

SOLID LIPID NANOPARTICLES: AN EFFECTIVE LIPID BASED TECHNOLOGY FOR POORLY WATER SOLUBLE DRUGS

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ABSTRACT

Solid lipid nanoparticles (SLNs) are the effective lipid based colloidal carriers which were introduced as an alternative to the conventional carriers such as microemulsions, liposomes, microparticles and nanoparticles based on synthetic polymers or natural macromolecules. Typically they enhance the oral bioavailability of the low aqueous soluble drugs due to their potential to enhance gastrointestinal solubilization and absorption via selective lymphatic uptake. These properties can be harvested to improve the therapeutic efficacy of the drugs with low bioavailability, as well as to reduce their effective dose requirement. This paper presents an overview about the choice of the drug candidates, advantages, methods of preparation such as high pressure homogenization, ultrasonication/high speed homogenization, solvent evaporation/emulsification, supercritical fluid method, microemulsion based method and spray drying method are discussed. Appropriate analytical techniques for characterization of solid lipid nanoparticles such as photon correlation spectroscopy, scanning electron microscopy, differential scanning calorimetry etc. are discussed. Applications with respect of routes of administration such as oral, parenteral, topical, pulmonary etc are elaborated in detail. References of the most relevant literature published by various research groups around the world are provided.

Keywords: solid lipid nanoparticles, colloidal carriers, bioavailability enhancement, homogenization.

INTRODUCTION

It is well known that the majority of the new chemical entities (more than 60% of drugs) coming directly from synthesis are poorly soluble. Consequently, many of these substances have bioavailability problems after oral administration (1, 2). Frequent approaches to enhance solubility and subsequently oral absorption are the use of cyclodextrin (3, 4) microemulsions such as cyclosporine A (CycA)- loaded microemulsions used as commercial product (5, 6), microparticles and nanoparticles based on synthetic polymers or natural macromolecules. Despite the excellent tolerability of these carrier systems, the number of products on the market is relatively low due to problems such as: (i) the size of drugs that need to be fit in the cyclodextrin rings, (ii) limited physical stability, (iii) large scale production method yielding a product of a quality accepted by the regulatory authorities, (iv) presence of solvent residues left over from production, (v) the cytotoxicity of the polymers (7- 13).

Lipid based drug delivery systems are introduced to overcome the limitations associated with traditional formulations. These systems offer large variety of options such as solutions, suspensions, emulsions, microemulsions, self-emulsifying drug delivery systems (SEDDS), dry emulsions and solid lipid nanoparticles (14). It is also possible to form blends that are composed of several excipients: they can be pure triglyceride (TG) oils or blends of different TG, diglyceride (DG) and monoglyceride (MG). In addition, different types of surfactants (lipophilic and hydrophilic) can be added (15).

Solid lipid nanoparticles (SLNs) are considered to be the most effective lipid based colloidal carriers (fig 1), introduced in early nineties (16, 17). This is the one of the most popular approaches to improve the oral bioavailability of the poorly water soluble drugs. SLNs are in the range of submicron size (50-1000 nm) and are composed of physiologically tolerated lipid components which are in solid state at room temperature. The schematic representation of different particulate drug carriers such as emulsions and liposomes and their advantages are compared with SLNs in Fig. 2. SLNs combine all the advantages of polymeric nanoparticles, fat emulsions and liposomes (Table 1) (18- 21).

Figure 1: Solid lipid nanoparticle containing lipophilic drug in lipid core comparing with emulsion

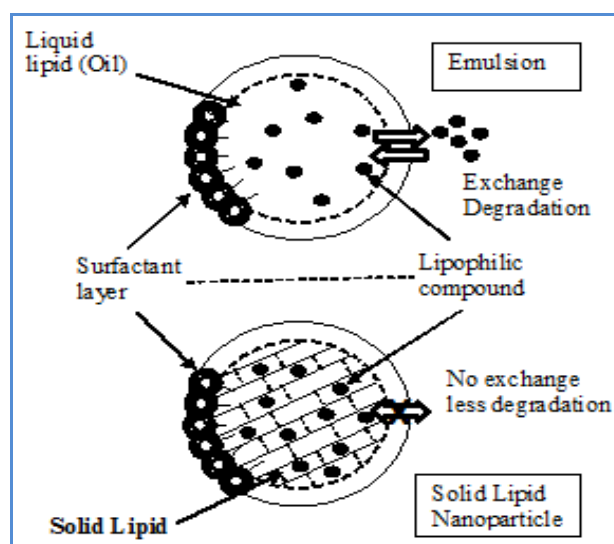


Table 1: Advantages of solid lipid nanoparticles:
➤ Better control over release kinetics of encapsulated compounds
• Engineering via size and lipid composition
• Melting serve as trigger
➤ Enhanced bioavailability of entrapped bioactive compounds
➤ Chemical protection of labile incorporated compounds
➤ Much easier to manufacture than biopolymeric nanoparticles
• No special solvent required
• Wider range of lipids
• Conventional emulsion manufacturing methods applicable
➤ Raw materials essential the same as in emulsions
➤ Very high long-term stability
➤ Application versatility:
• Can be subjected to commercial sterilization procedures
• Can be freeze dried to form powdered formulation

candidates for the SLNs (Table 2). Based on this classification, chemical compounds are divided in four classes where in class II compounds have high solubility and low permeability whereas class IV compounds have low solubility and low permeability. Hence compounds from class II and class IV are most likely the suitable candidates of choice for preparing solid lipid nanoparticles (22, 23).

Table 2: Biopharmaceutical Classification System (BCS) and Potential advantages of Lipid based systems²⁰

		← Membrane permeability	
↑ Aqueous Solubility	BCS Class I	BCS Class III	
	• Enzymatic degradation	• Enzymatic degradation	
	• Gut wall efflux	• Gut wall efflux	
		• Bioavailability	
	BCS Class II	BCS Class IV	
	• Solubilization	• Enzymatic degradation	
	• Bioavailability	• Gut wall efflux	
		• Bioavailability	

CHOICE OF DRUG CANDIDATES

Biopharmaceutical Classification System (BCS) can serve as a useful preliminary guide for the selection of the

Figure 2: Schematic representation of different particulate systems along with their advantages

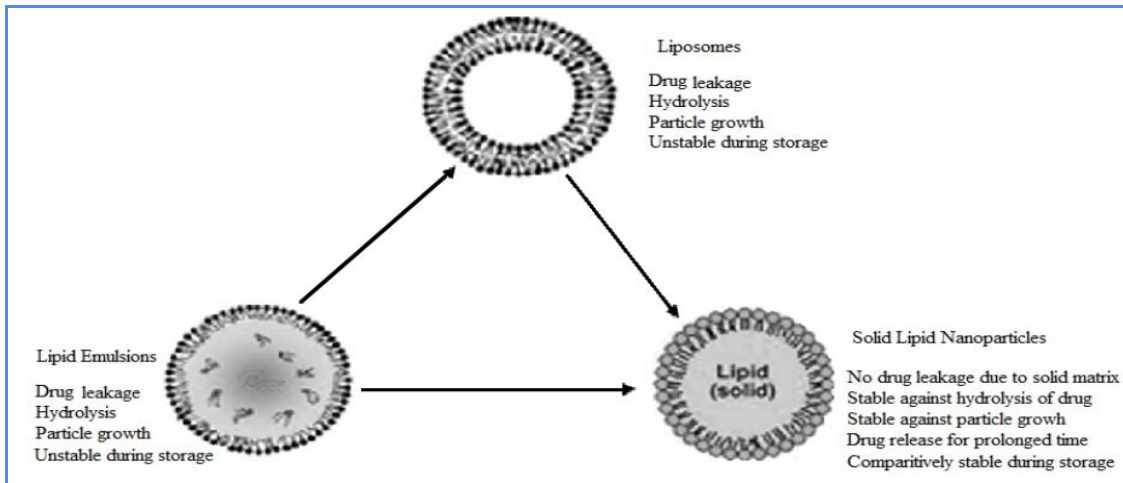


Figure 3: Various mechanisms of enhancement of drug bioavailability in the presence of lipids

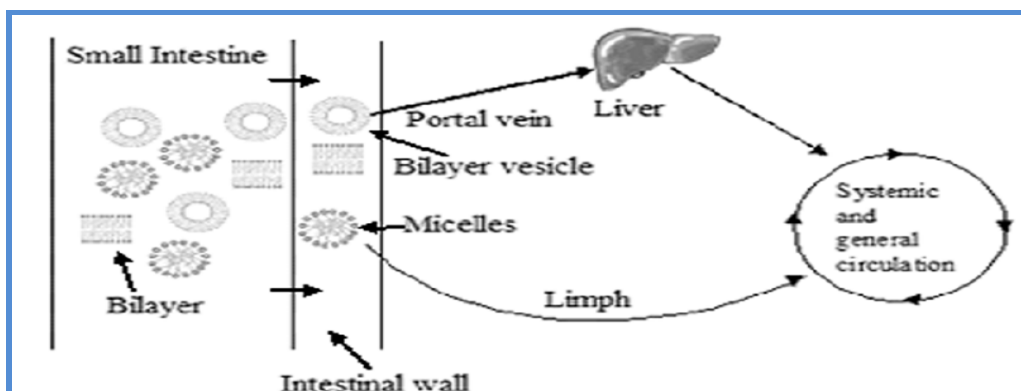


Figure 4: Solid lipid nanoparticles preparation by hot homogenization process

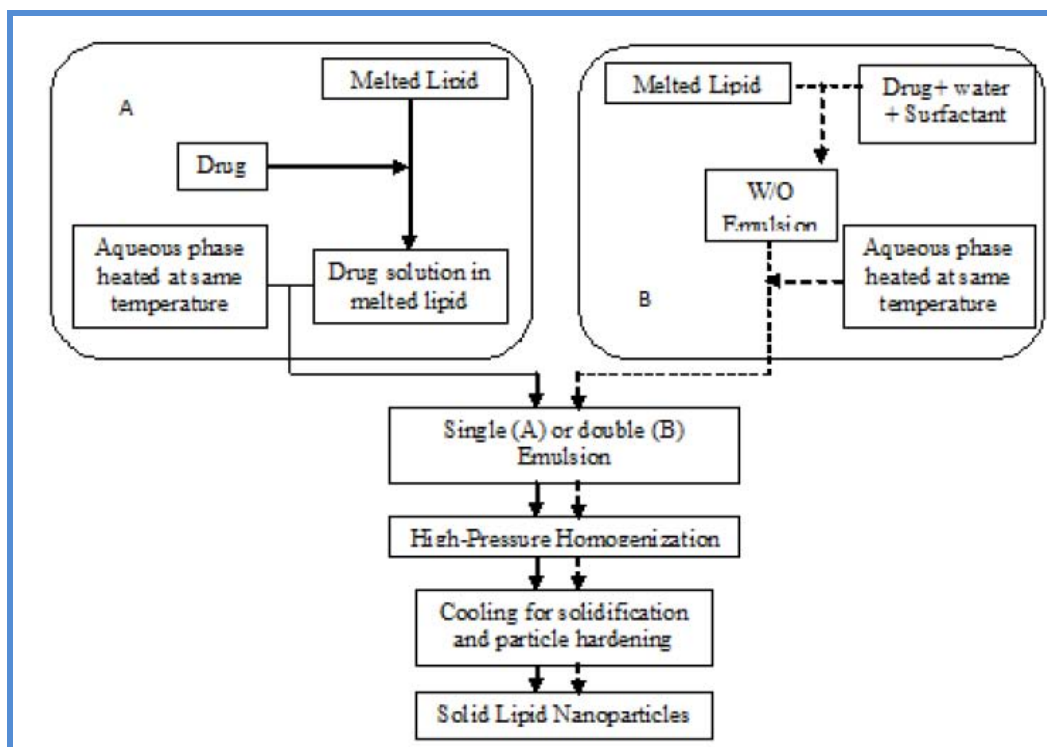


Table 3: Lipids and emulsifiers used for preparation of solid lipid nanoparticles

Lipids	Hard fats	Emulsifiers/Coemulsifiers
Non- Digestible lipids	Witepsol W 35	Soy lecithin
Mineral oils	Witepsol S 35	Egg lecithin
Sucrose polyesters	Witepsol H 42	Phosphatidylcholine
Digestible lipids	Witepsol E 85	Poloxamer 188
Triglycerides	Glyceryl mono stearate (Imwitor 900)	Poloxamer 182
Tricaprin	Glyceryl behenate (Compritol 888 ATO)	Poloxamer 407
Trilaurin	Glyceryl palmitostearate (Precirol ATO 5)	Poloxamine 908
Trimyristin	Cetyl palmitate	Tyloxapol
Triopalmitin	Stearic acid	Polysorbate 20
Tristearin	Palmitic acid	Polysorbate 60
Hydrogenated coco-glycerides (Softisan)	Decanoic acid	Polysorbate 80
Diglycerides	Behenic acid	Sodium cholate
Fatty acids	Acidan N12	Sodium glycocholate

ENHANCEMENT IN ORAL ABSORPTION

Few mechanisms are described in enhancing the oral bioavailability of drug molecules by SLNs:

Dissolution/solubilization:

SLNs entering into the GI tract, stimulates the gallbladder contractions and biliary and pancreatic secretions, including bile salts (BS), phospholipids (PL) and cholesterol, due to the lipids presence in the formulation (24, 25). These products, along with the gastric shear movement, form a crude emulsion which promotes the solubilization of the coadministered lipophilic drug (26,

27). Moreover, the esters are rapidly hydrolyzed in the presence of pancreatic lipase, and the lipolytic products upon interaction with BS/PL form different micellar species that prevent the co-administered lipophilic drug precipitation. Further, the surface active agents present in the SLNs may further stimulate the solubilization of the lipophilic compound.

Stimulation of lymphatic transport:

Bioavailability of lipophilic drugs could also be enhanced by the stimulation of the intestinal lymphatic transport pathway.



Gastric residence time:

Lipids in the GI tract provoke delay in gastric emptying which results in increased residence time of the co-administered lipophilic drug in the small intestine. This enables better dissolution of the drug at the absorptive site, and thereby improves absorption (28).

Affecting intestinal permeability:

A variety of lipids have been shown to change the physical barrier function of the gut wall and hence, enhance the permeability (29).

Reduced metabolism and efflux activity:

Certain lipids and surfactants have been shown to reduce the activity of efflux transporters in the GI wall, and hence, to increase the fraction of drug absorbed (30, 31).

Prevent first pass metabolism:

Solid lipid nanoparticles have been reported to enhance oral bioavailability of certain highly lipophilic drugs by accessing to systemic circulation via lymphatic route hence preventing their first pass metabolism (32). Solid lipid nanoparticles can be given by pulmonary and parenteral routes which is also an important factor to prevent hepatic first pass metabolism of certain drugs.

LIPIDS AND EMULSIFIERS FOR SOLID LIPID NANOPARTICLES

The drug absorption capability from the prepared SLNs is mainly depends upon the types of lipids. Various types of lipids have been used by the scientists for the preparation of solid lipid nanoparticles (Table 3). Non-digestible lipids include mineral oils, sucrose polyesters, which can't absorbed from the gut lumen, tend to retain the lipophilic drugs within the oil, and thus, may limit the absorption of the drug (33, 34). Digestible lipids, including triglycerides (TG), diglycerides (DG), phospholipids (PL), fatty acids (FA), cholesterol and other synthetic derivatives, are suitable oils for drug delivery systems of lipophilic compounds. These lipids are usually defined according to their carbon chain length, i.e., long chain triglyceride (LCT) or medium chain triglyceride (MCT), lipid class, i.e., TG, DG, MG or FA, degree of saturation and their interaction with water. For successful oral absorption enhancement, the lipid based delivery system needs to maximize the rate and extent of drug dissolution and maintain the drug in solution during its transit throughout the GI tract. Hence, methods for tracking the solubilization state of the drug after the dispersion of different lipid based delivery systems in the GI tract are highly needed.

Hoffman, A. and Dahan, A. explained the theory behind the bioavailability enhancement by using the solid lipid nanoparticles (33). According to them after oral administration, the lipidic component is subjected to enzymatic hydrolysis. Salivary glands secrete lingual lipase together with gastric lipase, secreted from the gastric mucosa. These secretions are playing an important role in the hydrolysis of triglycerides (TG) in the stomach and

leads to formation of diglycerides (DG) and fatty acids (FA). These diglycerides and fatty acids during passing through the pyloric sphincter in to duodenum and also along with the shear movement of the stomach cause the formation of crude emulsion (35). Lipids facilitate the secretion of bile salts (BS), biliary lipids (phospholipid (PL) and cholesterol ester) and pancreatic fluids into the duodenum. These agents absorb to the oil/water interface and produce a more stabilized emulsion with reduced droplet size. The enzymatic hydrolysis is completed by the action of pancreatic lipase, which upon complexation with co-lipase acts at the surface of the emulsified TG droplets to produce the corresponding 2-MG and two FA (24). Upon interaction with the endogenous BS and PL, these amphiphilic lipid digestion products form colloidal structures holding different levels of surface activity, which enables the solubilization of the co-administered poorly water soluble compound, and prevents their precipitation in the aqueous GIT milieu. In most cases, this process, which maintains the poorly water soluble drug in solution and prevents its precipitation, is thought to be the primary mechanism by which lipid based drug delivery systems augment the oral absorption of lipophilic drugs.

UPTAKE OF SOLID LIPID NANOPARTICLES

The majority of orally administered drugs gain access to the systemic circulation by absorption into the portal blood. However, some extremely lipophilic drugs ($\log P > 5$, solubility in TG > 50 mg/ml) gain access to the systemic circulation via lymphatic route, which avoids hepatic first-pass metabolism. Therefore, highly metabolized lipophilic drugs may be potential candidates for solid lipid nanoparticles, a lipid based delivery. Compounds showing increased bioavailability in the presence of lipids (dietary or lipid-based formulation) are absorbed via the intestinal lymph as they are generally transported in association with the long-chain TGs lipid core of intestinal lipoproteins formed in the enterocyte after re-esterification of free FAs and MGs. Short-chain TGs are primarily absorbed directly in the portal blood. Hence it is likely that the drug transport via the lymphatics requires coadministration of lipid to stimulate lipoprotein formation (32, 36- 40).

The lymph fluid is emptied (average 3 L per day) via thoracic duct into the subclavian vein, thus protecting the drug from hepatic first-pass metabolism (41- 45). The lymphatic system, being the principal systemic transport pathway for B and T lymphocytes as well as the primary route of metastatic spread of a number of solid tumours and several viruses (46, 47), is a potential drug delivery target for immunomodulatory, anticancer compounds and other related drugs (48- 59). The drug being transported in the circulatory system, in the form of either micelles or mixed micelles, may then be available in its free form, since upon dilution with a large volume of the lymph/blood, surfactant concentration may reduce below its cmc value and micelle may dissociate into monomers (60). The drug transported as lipid vesicles may



remain intact for extended periods and, thereby, can result in prolonged release of the encapsulated drug (61). Fig. 3 represents is the diagrammatic presentation of the various mechanisms by which solid lipid nanoparticles enhance the bioavailability of drugs such as: colloidal micelles formation, selective lymphatic uptake which reduces first-pass drug metabolism as intestinal lymph travels directly to the systemic circulation

PREPARATION OF SOLID LIPID NANOPARTICLES

SLNs are prepared from lipid, emulsifier and water/solvent by using different methods (Table 4) and are discussed below.

Table 4: Methods of Preparation of Solid Lipid Nanoparticles
• High pressure homogenization
○ Hot homogenization
○ Cold homogenization
• Ultrasonication/high speed homogenization
○ Probe ultrasonication
○ Bath ultrasonication
• Solvent evaporation/ emulsification
• Supercritical fluid method
• Microemulsion based method
• Spray drying method

High pressure homogenization (HPH):

It is a reliable and powerful technique, which is used for the production of SLNs. High pressure homogenizers push a liquid with high pressure (100–2000 bar) through a narrow gap (in the range of a few microns). The fluid accelerates on a very short distance to very high velocity (over 1000 km/h). Very high shear stress and cavitation forces disrupt the particles down to the submicron range. Generally 5-10% lipid content is used but up to 40% lipid content has also been investigated (62, 63).

Two general approaches of HPH are hot homogenization and cold homogenization; work on the same concept of mixing the drug in bulk of lipid melt (64- 66).

Hot homogenization:

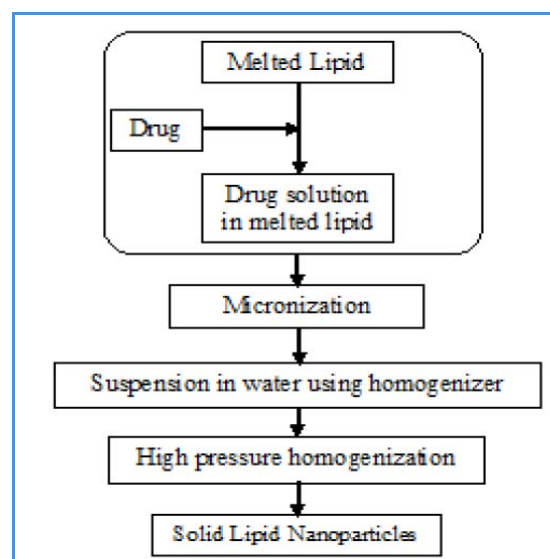
Hot homogenization is carried out at temperatures above the melting point of the lipid and can therefore be regarded as the homogenization of an emulsion. A pre-emulsion of the drug loaded lipid melt and the aqueous emulsifier phase (same temperature) is obtained by high-shear mixing device. HPH of the pre-emulsion is carried out at temperatures above the melting point of the lipid. In general, higher temperatures result in lower particle sizes due to the decreased viscosity of the inner phase (67). However, high temperatures increase the degradation rate of the drug and the carrier. Further, one should remember that the high pressure homogenization increases the temperature of the sample (approximately 108°C for 500 bar) (68). Increasing the homogenization pressure or the number of cycles often results in an

increase of the particle size due to high kinetic energy of the particles (fig. 4) (69).

Cold homogenization:

Cold homogenization has been developed to overcome various problems associated with hot homogenization such as: Temperature-induced drug degradation, drug distribution into the aqueous phase during homogenization, Complexity of the crystallization step of the nanoemulsion leading to several modifications and/or supercooled melts (62). In this technique the drug containing lipid melt is cooled, the solid lipid ground to lipid microparticles and these lipid microparticles are dispersed in a cold surfactant solution yielding a pre-suspension. Then this pre-suspension is homogenized at or below room temperature, the cavitation force is strong enough to break the lipid microparticles directly to solid lipid nanoparticles (Fig. 5).

Figure 5: Solid lipid nanoparticles preparation by cold homogenization process.



Ultrasonication/high speed homogenization:

SLNs are also prepared by ultrasonication or high speed homogenization techniques (70- 72). For smaller particle size combination of both ultrasonication and high speed homogenization is required. Potential metal contamination and physical instability like particle growth upon storage are the major drawbacks of this technique.

Solvent evaporation/ emulsification:

SLNs are also prepared by solvent evaporation method. Sjostrom and Bergenstahl described a production method to prepare nanoparticle dispersions by precipitation in o/w emulsions (73). 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, nanoparticles dispersion is formed by precipitation of the lipid in the aqueous medium by giving the nanoparticles of 25 nm mean size. Siekmann and Westesen also prepared solid lipid nanoparticles of 30 to 100 nm by dissolving tripalmitin in chloroform. This

solution was emulsified in an aqueous phase by high pressure homogenization. The organic solvent was removed from the emulsion by evaporation under reduced pressure (40–60 mbar) (74).

Supercritical fluid method:

This is an alternative method of preparing SLNs by particles from gas saturated solutions (PGSS). This technique has several advantages (75- 76) such as (i) avoid the use of solvents; (ii) Particles are obtained as a dry powder, instead of suspensions, (iii) mild pressure and temperature conditions. Carbon dioxide solution is the good choice as a solvent for this method (77).

Microemulsion based method:

This method is based on the dilution of microemulsions (78). As 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 65-70°C which is typically composed of a low melting fatty acid (e.g. stearic acid), an emulsifier (e.g. polysorbate 20, polysorbate 60, soy phosphatidylcholine and taurodeoxycholic acid sodium salt), co-emulsifiers (e.g. butanol, sodium monoctylphosphate) and water. The hot microemulsion is dispersed in cold water (2-3°C) under stirring (79- 80). Excipients like butanol are less favorable with respect to regulatory aspects. From the technical point of view precipitation of the lipid particles in water is the dilution of the system that leads to reduction of solid content of SLN dispersion. For some technological operations it is desirable to have a high lipid solid content, e.g. 30%. The SLN dispersion can be used as granulation fluid for transferring in to solid product (tablets, pellets) by granulation process, but in case of low particle content too much water needs to be removed (81). 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 (82, 83). Due to the dilution step; achievable lipid contents are considerably lower compared with the HPH based formulations.

Spray drying method:

It is an alternative technique to the liophilization. Freitas and Mullera recommended the use of lipid with melting point more than 70° C by using this method. The best results were obtained with SLN concentration of 1% in a solution of trehalose in water or 20% trehalose in ethanol-water mixture (84).

CHARACTERIZATION OF SLNS

Characterization of solid lipid nanoparticles is a serious challenge due to the small size of the particles and complexity of the system. Various parameters need to be considered as (62):

Particle size and zeta potential:

Particle size may be determined by photon correlation spectroscopy (PCS), transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM), scanning tunneling microscopy (STM), freeze fracture electron microscopy (FFEM) and laser diffraction (LD). The coulter counter method is not preferred due to the difficulties in the assessment of small nanoparticles and the need for electrolytes which may destabilize colloidal dispersions (81).

PCS works on the principle of measurement of fluctuation of the light intensity, cause by particle movement. It is a good tool for particle size measurement as it covers a size range from few nanometers to 3 µm but unable to cover larger microparticles. This can be overcome by using laser diffraction (LD) which based on the dependency of the diffraction angle on the particle radius. 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. It should be kept in mind that both methods are not measuring particle sizes; rather they detect light scattering effects which are used to calculate particle sizes. Difficulties may arise both in PCS and LD measurements for samples which contain several populations of different size. Therefore additional techniques may also be used. For example light microscopy is used. SEM and TEM provide a way to directly observe nanoparticles, physical characterization of nanoparticles with the former method being better for morphological examination (62). TEM has a smaller size limit of detection, a good validation for other methods, and affords structural required and one must be cognizant of the statistically small sample size and the effect that vacuum can have on the particles. However, the investigator should pay special attention to possible artifacts which may be caused by the sample preparation. For example, solvent removal may cause modifications which will influence the particle shape (85). Atomic force microscopy (AFM) is attracting 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.

The measurement of the zeta potential allows for predictions about the storage stability of colloidal dispersion (86). In general, particle aggregation is less likely to occur for charged particles (high zeta potential) due to electric repulsion. However, this rule cannot strictly be applied for systems which contain steric stabilizers, because the adsorption of steric stabilizers will decrease the zeta potential due to the shift in the shear plane of the particle (62).



Degree of lipid crystallinity and lipid modification:

These parameters are strongly correlated with drug incorporation and release rates so special attention must be paid to the characterization of the degree of lipid crystallinity and the modification of lipid. Due to the small size of the particles and the presence of emulsifiers, lipid crystallization and modification changes might be highly retarded.

Lipid status is mainly investigated by Differential scanning calorimetry (DSC) and X-ray scattering techniques. 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 spacings of the lipid lattice. Solvent removal will lead to modification changes so it is highly recommended to measure the SLN dispersions themselves. Structural properties of lipids are also investigated by Infrared and Raman spectroscopy techniques (87).

Co-existence of additional colloidal structures and dynamic phenomena:

The coexistence of additional colloidal structures (micelles, liposomes, mixed micelles, supercooled melts and drug nanoparticles) has to be taken into account for all SLN dispersions. The characterization and quantification are a serious challenge due to the similarities in size combined with the low resolution of PCS to detect multimodal distributions. Furthermore the sample preparation will modify the equilibrium of the complex colloidal system. Crystallization or changes of the lipid modification may be induced due to dilution of the original SLN dispersion with water which might cause the removal of surfactant molecules from the particle surface. Therefore, it would be highly desirable to use methods which are sensitive to the simultaneous detection of different colloidal species and which do not require preparatory steps. The magnetic resonance techniques, NMR and ESR, are powerful tools to investigate dynamic phenomena and the characteristics of nanoparticles in colloidal lipid dispersions. Repeated measurements of the same sample are possible due to the non-invasiveness of both methods.

NMR active nuclei of interest are ^1H , ^{13}C , ^{19}F and ^{31}P . Due to the different chemical shifts it is possible to attribute the NMR signals to particular molecules. For example; lipid methyl protons give signals at 0.9 ppm while protons of the poly ethylene glycol chains give signals at 3.7 ppm. Simple ^1H -NMR spectroscopy permits an easy and rapid detection of supercooled melts due to the low line widths of the lipid protons (88, 89). 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. It also allows for the characterization of liquid nanocompartments in recently developed lipid particles, which are made from blends of

solid and liquid lipids (90). The great potential of NMR with its variety of different approaches (solid-state NMR, determination of self-diffusion coefficients etc.) has scarcely been used in the SLN field, although it will provide unique insights into the structure and dynamics of SLN dispersions.

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 in to the aqueous phase. Furthermore, using an ascorbic acid reduction assay it is possible to monitor the time scale of the exchange between the aqueous and the lipid phase (91). The development of low-frequency ESR permits non-invasive measurements on small mammals. ESR spectroscopy and imaging will give new insights about the fate of SLN in vivo.

APPLICATIONS OF SOLID LIPID NANOPARTICLES

Oral delivery:

Oral administration of SLNs is possible as aqueous dispersion or after transforming in to dosage form i.e. tablets, pellets, capsules or powder in sachets (8, 13). For the production of the 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 tableting powder mixture. For the production of pellets the SLN dispersion can be used as wetting agent in the extrusion process (92). SLN powder can be used for the filling of hard gelatin capsules; or the SLN can be produced directly in liquid PEG 600 and filled in to soft gelatin capsules. Sachets are also possible using spray dried or lyophilized powders. In both cases it is beneficial to have a higher solid content to avoid the necessity of having to remove too much water. For cost reasons spray drying might be the preferred method for transferring SLN dispersions in to powder form (7).

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.

Camptothecin (CA)-containing SLN were produced from stearic acid (2%), lecithin (1.5%) and poloxamer 188 (0.5%). The encapsulation efficiency of CA was determined 99.6%. The zeta potential (245 mV) was remarkably high for poloxamer stabilized dispersions. The plasma levels and body distribution were determined



after administration of CA–SLN suspension versus a CA solution (CA-SOL). Two plasma peaks were observed after administration of CA–SLN. The first peak was attributed to the presence of free drug; the second peak can be attributed to controlled release or potential gut uptake of SLN. These two peaks were also found in the total CA concentration–time profiles of all measured organs. It was also found that the incorporation into SLN protected CA from hydrolysis (93).

Peribedil (PD), whose aqueous solubility is low and elimination half life is short, was incorporated in to lipid matrix to give prolonged release and high oral bioavailability. Therefore preventing known side effects and high frequency of oral administration.

Parenteral delivery:

SLNs can be administered intravenously, intramuscularly, subcutaneously or to the target organ, because of their small size. The particles are cleared from the circulation by the liver and the spleen. SLN formulations can be used for systemic body distribution with a minimized risk of blood clotting and aggregation leads to embolism. SLNs formulations also provide a sustained release depot of the drug when administered subcutaneously or intramuscularly (13, 94).

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 (95).

Camptothecin (CA)-loaded SLN were produced by HPH. The SLNs employed were composed of stearic acid, soy lecithin and poloxamer 188. The distribution of CA in the body was studied following intravenous administration showed increased uptake in some organ especially in brain (8, 96).

Paclitaxel containing solid lipid nanoparticles (SLNs) were prepared and studied in culture of macrophage (97- 100). They were loaded with paclitaxel and assessed in-vivo. The i.v. administered SLN led to higher and prolonged plasma levels of paclitaxel. Allow uptake by the liver and spleen macrophages and an increased uptake in the brain were observed (8).

Vaccine adjuvant:

Adjuvants have shown their applications in vaccines to enhance the immune response. Aluminum hydroxide particles, emulsion systems like SAF 1 and MF 59, Freund's complete adjuvant (FCA), Freund's incomplete adjuvant (FIA) are frequently used adjuvants; however they exhibit side effects or rapidly degrade in the body. The lipid components of SLN will degrade more slowly due to their solid state thus providing a longer lasting exposure to the immune system. Degradation of SLN can

be slowed down even more when using stearyl stabilizing surfactants that hinder the anchoring of enzyme complexes (8).

SLN have been tested as a vaccine adjuvant in hens. Hens were vaccinated under the addition of SLN and the egg yolk concentrations of IgY were determined. The adjuvant effect was compared to FCA/FIA and to the vaccine without adjuvant. The SLN induced characteristics changes of the chronological titer development. This is an indication of an adjuvant effect. The tissue tolerability was very good. Antibody titers were enhanced only slightly (101).

Transfection agent:

Cationic SLNs for gene transfer are formulated using the same cationic lipid as for liposomal transfection agents. The differences and similarities in the structure and performance between SLN and liposomes were investigated. A SLN preparation, its counterpart formulation without matrix lipid, a commercially available liposomal preparation – all based on the cationic lipid, 1, 2- Dioleoyl-snglycero- 3- trimethylammoniumpropane (DOTAP) and a liposomal formulation that additionally contained the helper lipid dioleoylphosphatidylethanolamine (DOPE) (Escort™) were compared. PCS showed that the prepared SLNs were smaller in diameter than the corresponding liposomes while AFM supported the expected structural differences. DNA binding differed only marginally. Cationic lipid composition governs the in vitro transfection performance than the colloidal structure it is arranged in. Hence, cationic SLN extends the range of highly potent nonviral transfection agents by one with favorable and distinct technological properties (102). Combination of cationic SLN with the nuclear localization signal TAT2 increased transfection efficiency hundredfold (100).

Topical delivery:

Topical applications of lipid nanoparticles have been used with promising results either for therapeutic or cosmetic purposes. SLN have shown some protective activity on skin surface, such as a UV-blocking potential (103). SLN may be formulated in creams, gels, sprays. 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 administration. 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 incorporation step implies a further reduction of the lipid content. An increase of the solid lipid content of the SLN dispersion results in semisolid, gel-like systems, which might be acceptable for direct application on the skin. Unfortunately, in most cases, the increase in lipid content is connected with a large increase of the particle size. Surprisingly it has been found that very high concentrated (30–40%), semisolid cetyl palmitate formulation



preserves the colloidal particle size. A dramatic increase of the elastic properties was observed with increasing lipid content. 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 (62).

Pulmonary delivery:

Unique features that can facilitate systemic delivery via pulmonary administration of drugs are large surface area, good vascularisation, large capacity for solute exchange, ultra-thinness of the alveolar epithelium (0.1-0.5 mm) and first-pass metabolism is avoided. To demonstrate the suitability in principle of SLN for pulmonary delivery, aqueous SLN dispersions were nebulized with a Pari-Boy, the aerosol droplets were collected and the size of SLN analyzed. It could be shown that the particle size distributions of SLN before nebulization and after nebulization were almost identical, only very little aggregation could be detected which is of no significance for pulmonary administration (81). Alternatively the SLN powders might be used in dry powder inhalers. SLN could be spray-dried using, e.g. lactose as excipient in the spray-drying process.

Controlled release of perfumes and repellents:

SLNs are used to incorporate perfumes (103). The perfume Allure was incorporated in SLN and the release studied was compared to a nanoemulsion of identical lipid content and surfactant composition. The initial release was similar; this could be due to the presence of perfume in the outer shell of the SLN. The release of the perfume from SLN was delayed further to 8 h. This opens the prospect of developing long lasting perfume formulations based on the prolonged release of the perfume from lipid matrix (104).

Prolonged release is also desired for insect repellents while simultaneously the releasing carrier systems should firmly adhere to the skin. SLN as carrier systems are therefore preferred for incorporating insect repellents. The insect repellent DEET (N, N- diethyltoluamide) was incorporated in different SLN formulations. A loading of 10% stearic acid SLN stabilized with Tween 80 as surfactant. The particles were observed to be physically stable for a long term after incorporation in a ready to use gel (105).

SUMMARY AND OUTLOOK

Lipid carriers have bright future due to their inherent property to enhance the bioavailability of lipophilic drugs with poor aqueous solubility. These are the types of carriers which not only combines the advantages of other colloidal systems but also avoid the disadvantages of them. Clear advantages of SLN include the presence of physiologically tolerable lipids, the rapid and effective production processes including the possibility of large scale production, avoidance of organic solvents and the

possibility to produce high concentrated lipid suspensions. Lipophilic drugs are the potential candidates for solid lipid nanoparticles as these gain access to the systemic circulation via lymphatic route, which avoids hepatic first-pass metabolism. These compounds showing increased bioavailability in the presence of lipids (dietary or lipid-based formulation) are absorbed via the intestinal lymph and avoid the absorption in portal blood supply. The lymph fluid is emptied via thoracic duct into the subclavian vein, thus protecting the drug from hepatic first-pass metabolism and leads to bioavailability enhancement. The appropriate characterization of the complex surfactant/lipid dispersions requires several analytical methods. NMR, ESR and synchrotron irradiation will help in answer the question whether the drug is really incorporated in the solid lipid or whether lipid and drug nanosuspensions coexist in the sample.

Certainly, works need to be carried out to understand the interaction of SLN with their biological surrounding (absorption/desorption processes, enzymatic degradation, agglomeration, interaction with endogenous lipid carrier systems). several routes of administration are feasible for SLN administration. The most challenging route will be i.v. injection which requires absolute control of the particle size. The results obtained with the dermal application are encouraging and probably this will be the main application of the SLN.

In summary, solid lipid nanoparticles offer a promising delivery system for the enhancement of the bioavailability of poorly soluble drugs and are suitable for large-scale production but there is a need of further work to be done to understand the structure and dynamics of SLN on a molecular level in vitro and in vivo.

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