should be resistant to temperature changes which will occur during shipping 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 protective effect of the surfactant can be compromised by lyophilization. It has been found, that the lipid content of the SLN dispersion should not exceed 5% to prevent an increase in particle size. 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.[44, 45]

Again, best results were obtained with samples of low lipid content and with the cryoprotector trehalose. Slow freezing in a deep freeze (-$70^0$C) was superior to rapid cooling in liquid nitrogen. Furthermore, introduction of an additional thermal treatment to the frozen SLN dispersion (2 h at -$22^0$C followed by a 2-h temperature decrease to -$40^0$C) was found to improve the quality of the lyophilizate.

### Spray drying

Spray drying might be an alternative procedure to lyophilization in order to transform an aqueous SLN dispersion into a dry product. This method has been used scarcely for SLN formulation, although spray drying is cheaper compared to lyophilization. Spray drying might potentially cause particle aggregation due to high temperatures, shear forces and partial melting of the particles.[46]

### Characterization of SLN Quality and Structure

### Defining the goals

An adequate characterization of the solid lipid nanodispersion is a necessity for the control of the quality of the product. The characterization methods should be sensitive to the key parameters of SLN performance and should avoid artifacts. One statement of Laggner about lipids should always be kept in mind: ''Lipids and fats, as soft condensed material in general, are very complex

systems, which not only in their static structures but also with respect to their kinetics of supra molecular formation. Hysteresis phenomena or super cooling can gravely complicate the task of defining the underlying structures and boundaries in a phase diagram.''

What are the most important key factors which have a direct impact on the stability and release kinetics? Several parameters have to be considered:

1. Particle size and zeta potential

2. Degree of crystallinity and lipid modifications

3. Coexistence of additional colloidal structures (micelles, liposomes, super cooled melts, drug nanoparticles) and time scale of distribution processes.

### Measurement of 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 that PCS is a good tool to characterize nanoparticles, but it is not able to detect larger micro particles. They can be visualized by means of LD measurements. This method is based on the dependence of the diffraction angle on the particle radius (Fraunhofer spectra). Smaller particles cause more intense scattering at high angles compared to the larger ones. It should be kept in mind that both methods do not 'measure' particle sizes. Rather, they detect light scattering effects which are used to calculate particle sizes. For example, uncertainties may result from non-spherical particle shapes. [47, 48]

Atomic force microscopy (AFM) is attracting increasing attention. For example, imaging of fibrinogen polymerization, the budding of a virus of an infected cell, the in vitro degradation of polymer surfaces and polymer nanoparticles were performed

Rapid progress in the development of field–flow-fractionation (FFF) has been observed during recent years. The separation principle of FFF is based on the different effect of a perpendicular applied field on particles in a laminar flow. 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). A combination of different FFF separation principles may give unique resolution. [49, 50]

### Measurement of crystallinity, lipid

Particle size analysis is a necessary, but not a sufficient step to characterize SLN quality. Special attention must be paid to the characterization of the degree of lipid crystallinity and the modifications of the lipid, because these parameters are strongly correlated with drug incorporation and release rates.

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. [174] Unfortunately, this source has limited accessibility for most investigators.

Infrared and Raman spectroscopy are useful tools for investigating structural properties of lipids.

Both nuclear magnetic resonance (NMR) and electron spin resonance (ESR) meet these requirements. They are powerful tools for investigating dynamic phenomena and the characteristics of nano compartments in colloidal lipid dispersions. Due to the non-invasiveness of both methods, repeated measurements of the same sample are possible.

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. [51]

### Possible problems in SLN preparation and SLN performance

SLN offer several advantages compared to other systems (easy scaling up, avoidance of organic solvents, high content nanoparticles). These advantages have been discussed in a variety of papers. However, less attention has been paid to the detailed and appropriate investigation of the limitations of this carrier system. Therefore, these aspects will be discussed in the following part of the article.

### High pressure-induced drug degradation

HPH has been shown to decrease the molecular weight of polymers. High shear stress has been assumed to be the major cause and evidence of free radical formation was reported. This study also indicated that cavitations is less important for the mechanism of polymer degradation. Cavitation can be suppressed by the application of back pressure without significant changes of the homogenization efficiency. However, HPH might be not suitable for the processing of shear sensitive compounds (DNA, albumin, and erythropoietin). [52]

## Lipid crystallization and drug incorporation

Lipid crystallization is an important point for the performance of the SLN carriers. The relation between lipid modifications and drug incorporation has been investigated for decades. The characterization of lipid modifications is well established. Methods are mainly based on X-ray and DSC measurements. The following four key aspects should be considered in the discussion of drug incorporation into SLN:

1. The existence of super cooled melts

2. The presence of several lipid modifications

3. The shape of lipid nanodispersion

4. Gelation phenomena

### Super cooled melts

Super cooled melts are not unusual in SLN systems. They describe the phenomenon that lipid crystallization may not occur although the sample is stored at a temperature below the melting point of the lipid. Super cooled melts are not lipid nanosuspensions but emulsions. Special attention should be paid to super cooled melts, because the potential advantages of SLN over nano emulsions are linked to the solid state of the lipid. The main reason for the formation of super cooled melts is the size dependence of crystallization processes.

### Lipid modifications

It is not sufficient to describe the physical state of the lipid as crystallized or non-crystallized, because the crystallized lipid may be present in several modifications of the crystal lattice. In general, lipid molecules have a higher mobility in thermodynamically unstable configurations. Therefore, these configurations have a lower density and ultimately, a higher capability to incorporate guest molecules (e.g. drugs). The advantage of higher incorporation rates in unstable modifications is paid off by an increased mobility of the drug. [53]

### Particle shape

The shape of lipid nanoparticles may significantly differ from a sphere. Lipids prefer to crystallize in the platelet form. Differences in shape between nanoemulsion (spheres) and SLN (platelets) have been observed by TEM. The shape of the lipid crystals is not only a theoretical matter of particle sizing (PCS and LD assume spherical shapes). Platelet shapes have much larger surface areas compared to spheres; therefore, higher amounts of surfactants are needed for stabilization. Particle sizes of 100 nm (measured by PCS or LD) translate into 20 lipid layers assuming if a spherical shape. [54,55]

### Gelation phenomena

Gelation phenomena describe the transformation of low-viscosity SLN dispersion into a viscous gel. This process may occur very rapidly and unpredictably. In most cases, gel formation is an irreversible process which involves the loss of the colloidal particle size

The surfactant molecules cannot longer provide sufficient coverage of the new surfaces and therefore, particle aggregation is observed. Gelation can be retarded or prevented by the addition of co-emulsifying surfactants with high mobility (e.g. glycocholate). [56]

### Coexistence of several colloidal species

The presence of several colloidal species is an important point to consider. Unfortunately, this aspect has been ignored in the majority of the SLN literature. Stabilizing agents are not localized exclusively on the lipid surface, but also in the aqueous phase. Therefore, micelle forming surfactant molecules (e.g. SDS) will be present in three different forms, namely: (i) on the lipid surface; (ii) as micelle; and (iii) as surfactant monomer. Lecithin will form liposomes, which have also been detected in nano emulsions for parenteral nutrition. Only the detection of the presence of several colloidal species is not sufficient to describe the structure of colloidal lipid dispersions, because dynamic phenomena are very important for drug stability and drug release. Therefore, the kinetics of distribution processes has to be considered. For example, the degradation of hydrolysable drugs will be faster for water solubilized and surface localized molecules compared to molecules in the lipid matrix. The kinetics of the degradation will be determined by: (i) the chemical reactivity of the drug; and

(ii) The concentration of the drug in the aqueous medium or at the lipid/water interface. These results indicates that the protection of the nitroxide was best in the liposome system. In both studies, nitroxide reduction took place a matter of minutes and not hours or days. An interesting finding of Ahlin was that the reduction rate decreases with storage time. This change represents most likely distribution phenomena of the nitroxide from the SLNs into the liposomes.

### Drug incorporation and drug release

A large number of drugs with a great variety of lipophilicity and the general structure have been studied with regard to their incorporation into SLNs, e.g. oxazepam, diazepam, cortisone, beta-methasone valerate, retinol, prednisolone, retinol, menadione, ubidecarenone, timolol, desoxycortisone, pilocarpine, progesterone, hydrocortisone, idarubicin, doxorubicin, thymopentin, [D-Trp-6]LHRH, gadolinium (III) complexes, 39-azido-39-deoxythymidine palmitate, azidothymidine palmitate, camptothecin, aciclovir, cyclosporine, vitamin E palmitate, etomidate, tetracaine. [214-219,221-229,134,184] The balance between steric stabilizers and other surfactants can possibly be used to modify the release and particle degradation, because lipases ¨ W. Mehnert, K. Mader / Advanced Dru need a lipid interface for activation of the enzyme (lid opening). Therefore, PEG-coated SLN are not easily recognized by these enzymes.

## Dose

The dose to be incorporated into a nano particulate system depends on the extent of particle uptake. This in turn depends on the particle size (smaller particles are taken up more readily and in greater proportions than the larger ones), surface hydro- phobicity/hydrophilicity (an optimum balance is required. Also, a major proportion of the NPs administered orally can be excreted without absorption, depending on the particle size and surface characteristics. For a given dose, an amount of particles taken up in excess of the expected figure will result in toxicity and the lower absorption will end up in failed therapy as a consequence of the sub therapeutic drug levels attained.

## Dosage Form

NPs are normally given orally to experimental animals in the form of suspension. An oral multiple-unit dosage form, which overcame many of the problems commonly observed during the compression of micro particles into tablets, was developed by Bodmeier et al. [58]

## Formulation Evaluation

## Degradation Studies

Degradation in NPs is indicative of their stability and the possible time period and kinetics of release of incorporated drug. The dose of the drug to be incorporated can be calculated by correlating the in vivo detectable levels of NPs with the degra- dation kinetics over a period of time. Thus, the effective delivery period of the drug from the NPs becomes dependent on the combined effect of polymer degradation and natural scaven- ging mechanisms of the body. It was suggested that the surface- associated PVA rather than the particle size plays a dominant role in controlling the degradation of NPs.

## Toxicity aspects and in vivo fate

One can anticipate that SLNs are well tolerated in living systems because they are made from physiological compounds and therefore, metabolic path ways exist. Of course, the toxicity of the emulsifiers has to be considered, but their potential toxicity is relevant for other carrier systems, too. Problems may arise, because SLNs may interfere with the pyrogen tests (limulus test) and cause gelation in any case.   Obviously, a certain amount of larger micro droplets is tolerated by the human body. This statement should not be transferred to SLNs, because a solid lipid is not deformable as oil, and therefore, in contrast to nanoemulsion, capillary blockage will occur if the particle size exceeds the size of the diameter of the blood vessel. [58]

The results of cytotoxicity studies (MTT test) indicated that SLNs are less toxic than polymeric nanoparticles. The experimental data of the toxicity studies on cells in vitro confirmed the expected low toxicity of the carrier.[58]

## Administration routes and in vivo fate

The in vivo fate of the SLN particles will depend mainly on the following points:

(a) Administration route

(b) Interactions of the SLNs with the biological surroundings including:

(b1) distribution processes (adsorption of biological material on the particle surface and desorption of SLN components into the biological surrounding).

(b2) enzymatic processes (e.g. lipid degradation by lipases and esterases)

SLN degradation are lipases, which are present in various organs and tissues. Lipases split the ester linkage and form partial glycerides or glycerol and free fatty acids. Most lipases require activation by an oil /water interface, which opens the catalytic center (lid opening).[253]

## Peroral administration

Peroral administration forms of SLNs may include aqueous dispersions or SLN loaded traditional dosage forms, e.g. tablets, pellets or capsules.

The microclimate of the stomach favors particle aggregation due to the acidity and high ionic strength. It can be expected, that food will have a large impact on SLN performance, and however, no experimental data have been published on this issue to our knowledge. The question concerning the influence of the stomach and pancreatic lipases on SLN degradation in vivo remains open, too.
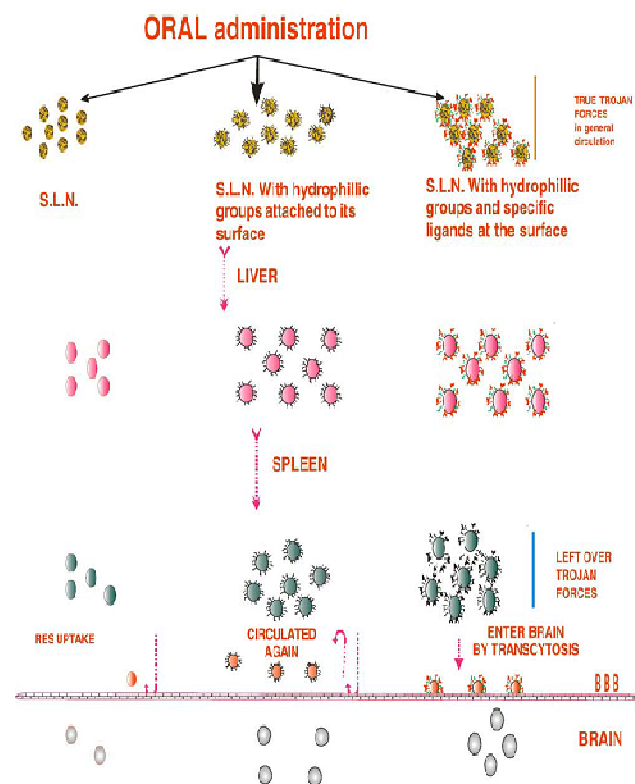


**Figure 7:** Fate of SLNs after oral administration

## Parenteral administration

SLNs have been administered intravenously to animals. Pharmacokinetic studies of doxorubicin incorporated into SLNs showed higher blood levels in comparison to a commercial drug solution after i.v. injection in rats. Concerning the body distribution, SLNs were found to cause higher drug concentrations in lung, spleen and brain, while the solution led to a distribution more into liver and kidneys.

The adsorption of a blood protein onto particle surfaces is supposed to be responsible for the uptake of SLNs by the brain by mediating the adherence to the endothelial cells of the blood–brain barrier.[60]

## Transdermal application

The smallest particle sizes are observed for SLN dispersions with low lipid content (up to 5%). Both the low concentration of the dispersed lipid and the low viscosity are disadvantageous for dermal ad- ministration. 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. The rheological properties are comparable to typical dermal formulations. The results indicate that it is possible to produce high concentrated lipid dispersions in the submicron size range in a one-step production. Therefore, further formulation steps (e.g. SLN dilution in cream or gel) can be avoided.

## Steps towards the pharmaceutical market

Over recent years, the first cosmetic products based on NCLs have appeared on the market. Their introduction into the lipid-based cosmetic industry may be considered as a step towards the pharmaceutical market[61]

Some of the most important requirements are:

• Regulatory acceptance of the excipients

• proven feasibility of large-scale production

• Sufficient drug loading to achieve therapeutic levels

## CONCLUSION

Many steps have been taken in these directions, but research must continue to provide ever better controls, improving efficacy, targeting, drug loading, lowering the drug dose (thus diminishing side effects and toxicity). Lipid nanoparticles based on optimised formulations should have a place among modern pharmaceutical products, since they have the capability of enhancing therapy. An innovative reformulation of a drug could extend its patent life. New delivery systems for old molecules, whether natural or out of patent, could lead to reduce side effects, achieving a more effective therapy. These perspectives could be socially very useful, in particular for elderly, often subjected to chronic drug uses.

## Summary and Outlook

Solid lipid nanoparticles do not, as proposed, ''combine the advantages of other colloidal drug carriers and avoid the disadvantages of them''. They cannot simply be regarded as nano emulsions with a solid core. Clear advantages of SLNs include the composition (physiological compounds), the rapid and effective production process including the possibility of large scale production, the avoidance of organic solvents and the possibility to produce high concentrated lipid suspensions. Disadvantages include low drug-loading capacities, the presence of alternative colloidal structures (micelles, liposomes, mixed micelles, drug nanocrystals), the complexity of the physical state of the lipid (transformation between different modifications, possibility of super cooled melts) which cause stability problems during storage or administration (gelation, particle size increase, drug expulsion). The appropriate characterization of the complex surfactant / lipid dispersions requires several analytical methods in addition to the determination of the particle size. Kinetic aspects have to be taken into account. Further work needs to be done to characterize SLNs on the molecular level. NMR, ESR and synchrotron irradiation will help to answer the question whether the drug is really incorporated in the solid lipid or whether lipid and drug nanosuspensions coexist in the sample. Un- fortunately, these aspects have not always been considered and the terminus 'drug incorporation' in the SLNs literature is often misleading. The lipid matrix of solid lipid nano dispersions is more mobile compared to polylactide–co-glycolide based nanoparticles and therefore, controlled release due to restricted diffusion of the drug within the lipid matrix is questionable because of the drug mobility and the short way length. Controlled release is, however, not impossible to achieve, but other factors will determine the release rate (e.g. kinetics to reach equilibrium of the drug between lipid and aqueous phase might be slow). Further work needs to be done to understand the interaction of SLNs with their biological surrounding (adsorption/desorption processes, enzymatic degradation, agglomeration, interaction with endogenous lipid carrier systems). Several administration routes are feasible for SLNs administration.

Further work needs to be done to understand the structure and dynamics of SLNs on a molecular level in vitro and in vivo.

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