CHAPTER II
LITERATURE REVIEWS

1. Solid lipid nanoparticles (SLN)

In the middle of the 1990s, the attention of different research groups has focused on alternative nanoparticles made from solid lipids, the so-called solid lipid nanoparticles (SLN or lipospheres or nanospheres). The SLN combine the advantages of other innovative carrier systems (e.g. physical stability, protection of incorporated labile drugs from degradation, controlled release, excellent tolerability) while at the same time minimizing the associated problems. SLN formulations for various application routes (parenteral, oral, dermal, ocular. pulmonary, rectal) have been developed and thoroughly characterized *in vitro* and *in vivo*. A first product has recently been introduced to the Polish market (Nanobase, Yamanouchi) as a topically applied moisturizer (Wissing et al, 2004).

The definitions and structural features

SLN are particles with a solid lipid matrix with an average diameter in the nanometer range. In addition to lipid and drug, the particle dispersions contain surfactants as stabilizers. All excipients are GRAS substances or have an accepted GRAS status, therefore, a wide variety of substances can be used for formulating purpose.

SLN are particles made from solid lipids (i.e. lipids solid at room temperature and also a body temperature) and stabilized by surfactant(s). By definition, the lipids can be highly purified triglycerides, complex glyceride mixtures or even waxes. Through the work of various research groups, the carrier system SLN has been characterized intensively.

The main features of SLN with regard to parenteral application are the excellent physical stability, protection of incorporated labile drugs from degradation, controlled drug release (fast or sustained) depending on the incorporation model, good

tolerability and site-specific targeting. Potential disadvantages such as insufficient loading capacity, drug expulsion after polymorphic transition during storage and relatively high water content of the dispersions (70-99.9%) have been observed (Wissing et al., 2004).

Solid lipids have been used for several years in the form of pellets in order to achieve a retarded drug release after peroral administration (e.g. Mucosolvan® Retard Capsules). It has been claimed that SLN combine the advantages and avoid disadvantages of polymeric nanoparticles and lipid emulsions. SLN possesses obvious advantages which is superior to other colloidal carriers. Their benefits are (i) Possibility of controlled drug release and drug targeting (ii) Increased drug stability (iii) High drug payload (iv) Incorporation of lipophilic and hydrophilic drugs feasible (v) No biotoxicity of the carrier (vi) Avoidance of organic solvents (vii) No problems with respect to large scale production and sterilization (Mehnert and Mäder, 2001).

General ingredients for SLN production

General ingredients include solid lipid(s), emulsifier(s) and water. The term lipid is used in a broader sense and includes triglycerides, partial glycerides, fatty acids, steroids and waxes. All classes of emulsifiers have been used to stabilize the lipid dispersion. It has been found that the combination of emulsifiers might prevent particle agglomeration more efficiently (Mehnert and Mäder 2001). An overview of ingredients which are commonly used is provided in Table 1 and 2.

SLN preparation

1. High shear homogenization and ultrasound

High shear homogenization and ultrasound are dispersing techniques which were initially used for the production of solid lipid nanodispersions. Both methods are widespread and easy to handle. However, dispersion quality is often compromised by the presence of microparticles (Mehnert and Mäder, 2001).

Table 1 Lipids used for preparation of solid lipid nanoparticles

Lipids	References
Triglycerides	
Trilaurin Trimyristin Tripalmitin Tristearin	(Westesen, Siekmann and Koch 1993; Domb 1995; Westesen and Bunjes 1995; Bunjes Westesen and Koch 1996; Siekmann and Westesen 1996; Westesen and Siekmann 1997; Westesen, Bunjes and Koch 1997)
Hard fat types	
Witepsol®W 35 Witepsol®H 35 Witepsol®H 42 Witepsol®E 85	(Westesen, Siekmann and Koch 1993; Westesen, Bunjes and Koch 1997; Almeida, Runge and Müller 1997)
Partial glycerides	
Glyceryl monostearate Glyceryl behenate Glyceryl palmitostearate	(Müller et al., 1996; Schwarz and Mehnert 1997; Freitas and Müller 1998b; 1999; zur Mühlen, Schwarz and Mehnert 1998; Cavalli et al., 1999)
Waxes	
Cetyl palmitate	(Müller, Rühl and Runge 1996; Freitas and Müller 1998a)
Fatty acids	
Stearic acid Behenic acid	(Morel, Gasco and Cavalli 1994; Morel et al., 1996; Cavalli et al., 1997; 1999; Yang et al., 1999)

Table 2 Stabilizers used for preparation of solid lipid nanoparticles

Stabilizers	References
Natural stabilizers	
Soybean lecithin Egg lecithin	(Westesen, Siekmann and Koch 1993; Westesen and Bunjes 1995; Bunjes, Westesen and Koch 1996; Siekmann and Westesen 1996; Westesen and Siekmann 1997; Westesen, Bunjes and Koch 1997; Schwarz and Mehnert 1997; zur Mühlen, Schwarz and Mehnert 1998; Cavalli et al., 1999)
Synthetic stabilizers	
Poloxamer 188 Poloxamer 182 Poloxamer 407 Poloxamine 908	(Westesen, Siekmann and Koch, 1993; Siekmann and Westesen 1996; Müller et al., 1996; Müller, Rühl and Runge 1996; Freitas and Müller 1998b; Almeida, Runge and Müller 1997; Schwarz and Mehnert 1997; zur Mühlen, Schwarz and Mehnert 1998; Yang et al., 1999)
Polysorbate 20 Polysorbate 60 Polysorbate 80	(Cavalli et al., 1996; Almeida, Runge and Müller 1997)
Sodium cholate Sodium glycocholate Taurocholic acid sodium salt Butanol Butyric acid Dioctyl sodium sulfosuccinate	(Morel, Gasco and Cavalli 1994; Westesen and Bunjes 1995; Morel, et al., 1996; Müller, Rühl and Runge 1996; Bunjes, Westesen and Koch 1996; Siekmann and Westesen 1996; Almeida, Runge and Müller 1997; Westesen and Siekmann 1997)

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 ultrasonication (Wissing et al, 2004).

Higher stirring rates did not significantly change the particle size, but slightly improved the polydispersity index. No general rule can be derived from differences in the established optimum emulsification and cooling conditions. In most cases, average particle sizes in the range of 100-200 nm were obtained in this method (Mehnert and Mäder, 2001).

2. 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 nanoemulsions for parenteral nutrition. In contrast to other techniques, scaling up represents no problem in most cases. 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 accelerated 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 submicron range. Typical lipid contents are in the range 5-10% and represent no problem to the homogenizer. Even higher lipid concentrations (up to 40%) have been homogenized to lipid nanodispersions. (Mehnert and Mäder, 2001)

The two basic production methods for SLN are the hot homogenization technique and the cold homogenization technique (Figure 1). For both techniques the drug is dissolved or solubilized in the lipid being melt at approximately 5-10°C above its melting point. For the hot homogenization technique the drug-containing melt is

dispersed under stirring in a hot aqueous surfactant solution of identical temperature. Then the obtained pre-emulsion is homogenized using a piston-gap homogenizer, the produced hot o/w nanoemulsion is cooled down to room temperature, the lipid recrystallizes and leads to solid lipid nanoparticles.

The hot homogenization technique is also suitable for drugs showing some temperature sensitivity because the exposure to an increased temperature is relatively short. In case of highly temperature sensitive compounds the cold homogenization technique can be applied. It is also necessary to use this technique when formulating hydrophilic drugs because they would partition between the melted lipid and the water phase during the hot homogenization process. For the cold homogenization technique the drug-containing lipid melt is cooled, the solid lipid ground to lipid microparticles (approximately 50-100 μm) 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 (Müller, 2000a). In general, compared to hot homogenization, larger particle sizes and a broader size distribution are observed in cold homogenized samples. The method of cold homogenization minimizes the thermal exposure of the sample, but it does not avoid it due to the melting of the lipid/ drug mixture in the initial step (Mehnert and Mäder, 2001).

The influence of homogenizer type, applied pressure, homogenization cycles and temperature on particle size distribution has been studied extensively. 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) (Wissing et al, 2004).

3. Solvent emulsification/evaporation

Different academic groups have attempted the production of SLN via precipitation. In the solvent emulsification-evaporation, the lipid is dissolved in a water-immiscible organic solvent (e.g. toluene, chloroform) which is then emulsified in an aqueous phase before evaporation of the solvent under reduced pressure. Upon

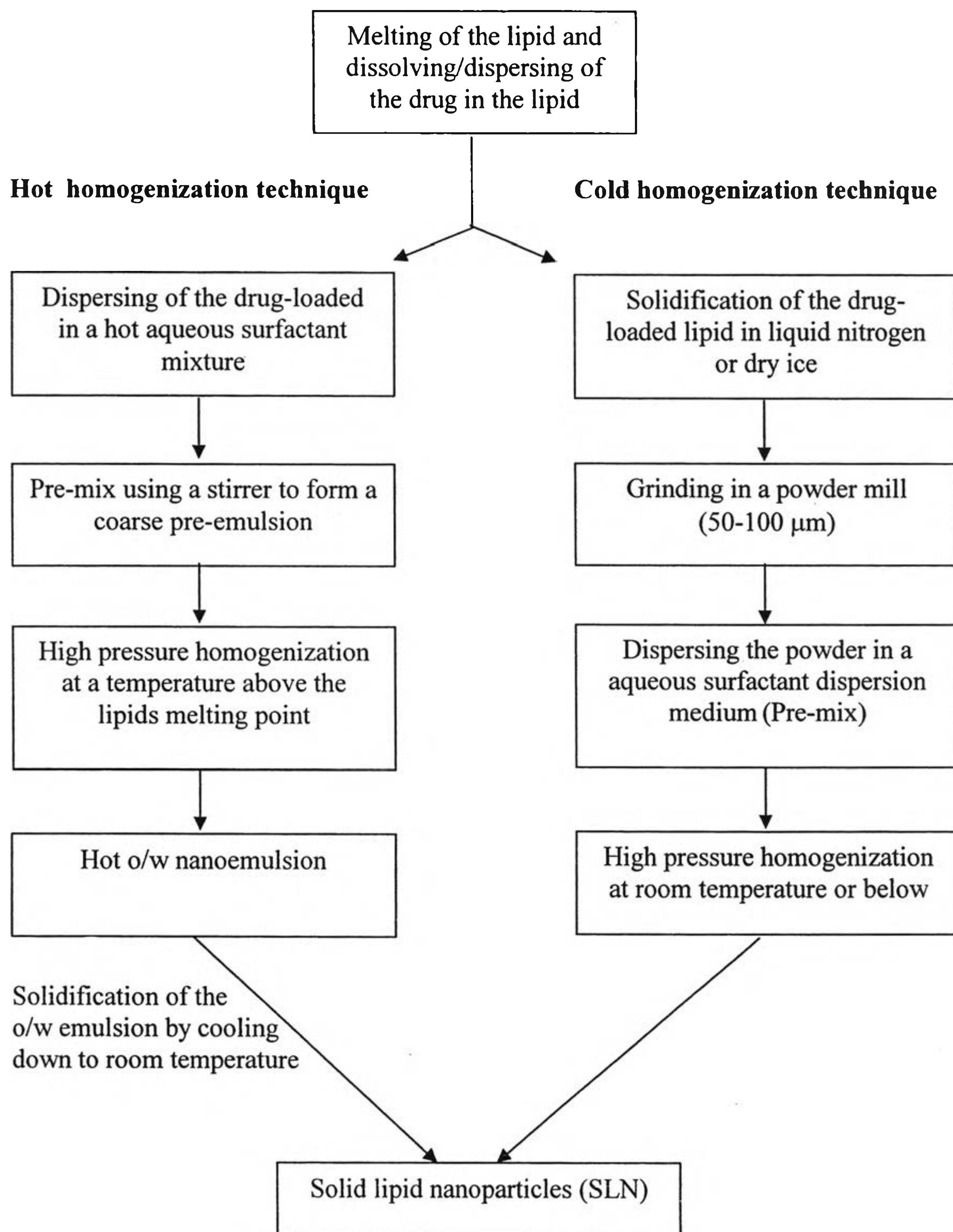

Figure 1 Schematic procedure of hot and cold homogenization techniques for SLN production

evaporation of the solvent, the lipid precipitates forming SLN. An important advantage of this method is the avoidance of heat during the preparation, which makes it suitable for the incorporation of highly thermolabile drugs. Problems might arise due to solvent residues as 20-100 ppm in final dispersions. Also, these dispersions are generally dilute, because of the limited solubility of lipid in the organic material. Typically, lipid concentrations in the final SLN dispersion range around 0.1 g/L, therefore, the particle concentration has to be increased by means of, e.g. ultra-filtration or evaporation (Wissing et al., 2004).

Comparable small particle size distributions are not achievable by melt emulsification of similar composition. The mean particle size depends on the concentration of the lipid in the organic phase. Very small particles could only be obtained with low fat loads (5% w/w) related to the organic solvent. With increasing lipid content the efficiency of the homogenization declines due to the higher viscosity of the dispersed phase (Mehnert and Mäder, 2001)

4. Microemulsion based SLN preparations

Gasco and co-workers developed SLN preparation techniques which are based on the dilution of microemulsions (Gasco 1993; Cavalli et al., 1995; 1997; 2000; 2003). To form a microemulsion with a lipid being solid at room temperature, the microemulsion needs to be produced at a temperature above the melting point of the lipid. The lipids (fatty acids and/or glycerides) are melted. A mixture of water, co-surfactant(s) and the surfactant is heated to the same temperature as the lipid and added under mild stirring to the lipid melt. A transparent, thermodynamically stable system is formed when the compounds are mixed in the correct ratio for microemulsion formation. This microemulsion is then dispersed in a cold aqueous medium (2-3°C) under mild mechanical mixing, thus ensuring that the small size of the particles is due to the precipitation and hot mechanically induced by a stirring process. Surfactants and co-surfactants include lecithin, biliar salts, but also alcohols such as butanol (Müller, 2000).

Experimental factors such as microemulsion composition, dispersing device, temperature and lyophilization on size and structure of the obtained SLN have been studied intensively. Due to the dilution step, achievable lipid contents are considerably lower compared with the HPH based formulations (Mehnert and Mäder, 2001). It has to be remarked critically that the removal of excess water from the prepared SLN dispersion is a difficult task with regard to the particle size. Also, high concentrations of surfactants and co-surfactants are necessary for formulation purposes, however less desirable with respect to regulatory purposes and application (Wissing et al., 2004).

Incoporation of drugs

An innovative and successful carrier system should allow a high loading capacity for incorporated drugs as well as long-term incorporation. Table 3 lists examples relevant for the parenteral application including the corresponding references (Wissing et al., 2004).

Models for incorporation of active compounds into SLN

There are basically three different models for the incorporation of active ingredients into SLN (Müller et al., 2002).

(I) Solid solution model
(II) Drug-enriched shell model
(III) Drug-enriched core model

The structure obtained is a function of the formulation composition (lipid, active compound, surfactant) and of the production conditions.

A homogeneous matrix with molecularily dispersed drug or drug being present in amorphous clusters is thought to be mainly obtained when applying the cold homogenization method and when incorporating very lipophilic drugs in SLN with the hot homogenization method. In the cold homogenization method, the bulk lipid contains the dissolved drug in molecularily dispersed form, mechanical breaking

Table 3 Examples of drugs relevant for parenteral application incorporated into SLN

Drug	References
AZT-P and derivatives	(Heiati et al., 1997; 1998a; 1998b)
Camptothecin	(Yang et al., 1999; Yang and Zhu 2002)
Clobetasol propionate	(Hu et al., 2002)
Cortisone	(Westesen, Bunjes and Koch 1997)
Cyclosporin A	(Ugazio, Cavalli and Gasco 2002)
Diazepam	(Westesen, Bunjes and Koch 1997; Sznitowska et al., 2001; Viriyaroj 2001)
Doxorubicin	(Cavalli, Caputo and Gasco 1993; Zara et al., 1999; Fundaro et al., 2000; Miglietta et al., 2000)
Etomidate	(zur Mühlen, Schwarz and Mehnert 1998)
Dioctanoyl-5-fluoro-2'-deoxuridine	(Wang, Sun and Zhang 2002)
Hydrocortisone	(Cavalli et al., 1999)
Idarubicin	(Cavalli, Caputo and Gasco 1993)
[D-Trp-6]LHRH	(Morel, Gasco and Cavalli 1994)
Mifepristone	(Hou et al., 2003)
Paclitaxel	(Cavalli, Schwarz and Gasco 2000; Miglietta et al., 2000; Chen et al., 2001)
Prednisolone	(Westesen, Bunjes and Koch 1997; zur Mühlen, Schwarz and Mehnert 1998)
Progesterone	(Cavalli et al., 1999)
Retinoic acid	(Lim and Kim 2002)
Sodium cromoglycate	(Cortesi et al., 2002)
Tetracaine	(zur Mühlen, Schwarz and Mehnert 1998)
Thymopentin	(Morel et al., 1996)
Tobramycin	(Cavalli et al., 2000)

by high pressure homogenization leads to nanoparticles having the homogeneous matrix structure (Figure 2 left). The same will happen when the oil droplet produced by the hot homogenization method is being cooled, crystallizes and no phase separation between lipid and drug occurs during this cooling process. This model is assumed to be valid for incorporation of, e.g. the drug prednisolone, which can show release from 1 day up to weeks.

An outer shell enriched with active compound can be obtained (Figure 2 middle) when phase separation occurred during the cooling process from the liquid oil droplet to the formation of a solid lipid nanoparticle. According to the diagram, the lipid can precipitate first forming a practically compound-free lipid core. At the same time, the concentration of active compound in the remaining liquid lipid increases continuously during the forming process of the lipid core. Finally, the compound-enriched shell crystallizes comparable to the eutecticum in the diagram. This model is assumed, for example, for coenzyme Q10 which the enrichment leads to a very fast release.

A core enriched with active compound can be formed when the opposite occurs, which means the active compound starts precipitating first and the shell will have distinctly less drug (Figure 2 right). This leads to a membrane controlled release governed by the Fick law of diffusion.

The three models presented each represent the ideal type. Of course, there can also be mixed types which can be considered as a fourth model.

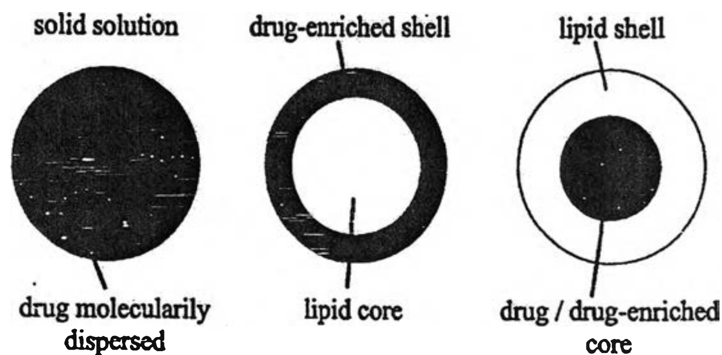

Figure 2 Models of incorporation of active compounds into SLN: solid solution model (left), core-shell models with drug-enriched shell (middle) and drug-enriched core with lipid shell (right).

Sterility and secondary production steps.

Sterility

Parenteral administration requires sterile formulations. Aseptic production, filtration, γ-irridation and heating are normally used to achieve sterility. Filtration sterilization of dispersed systems requires high pressure and is not applicable to particles > 0.2 μm. 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 γ-sterilization in all samples due to the high energy of the γ-rays. These radicals may recombine with no modification of the sample or undergo secondary reactions which might lead to chemical modifications of the sample. The degree of degradation depends on the general chemical reactivity. Furthermore, high molecular mobility (semisolid or liquid state) and the presence of oxygen favor γ-sterilization-induced degradation reactions (Mehnert and Mäder, 2001).

Autoclaving at 121°C can not be performed when using sterically stabilizing polymers, e.g. poloxamer series. 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°C to 110°C, but simultaneously prolonging the autoclaving time). Cavalli et al (1997) reported SLN prepared from oil-in-water microemulsion, whose internal phase was constituted of different lipid matrices could be sterilized by autoclaving. They were stable and maintained a spherical shape and narrow size distribution as confirmed by TEM analysis.

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. This technology is well known from parenteral emulsions and easy to apply to SLN. Alternatively, the SLN can produce aseptically, again identical to parenteral emulsions that already established techniques in the pharmaceutical industry (Müller, 2000).

These dispersions are intended for the investigations of tolerability of SLN on the cellular level and to provide dispersions for animal testing not requiring a terminal sterilization process. This is a great importance because some nanoparticle dispersions or the drug itself do not tolerate a terminal sterilization process, e.g. autoclaving or γ-irradiation is disliked by pharmaceutical companies due to the regulatory problems involved. Therefore a general aseptic process resulting in a sterile product is of general interest. Zimmermann et al (1999) prepared SLN dispersions consisted of Compritol and Poloxamer 188 underneath a laminar air flow cabinet (LAF). The LAF unit itself was placed into a clean room. They concluded that aseptic production of aqueous SLN dispersions is feasible when modifying accordingly the production equipment. The homogenization process itself proved to be an efficient sterilization process.

Secondary production steps

Lyophilization

Freeze drying, also known as lyophilization, is widely used for pharmaceuticals to improve the stability and long term storage stability of labile drugs. SLN dispersions can be freeze-dried to obtain dry products which can easily be stored and reconstituted before use by addition of an aqueous medium (Cavalli et al., 1997).

The intravenous use of SLN; especially, has strong demands with respect to microparticle contamination and general toxicological considerations. Average diameters above 5 μm might cause death due to embolism. For that reason, aggregation and particle growth have to be avoided during storage. The chemical stability of hydrolizable drugs is another problem. One possible solution for both stability problems is freeze-drying (Zimmermann et al., 2000).

Storage stability involved chemical and physical aspects and included the prevention of degradation reactions (e.g. hydrolysis) and the preservation of the initial particle size. Lyophilization 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 (Mehnert and Mäder, 2001). The process has been optimized with regard to operating conditions, lipid concentration, type and concentration of cryoprotectant and redispersing conditions (Wissing et al., 2004).

Many reseachers studied the protective effect of various types and concentrations of cryoprotectants (e.g. carbohydrates) on freeze-thaw cycles. The results were concluded that the sugar trehalose proved to be most effective in preventing particle growth during freezing and thawing and also in the freeze-drying process (Schwarz and Mehnert, 1997; Müller et al., 1995). Freeze-drying of drug-free SLN under optimized conditions leads to a lyophilisate with good reconstitution properties. As optimized SLN formulation, the reconstituted SLN are suitable for i.v. administration with regard to the size distribution. Drug loading of the particles impairs the reconstitution quality. However, the lyophilisates of drug-loaded SLN can be used formulations less critical with regard to the presence of microparticles than i.v. injectables. Further optimization of the lyophilization parameters to obtain an i.v. injectable product appears feasible.

The addition of cryoprotectors will be necessary to decrease SLN aggregation and to obtain a better redispersion of the dry product. Cryoprotectors are place holders which prevent the contact between discrete lipid nanoparticles. Furthermore, they interact with the polar head groups of the surfactants and serve as a kind of “pseudo hydration shell”. Average particle sizes of all lyophilized samples with cryoprotective agents were 1.5-2.4 times higher than the original dispersions.

Heiati et al. (1998) compared the influence of four cryoprotectors (trehalose, glucose, lactose and mannitol) on the particle size of azidothymidine palmitate loaded SLN lyophilizates. Trehalose was found to be the

most effective cryoprotector for preventing aggregation during lyophilization and subsequent reconstitution of SLN.

Yang and Zhu (2002) have investigated the effect of cryoprotective sugars on the physical properties, speed of redispersion and the turbidity ratio of reconstituted camptothecin-SLN suspension to initial suspension. They found that the combination of 10% mannitol and 5% glucose was optimized. The lyophilized product had excellent physical properties and could disperse in distilled water within 0.5 min without the change of turbidity compared with the initial ones.

The protective effect of the surfactant can be compromised by the lyophilization. It has been found that the lipid content of SLN dispersion should not exceed 5% to prevent an increase in particle size. Zimmermann et al (2000) found that optimization of the lyophilization parameters results in formulations which are i.v. injectable with regard to particle size. The best results were obtained with samples of low lipid content and with the cryoprotector trehalose. Slow freezing in a deep freeze (-70°C) was superior to rapid cooling in liquid nitrogen. Furthermore, introduction of an additional thermal treatment to the frozen SLN dispersion (2h at -22°C followed by a 2-h temperature decrease to -40°C) was found to improve the quality of the lyophilizate.

Spray-drying

As an alternative for SLN formulations that cannot be lyophilized successfully, spray-drying was investigated as additional method for converting the aqueous dispersion into a dry product (Müller et al., 1995). This method has been used scarcely for SLN formulation, although spray-drying is cheaper compared to lyophilization. Freitas and Müller (1997) obtained a redispersable powder by spray-drying, which complies with the general requirements of i.v. injections with regard to the particles size and the selection of the ingredients. A general difficulty was the risk of the SLN melting during spraying. Accordingly, attempts using lower melting lipids were not successful (e.g. Dynasan 114 melting at

57°C, cetylpalmitate at 47°C). The problem could be circumvented by using Compritol 888 ATO (melting point 72°C) or Syncrowax HRSC (melting range 105-115°C). Spray-drying might potentially cause particle aggregation due to high temperature, shear forces and partial melting of the particles. They recommend the use of lipids with the melting points > 70°C for spray drying. Furthermore, the addition of carbohydrates and low lipid content favor the preservation of the colloidal particle size in spray drying.

Analytical characterization of SLN

1. 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 (Heurtault et al., 2003). The Coulter counter method is rarely used to measure SLN particle size because of difficulties in the assessment of small nanoparticles and the need for electrolytes which may destabilize colloidal dispersions.

PCS (also known dynamic light scattering) measures the fluctuation the intensity of the scattered light which is caused by the 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. 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. 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 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. Platelet structures commonly occur during lipid crystallization and have also been suggested in the SLN literature. Furthermore, 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 range. It gives a fast indication of the presence and character of microparticles (microparticles of unit form or microparticles consisting of aggregates of a smaller particle). Electron microscopy provides, in contrast to PCS and LD, direct information on the particle shape. However, the investigator should pay special attention to possible artifacts which may be caused by the sample preparation. For example, solvent removal may cause modifications which will influence the particle shape.

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. The atomic force microscope obtains images quickly enough (about 20 seconds per image) to allow the observation of in situ processes occurring at interfaces. The size of the visualized particles was of the same magnitude compared with the results of PCS measurements. The AFM investigations revealed the disk-like structure of the particles. AFM images indicate that crystalline particles are surrounded by soft layers. The softness of these layers was proved by form alterations, which occurred if they were imaged successively by contact AFM.

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. A certain advantage of FFF over PCS is the high resolution of a small particle size differences. The high dilution of the sample by FFF may cause potential problems because it may disturb the sample characteristics (for example, dilution with pure water may cause removal of the surfactant from the particle surface).

The measurement of the zeta potential allows for predictions about the storage stability of colloidal dispersion. 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 stabilizers,

because the adsorption of steric stabilizers will decrease the zeta potential due to the shift in the shear plane of the particle (Müller et al., 2000). The greater the zeta potential the more likely the suspension is to be stable because the charged particles repel one another and thus overcome the natural tendency to aggregate. The measurement of the zeta potential allows predictions to be made about the storage stability of a colloidal dispersion. It is currently admitted that zeta potentials under |30| mV, optimum > |60| mV, are required for full electrostatic stabilization; potentials between |5| and |15| mV are in the region of limited flocculation; and between |5| and |3| mV of maximum flocculation (Heurtault et al., 2003).

2. Measurement of crystallinity, lipid modification and assessment of coexistence of additional colloidal structures

Differential scanning calorimetry is thermoanalysis of the SLN dispersions which provides information about crystallization behavior, the timing of polymorphic transitions, fusion temperature, enthalpy, and the degree of crystallinity of melted-homogenized glyceride nanoparticle dispersions.

The X-ray diffraction pattern is photographed on a sensitive plate arranged behind the crystal, and by such a method the structure of a crystal may be investigated. It has become possible to determine to the distances of the various compounds can be determined in this way. X-ray scattering (SAXS) often completes the study. These techniques were used to investigate crystallization tendency and polymorphic transitions of triglyceride nanoparticles (Heurtault et al., 2003).

The coexistence of additional colloidal structures (micelles, liposomes, mixed micelles, supercooled melts, drug nanoparticles) has to be taken into account for all SLN dispersions. The characterization and qualification 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. Dilution of the original SLN dispersion with water might cause the removal of surfactant molecules from the particle surface and induce further changes such as crystallization or changes of the

lipid modification. 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.

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

^{1}H-NMR spectroscopy permits an easy and rapid detection of supercooled melts due to the low linewidths of the lipid protons. 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 made from blends of solid and liquid lipids. 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. The development of low-frequency ESR permits non-invasive measurements on the small mammals. ESR spectroscopy and imaging will give new insights about the fate of SLN *in vivo* (Müller et al., 2000).

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 dispersion is generally more than 1 year and Müller et al (1995) could show stability of SLN made from glyceryl palmitostearate or tribehenate for up to 3 years by PCS, The average diameter of the main population remained between 160 and 220 nm for the investigated period (Wissing et al., 2004).

Freitas and Müller (1998) investigated the effect of light and temperature on the physical stability of SLN dispersions composed of 10% tribehenate and 1.2% poloxamer 188. They found that the 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 the darkness particle growth started after 4 months storage. The gelation was accompanied by a decrease in zeta potential 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 the elevated temperature and remained stable for more than 3 months when refrigerated. Again, particle growth could be correlated to a decrease in zeta potential from -24.7 to approximately -15 mV.

The crystallization process might lead to stability problems such as gelling or the expulsion of the incorporated drug. Polymorphism is one of the important physical degradation routes which affects the stability of solid dosage forms because, even through they are chemically identical, polymorphs generally have different thermodynamic properties such as melting points, X-ray diffraction patterns, and solubility. The main polymorph forms in glycerides are the α, β' and β forms. The α form has the tendency to be transformed quickly to a form with better chain

packing such as the β' form. The most stable form is the β form. Therefore, the transition of liquid (melt) from α to β via β' was the pathway for triglycerides to the optimum packing form of the molecules. This unstable form gradually transformed toward the most stable form during storage at elevated temperatures while losing the initial spherical surface structures. This led to crystalline aggregate growth (Heurtault, 2003).

Release of drug from SLN

The release profile characterization of SLN may play a significant role on the application. A great number of in vitro studies have examined the rate at which drug is released from SLN. Most frequently, biphasic release patterns are observed. Despite the abundance of work, little directly comparable work is available in the literature. Studies have implicated a wide variety of variables influencing the rate of release. Solubility of the drug in the lipid and drug/lipid interactions appears to influence the rate of release, perhaps through the partition coefficient. Even the temperature employed during preparation of the SLN has been suggested as influencing release by enhancing the solubility of the drug in the aqueous phase during particle formation and thereby promoting drug localization at the surface region. One of the most frequently mentioned characteristics influencing release appears to be particle size. Tetracaine loaded glyceryl behenate-SLN showed a burst release of up to 80% of the drug from particles 40 nm in diameter but only 40% when the particles were about 200 nm in diameter. The smaller particles have a larger specific area. The large specific area suggest that, compared to the 200-nm particles, a larger fraction of the drug may be located close to the surface of the 40-nm particles. This might help explain why the smaller particles initially release drug at a higher rate (Bummer, 2004).

Tissue distribution and drug targeting

The accumulation of SLN within the Kupffer cells of the liver is predominantly found after intravenous injection in case non-stealth SLN are injected. With the exception of liver diseases like hepatic neoplasms, liver infections like hepatitis and visceral leishmaniasis and systemic candidasis, and physiologic

disorders (e.g. hypercholesterinemia), passive liver targeting should be avoided. The systemic use of colloidal carriers is limited by the presence of mononuclear phagocytic system (MPS), and it is consequently necessary to avoid such recognition. SLN carriers are mostly recognized by macrophages due to the physicochemical characteristics in particle size, surface charge and surface hydrophobicity. Various attempts have been made to achieve long circulation times by avoiding MPS uptake as discussed for stealth SLN. One outstanding example of targeting specific organs is brain delivery. The uptake of SLN by the brain might be explained by adsorption of blood proteins like apolipoproteins on particle surfaces mediating the adherence to endothelial cells of the blood brain barrier (Wissing et al., 2004).

Applications of SLN for drug delivery

1. SLN for oral administration

Oral administration of SLN is possible as aqueous dispersion or alternatively after transform into a traditional dosage form, i.e. tablets, pellets, capsules or powders in sachets. 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 (Müller et al., 2000b).

An example for orally administered SLN is cyclosporin loaded particles. The cyclosporin-SLN were produced from stearic acid and stabilized by lecithin and poloxamer. The mean diameter of cyclosporin A stearic acid nanoparticles was 316.1 nm while the encapsulation ratio of cyclosporine A to stearic acid nanoparticles reached to 88.36%. The relative bioavailability of cyclosporin A stearic acid nanoparticles over reference, Sandimmun Neoral® was nearly 80%, and the time to reach maximum concentration of cyclosporin A after oral administration of cyclosporin A stearic acid nanoparticles was delayed significantly than the reference, suggesting an obvious sustained release effect. The conclusion from this

study was that the stearic acid nanoparticles might be a very potential drug carrier (Zhang et al., 2000).

Demirel et al (2001) investigated the bioavailability and plasma profiles of orally piribedil-loaded SLN. Oral bioavailability of piribedil from solid lipid microparticles increases when compared to pure piribedil. The *in vivo* release profile is longer than 24 h. The slower *in vivo* release may lead to prolonged activity and avoidance of frequent administration of piribedil. This is in agreement with the data of cyclosporin.

Hydrophillic macromolecules such as insulin could be loaded into tripalmitin nanoparticles. The w/o/w multiple emulsion technique was applied and conveniently modified for the production of tripalmitin nanoparticles. The surface of the particles could be modified through the incorporation of poloxamer 188 or the lipid derivative PEG 2000-stearate into the formulation. Results of the stability of the nanoparticles in gastric and intestinal media indicate that PEG-stearate coated nanoparticles were stable. Sterical stabilization significantly improved the resistance of these colloidal systems in the gastrointestinal fluids. It was showed that insulin, chosen as a model peptide, could be associated and released from PEG-stearate coated nanoparticles (Garci'a-Fuentes et al., 2002).

2. SLN for pulmonary administration

Until now the SLN system has not yet been fully exploited for pulmonary drug delivery, very little has been published in this area. Alternatively 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. 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. In addition, SLN proved to possess a high tolerability, one might also consider drug targeting to lung macrophages. Particles in the lung are easily accessed by lung macrophages, that means one could use the SLN system for treating infections of the MPS system (Müller et al., 2000).

In vivo toxicity of solid lipid microparticles (SLM) as carrier for pulmonary administration was studied by Sanna et al., (2003). They prepared SLM by using compritol and poloxamer. SLM were obtained by oil in water emulsification employing the phase inversion technique. The results of morphology and size after lyophilization and sterilization by autoclaving were shown that it was suitable for pulmonary delivery by a 1-step process. *In vivo* assessment was carried out in rats by intratracheal instillation of either placebo or SLM dispersion, and by bronchoalveolar lavage for cytological analysis. Total cell counts showed no significant differences between placebo and SLM 0.5% or 2.5% groups. Regarding cytology, percentage of polymorphonuclear neutrophils and macrophages did not significantly differ between groups. It was concluded that SLM might be a potential carrier for encapsulated drugs to be given by pulmonary route for local (e.g. antiasthma, antimicrobial) or systemic therapy.

3. SLN for topical administration

The smallest particle sizes are observed for SLN dispersions with low lipid content (up to 5%). 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 preserve the colloidal particle size. 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 (Mehnert and Mäder, 2001).

The cosmetic field offered interesting applications. It has been found in prednisolone incorporated into SLN for dermal use. Prednisolone-SLN appeared to induce a localizing effect in the epidermal layer which was pronounced at

6 h and declined later. It was concluded that it shows well tolerated lipid nanoparticles are suitable for glucocorticoid targeting to viable epidermis (Maia, et al., 2002).

Incorporation of active ingredients into the solid SLN matrix protected them against chemical degradation. Stability enhancement was reported for coenzyme Q10 and also for the very sensitive active ingredients. Incorporation of vitamin E, chemically labile compound into the solid lipid matrix protects against chemical degradation. The SLN are physically stable in aqueous dispersions and also after incorporation into a dermal cream. The occlusive effect of SLN promotes the penetration of active compounds into the upper layer of the epidermis, mainly into the stratum corneum. This improved penetration can enhance the cosmetic performance of ingredients (Dingler et al., 1999).

Recently discovered area of application is the use of SLN in poor water solubility substance delivery. The transdermal delivery capacity and anti-inflammatory activity of triptolide were evaluated by Mei et al (2003). The results indicated that SLN dispersions could serve as efficient promoters for the triptolide penetrating into skin. The anti-inflammatory activity of SLN dispersion was stronger than that of microemulsion in carrageenan induced rat paw edema.

4. SLN for parenteral administration

SLN can be used for intravenous application. The pharmacokinetics and tissue distribution of doxorubicin in SLN were compared to the commercial solution. After i.v. administration, doxorubicin was still present in the blood 24 h of stealth and non-stealth SLN, while it was not detectable after the injection of the commercial solution. This result showed the prolonged circulation time of the SLN compared to the doxorubicin solution (Fundaro et al., 2000).

Yang et al., (1999) reported on the pharmacokinetics and body distribution of camptothecin after i.v. injection in mice. In comparison to a drug solution, SLN were found to lead to much higher AUC/dose and mean residence times (MRT) especially in brain, heart and reticuloendothelial cells containing organs.

The highest AUC of SLN to drug solution among the tested organs was found in the brain.

Parenteral oxytetracycline (OTC) therapy in farm animals requires daily administration of drug over several days, usually 3-5 days, in order to provide prolonged therapeutic blood levels. Domb (1995) developed the liposphere system composed OTC, solid glyceride, phospholipids, buffer solution and preservatives for long acting of the drug. Serum OTC concentration after single intramuscular injection of OTC-liposphere formulation in turkeys showed an effective extended release for 3-5 days as compared to about 1 day for the commercial OTC solution. It was demonstrated the usefulness of the lipid particles encapsulation system for extending drug release after a single injection.

2. Nanostructured lipid carriers (NLC)

The major advantage of SLN is the possibility of production on large industrial scale (Dingler and Gohla, 2002). However, depending on the drug some potential problems can occur:

1. limitation in drug loading capacity
2. drug expulsion during storage
3. high water content of aqueous SLN dispersions (70-95%)

To overcome the limitations of SLN, nanostructured lipid carriers (NLC) have been developed. In contrast to SLN being produced from solid lipids, the NLC are produced by controlled mixing solid lipids with spatially incompatible liquid lipids leading to special nanostructures with improved drug incorporation and release properties. For the production of NLC, the resulting matrix of the lipid particles shows a melting point depression compared to the original solid lipid but the matrix is still solid at body temperature. Depending on the lipid blend, different types of NLC are obtained. The three types of NLC compared to the more or less highly ordered matrix of SLN are shown in Figure 3.

A prerequisite for good drug accommodation are larger distances between fatty acid chains of the glycerides and general imperfections in the crystal (e.g. to

accommodate amorphous drug cluster). Distances between fatty acid chains can be increased by using glycerides being composed of very different fatty acids (e.g. in the length of C chain, mixture of saturated and unsaturated acids). To achieve "highest incompatibility", instead of taking a solid lipid as for SLN, the novel lipid particle was produced by mixing solid lipids with chemically very different liquid lipids (oils). This leads to more imperfections in the crystal and higher drug load (Figure 3, upper right)

Drug loaded in SLN is limited due to the formation of the lipid crystal. Drug expulsion is caused by an ongoing crystallization process towards a perfect crystal. Thus, by avoiding crystallization, one can avoid these obstacles which are realized in the NLC type 2. The lipid matrix is solid but not crystalline-it is in an amorphous state (Figure 3, lower left). This can be achieved by mixing special lipids. The solid character of the particles was proven by NMR measurements and the lack of crystallinity by DSC analysis.

The third type of NLC is a multiple system, being comparable to w/o/w emulsions. In this case it is an oil-in-solid lipid-in-water dispersion. The solid lipid matrix contains tiny liquid oil nanocompartments (Figure 3, lower right). This NLC type uses the fact that for a number of drugs, the solubility in oils is higher than their solubility in solid lipids (Müller et al., 2002).

NLC can exploit all the advantages known from lipid nanoparticles for oral administration. Compared to the other systems, drug loading can be increased, drug inclusion is improved. NLC can easier be processed to traditional dosage forms well known by the patient, e.g. tablet, capsule or pellet. Because of the high particle concentration and cream-like consistency the NLC dispersions might be directly filled into capsules when producing the particles in a suitable medium, e.g. PEG 600, oil.

The high particle concentration facilitated the use of these dispersions for granulation or as wetting agent in the pellet production. It appears also feasible that the cream-like particle dispersion can be filled into tubes. The patient can dose the required amount of drug on a spoon by using a special dosing mechanism. This would

be a simple and versatile system for individual dosing of e.g. cyclosporin SLN. Another very attractive route is delivery of drug to the skin. High concentrated NLC are already cream-like and can directly be applied to the skin. Improved delivery could be achieved by effects like occlusion and creation of supersaturated systems similar to microemulsions but without a high surfactant content, e.g. for cyclosporin to treat psoriasis). A broad application is parenteral delivery ranging from s.c. depots to i.v. and also i.m. As an i.v. system, the NLC can be loaded with paclitaxel avoiding critical solubilizers like Cremophor EL. The smart NLC as the new generation offer much more flexibility in drug loading, modulating of release and improved performance in producing final dosage forms such as creams, tablets, capsules and injectables (Radtke and Müller, 2005).

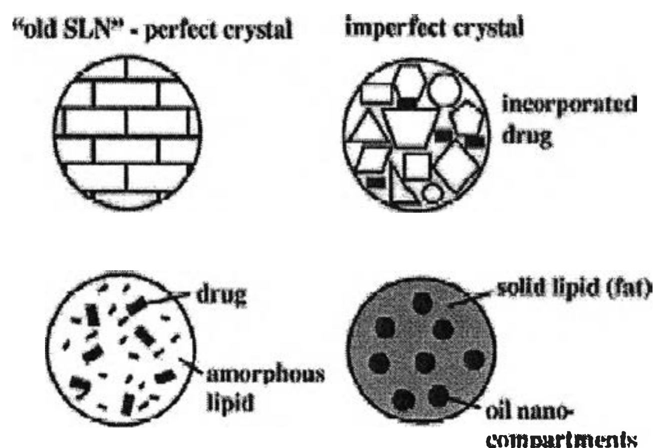

Figure 3 The three types of NLC compared to the relatively ordered matrix of SLN (upper left), NLC types: imperfect type (upper right), amorphous type (lower left), multiple type (lower right).

Drug incorporation

The first compound formulated in NLC was Retinol. The loading capacity of Retinol in SLN produced from Compritol was only approximately 1%. Producing NLC from a mixture of Compritol and Miglyol 812 increased the loading capacity to 5%. cyclosporin is also a very attractive molecule for incorporation into SLN. Cyclosporin could be incorporated into Imwitor 900 SLN reaching a total

cyclosporin 2%. Contrast to cyclosporine-loaded NLC were produced by admixing different oils to solid lipids, it was also possible to create semisolid NLC formulations containing a total of 4% cyclosporine (Müller et al., 2002).

3. Amphotericin B (AmB)

Over the past 40 years, AmB has been the mainstay of antifungal therapy for severe systemic infections (Hillery, 1997). Certainly, it rivals the venerable antibacterial penicillin G for its long-lived importance in its therapeutic niche and has proven far more resistant to microbial adaptability. AmB is still the drug of choice for many serious systemic fungal infections, which owing to AIDS and improved organ transplant immunosuppression drugs are becoming tragically frequent in immune-compromised individuals (Hartsel and Bolard 1996).

AmB is a natural product derived from *Streptomyces nodosus*, a soil actinomycete. AmB is an older drug of the polyene class similar to nystatin. AmB is named for its amphoteric properties, which are acquired from a carboxyl group on the main ring and a primary amino group on mycosamine (Clinical pharmacology, 2000). AmB is one of a family of some 200 polyene macrolide antibiotics. Those studied to date share the characteristics of four to seven conjugated double bonds, an internal cyclic ester, poor aqueous solubility, substantial toxicity on parenteral administration, and a common mechanism of antifungal action. AmB is a heptane macrolide containing seven conjugated double bonds in the trans positon and 3-amino-3,6-dideoxymannose (mycosamine) connected to the main ring by a glycosidic bond (Figure 4). The amphoteric behavior for which the drug is named derives from the presence of a carboxyl group on the main ring and a primary amino group on mycosamine; these groups confer aqueous solubility at extremes of pH. X-ray crystallography has shown the molecule to be rigid and rod-shaped, with the hydrophilic hydroxyl groups of the macrolide ring forming an opposing face to the lipophilic polyenic portion (Bennett, 2001).

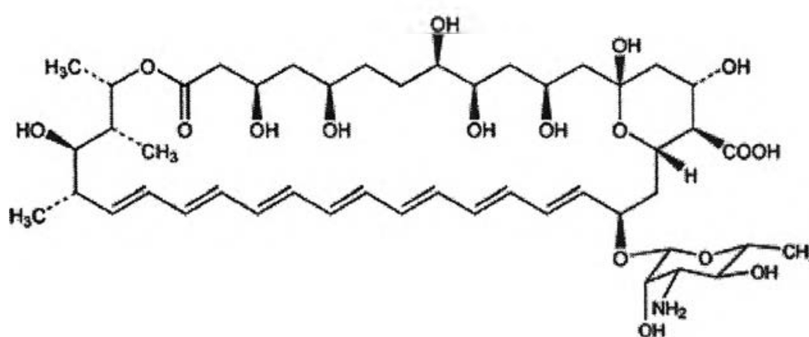

Figure 4 The chemical structure of AmB

The major drawback to the use of AmB is its insolubility in water. To become biologically active, it must be solubilized in an aqueous mulieu, and the manner in which that is done, as well as the route by which is administered to humans or experimental animals, determines its effectiveness (Bennett, et al., 1963). The formulation licenced for routine clinical use, which is produced and marketed by Bristol-Myers Squibb, Co., Ltd as Fungizone®, is a mixture of AmB with a detergent, deoxycholate, in a phosphate buffer. Under this condition, AmB forms a colloidal dispersion suitable for intravenous administration. Despite its proven efficiency, use of the conventional formulation of AmB (AmB deoxycholate, AmBD) is limited by potentially severe adverse reactions, often causing decreased renal function, anaphylaxis, chills, high fever, nausea, phlebitis, anorexia and a host of other unpleasant effects. The harmful side effects increase with increasing dosage; therefore, the amount of AmB that can be administered safely is limited. Dosage is an even greater limiting factor in the treatment of immunocompromised patients, including those with AIDS or cancer patients undergoing intensive cytoreductive therapy. To compound the difficulties, some of these patients are infected with fungal strains somewhat resistant to AmB.

Several strategies, including modification of the AmB molecule and changes in delivery systems, have been used to improve the therapeutic effectiveness of AmB and reduce its toxicity. For example, semisynthetic derivatives prepared by substitution of one or both of the functional amino or carboxyl groups were attempted.

Of these derivatives, the methyl ester of AmB has been investigated most extensively, but other compounds are under study as well. Several derivatives of AmB which were modified solely at the C-13 hemiketal position have been prepared. Modifications in delivery have involved the administration of AmB in combination other antifungal drugs in attempts to identify synergistic interactions and have also involved the delivery AmB directly to the target organ by aerosolization or intranasal deposition. Perhaps the most promising approach has involved modification of the physical state of AmB, and the first reports of greater efficacy of liposomal AmB with Fungizone® appeared more than a decade ago (Brajtburg and Bolard, 1996).

Mechanism of action

AmB binds to sterols in the cell membranes of both fungal and human cells. It is usually fungistatic *in vivo* but can have fungicidal activity at high concentrations or against extremely susceptible organisms. Its higher affinity for ergosterol, the sterol found in fungal cell membranes, over cholesterol, the sterol found in human cell membranes, allows AmB to be used systemically. As a result of this binding, membrane integrity is impaired, causing the loss of intracellular potassium and other cellular contents. Some adverse reactions to AmB, such as electrolyte loss and nephrotoxicity, are an extension of its pharmacologic action, while anaphylactoid infusion-related reactions may be related to stimulation and release of prostaglandin synthesis. Anemia may be secondary to an inhibition of erythropoietin production.

Fungi that are typically sensitive to AmB include: *Acremonium* sp., *Aspergillus fumigatus, Blastomyces dermatitidis, Candida albicans, Candida guilliermondi, Candida tropicalis, Coccidioides immitis, Cryptococcus neoformans, Fusarium* sp., *Histoplasma capsulatum, Mucor mucedo, Rhodotorula* spp., and *Sporothrix schenckii*. In an *in vitro* study, the combination of AmB and azithromycin has been shown to be synergistic against *Fusarium*. The combination of amphotericn B and rifabutin has been shown to be synergistic against *Fusarium* and *Aspergillus*. Protozoa that are typically sensitive include: *Leishmania braziliensis, L donovani, L. mexicana,* and *Naegleria fowleri*. Occasionally, *Acanthamoeba castellanii* and *A.*

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polyphaga are susceptible to AmB. AmB is not active against bacteria, *Rickettsiae*, or viruses (Clinical pharmacology, 2000).

Pharmacokinetics

AmB may be given orally, by inhalation, and intravenously; however it is poorly absorbed from the gastrointestinal tract, so parenteral administration is required to treat systemic fungal infections. High doses of AmB oral suspension produce inconsistent and low blood concentrations. In both pediatric and adult patients, the average serum concentration following AmB oral suspension 100 mg po four times daily was 0.05 μg/ml. There is no evidence of accumulation of AmB after 2 weeks or more of therapy. Intravenous administration of 30 mg of AmB over several hours produces average peak serum concentrations of about 1 μg/ml. Doses of 50 mg over the same time period produce average peak serum concentrations of about 2 μg/ml. AmB can not be given intramuscularly.

Distribution of AmB is believed to be multicompartmental. Low concentrations of AmB are achieved in aqueous humor, pleural, pericardial, peritoneal, and synovial fluids. Because CSF concentrations are approximately 3% of those in serum, AmB must be given intrathecally to achieve fungistatic concentrations within the CSF. Arachnoid villi remove AmB from the CSF, and the drug is stored in the extracellular compartment of the brain, which may act as a reservoir for the drug. AmB is 90-95% protein-bound, primarily to lipoproteins.

Following inhalation of 4 mg AmB radiolabelled with technetium-99m inhaled over 10 minutes, between 3.5% and 4% of the total activity of radiolabelled AmB was delivered to the lungs. As a suspension of AmB without deoxycholate was used, the actual distribution cannot be determined. However, since particles of AmB deoxycholate suspension are smaller than the radiolabelled particles, a greater percentage of AmB delivered to the lungs would be expected with this formulation.

Metabolism of AmB is unknown. Small amounts of drug are excreted through the biliary system. Approximately 2-5% of a dose is eliminated renally at an

extremely slow rate, with 40% being eliminated over 7 days. The elimination half-life in adults with normal renal function averages 24 hours, but after prolonged administration, elimination half-life can be as long as 15 days, possibly due to slow release of the drug from peripheral compartments. Elimination half-life of AmB is extremely variable in both neonates and pediatric patients. AmB can be detected for up to 4 weeks in blood and 4-8 weeks in urine after discontinuation of intravenous therapy. AmB is poorly hemodialyzable (Clinical pharmacology, 2000).

Effect on the immune system

AmB has been shown to posses immunostimulatory properties, which raises the question of how relevant are *in vitro* susceptibility testing data alone, since the role of the host's response to the disease process is not taken into consideration. Depending upon the concentration of AmB, the drug can stimulate macrophages to produce increase levels of prostaglandin E_2 and kill bacteria, parasites, or tumor cells. At higher concentrations, AmB can result in a decrease in chemotaxis of polymorphonuclear leukocytes and phagocytosis, and killing by macrophages.

Humoral immunostimulation of mice immunized with hapten-protien-conjugate trinitrophenylated human serum albumin has resulted in an increased frequency of antibody-producing cells in the spleen and lymph nodes. AmB enhances the switching of lymphoid cells from producing IgM to IgG antibody, an effect that is dependent upon T-cells being stimulated. The magnitude of the observed effect is greater for the second response. AmB has similar potency to BCG, complete Freund's adjuvant, and Pertussive-Maalox.

AmB has an effect upon the delayed-type hypersensitivity and cell-mediated immunity, which confirm its interaction with T-cells or specific T-cell subsets. It appears that the immunoadjuvant effect may be dependent upon a specific T-cell population. It is clear that AmB enhances the immune reactivity of lymphoid cells. Medoff and Kobayashi have suggested that AmB acts in two ways. First, it kills fungi, and second, it interacts with the suppressor T-cells that may cause the anergy that is often associated with disseminated fungal infections. Because AmB is selectively toxic for suppressor cells, this could result in an immunosuppressive

effect. The interaction of AmB and the host's immune system is extremely complex (Lorian, 1991).

Dosage admininistrations

Intravenous dosage - rapid escalation:

Adults: The full maintenance dosage may be administered on day 1, monitoring the patient closely during the first 15-30 minutes for shaking chills, rigors, or other evidence of anaphylactoid reactions. Most clinicians would select a maintenance dose of 0.5-1.0 mg/kg/day i.v. (range: 0.25-1.5 mg/kg/day). Daily dosages should not exceed 1.5 mg/kg. If shaking chills, or other infusion-related reactions occur, administer meperidine i.v. to abort the reaction, premedicate the patient with ibuprofen po or hydrocortisone i.v., and attempt to administer the first full dose, monitoring the patient closely for any reaction. Premedicate patients who demonstrate infusion-related reactions with ibuprofen po or hydrocortisone i.v. prior to each AmB dose, using meperidine i.v. to abort any subsequent infusion-related reactions. The maintenance dosage can be administered once daily, according to patient tolerance. Doubling the daily dose and administering this dose every other day has also been done in the treatment of some infections.

Children: Initiate therapy with 0.25 mg/kg i.v. over 6 hours, with frequent observation during the first several hours of the infusion. If no reaction occurs with the initial dose, a full maintenance dose may be administered. A maintenance dose of 0.5 mg/kg i.v. is frequently used; however, the dosage range is 0.25-1 mg/kg/day. In some cases, doses of 1-1.5 mg/kg/day may be required for short-term use. Once therapy has been established, AmB can be administered on an every other day basis at 1-1.5 mg/kg/dose.

Intravenous dosage - gradual escalation:

Adults: Most clinicians begin systemic therapy at 0.1-0.25 mg/kg/day i.v. and gradually increase the dose to 0.25-1.0 mg/kg/day over a period of several days. Daily dosages should not exceed 1.5 mg/kg. In patients with life-threatening

infection, the full maintenance dose may be administered on day 1 of therapy. If shaking chills, or other infusion-related reactions occur, administer meperidine i.v. to abort the reaction, premedicate the patient with ibuprofen po or hydrocortisone i.v., and attempt to administer the first full dose, monitoring the patient closely for any reaction. Premedicate patients who demonstrate infusion-related reactions with ibuprofen po or hydrocortisone i.v. prior to each AmB dose, using meperidine i.v. to abort any subsequent infusion-related reactions. The maintenance dosage can be administered once daily, according to patient tolerance. Doubling the daily dose and administering this dose every other day has also been done in the treatment of some infections.

Children: Initially 0.25 mg/kg/daily i.v. in 5% dextrose injection over 2-6 hours. Gradually increase dosage by increments of 0.125-0.25 mg/kg every day or every other day up to maximum of 1 mg/kg/day or 30 mg/m^2/day. Once therapy has been established, AmB can be administered on an every other day basis at 1-1.5 mg/kg/dose.

Adverse Reactions

Infusion-related reactions (i.e., reactions occurring during or shortly after the infusion) are commonly seen with conventional i.v. AmB including headache, chills, fever, rigors, flushing, hypotension, and nausea/vomiting. With subsequent doses, the intensity of these reactions usually decreases. AmB has been shown to stimulate prostaglandin synthesis, which may account for the infusion-related reactions. Although several medications frequently are prescribed to suppress these reactions prior to administration of an AmB dose, only hydrocortisone, meperidine, and ibuprofen have been shown to be effective. Variable success has been demonstrated with dantrolene. Slowing the rate of infusion is not helpful. Infusion-related reactions can be more severe if administration occurs shortly after platelet or granulocyte transfusions.

Nephrotoxicity occurs in more than 80% of patients receiving large cumulative doses (e.g., up to 3-4 g) of conventional i.v. AmB. Nephrotoxicity is

manifest in many forms including azotemia, hypokalemia, hypomagnesemia, hypocalcemia, hyposthenuria, nephrolithiasis (specifically nephrocalcinosis), renal tubular acidosis, and frank renal failure. Renal tubular acidosis may be present without concurrent systemic acidosis. The mechanism is due, in part, to lysis of cholesterol-rich lysosomal membranes of renal tubular cells, causing renal tubular necrosis. Tubuloglomerular feedback (TGF) provokes constriction of the afferent arteriole, which, in turn, causes azotemia. It is believed that TGF is augmented by renal electrolyte loss during a hyponatremic state and that sodium "loading" prior to administering i.v. AmB may attenuate the process. Azotemia can develop after only a few doses. Although renal function can return to baseline in several days if AmB therapy is held, irreversible renal tubular necrosis can develop, especially after prolonged therapy, large cumulative doses, or concomitant therapy with other nephrotoxic drugs. It appears that patients with higher serum low-density lipoprotein (LDL) concentrations are more susceptible to AmB-induced nephrotoxicity than those with lower concentrations. Hyperkalemia also has been reported after rapid administration of large doses of conventional i.v. AmB to patients with renal insufficiency. Other adverse renal effects reported in patients receiving conventional i.v. AmB include anuria, decreased renal function, dysuria, hematuria, oliguria, and urinary incontinence.

A normocytic, normochromic anemia occurs in most patients receiving conventional i.v. AmB. This reaction is believed to be caused by a suppressive effect on erythropoietin production. Usually, this condition does not require transfusions and generally returns to baseline within several months following discontinuation of therapy. Anemia has also been reported rarely in patients receiving AmB lipid formulations. Other hematologic effects, including agranulocytosis, coagulation disorders, decreased or increased prothrombin, eosinophilia, leukopenia, leukocytosis, or thrombocytopenia have been reported rarely in patients receiving conventional i.v. AmB.

Intravenous administration of conventional AmB can cause an injection site reaction including erythema, inflammation at the injection site, or pain. Phlebitis or thrombophlebitis has also been reported with conventional AmB. The manufacturer of conventional AmB suggests that the addition of 1200-1600 units of

heparin to the i.v. fluid, alternate-day therapy, or use of a scalp-vein needle may decrease these reactions. Extravasation of AmB causes local irritation.

Gastrointestinal adverse reactions, such as abdominal pain, anorexia, cramping, diarrhea, dyspepsia, epigastric pain, GI bleeding, hematemesis, hemorrhagic gastroenteritis, melena, nausea/vomiting (in addition to the nausea/vomiting caused by i.v. infusion of AmB), stomatitis, weight loss, xerostomia, can occur in some patients receiving i.v. AmB.

Elevated hepatic enzymes, hyperbilirubinemia, and increased LDH have been reported in patients receiving conventional i.v. AmB. Rarely, acute hepatic failure, hepatitis, jaundice, hyperglycemia, and hypoglycemia have been reported.

Adverse neurologic reactions have been reported in patients receiving conventional i.v. AmB. The adverse reactions include abnormal thinking, agitation, anxiety, cerebral vascular accident, coma, confusion, depression, diplopia, dizziness, drowsiness, encephalopathy, extrapyramidal syndrome, hallucinations, hearing loss, insomnia, leukoencephalopathy, malaise, myasthenia, nervousness, peripheral neuropathy, seizures, tinnitus, transient vertigo, tremor, and visual impairment. Headache can also occur in some patients receiving AmB, while other patients can experience diplopia or blurred vision. Other adverse effects reported in patients receiving conventional i.v. AmB include alopecia, arthralgia, bone pain, diaphoresis, dystonic reaction, erythema multiforme, exfoliative dermatitis, generalized pain, myalgia, pruritus, purpura, rash (including maculopapular rash or vesicular rash), skin discoloration, skin ulcer, urticaria, and xerosis.

Anaphylaxis or anaphylactoid reactions, angioedema, bronchospasm, and wheezing have been reported in patients receiving conventional i.v. AmB. If severe respiratory distress, anaphylaxis or an anaphylactoid reaction occurs, the drug should be discontinued immediately and the patient given appropriate therapy as indicated. Less frequent adverse effects associated with conventional i.v. AmB consist of cardiac arrest (primarily in cases when infusion is too rapid), cardiac arrhythmias (including ventricular fibrillation), cardiomyopathy, dyspnea, heart failure, hypertension, hypersensitivity pneumonitis, peripheral neuropathy, pulmonary edema,

seizures, shock, and tachypnea. Cardiac enlargement with congestive heart failure occurred in a few patients receiving conventional i.v. AmB with 20-40 mg of hydrocortisone sodium succinate added to each infusion. Congestive heart failure was considered to be due to AmB-induced hypokalemic cardiopathy and corticosteroid-induced salt and fluid retention. Following discontinuance of hydrocortisone and administration of oral potassium supplements, cardiac status returned to normal although conventional AmB therapy was continued (Clinical pharmacology, 2000).

Despite its proven efficacy, use of the conventional formulation of AmB (AmB deoxycholate [AmBD]) is limited by potentially severe adverse reactions which described above. Over the past 15 years azoles, primarily fluconazole and itraconazole, have become attractive, less toxic alternatives to AmB. However, there are problems, namely, their relatively poor efficacy against invasive mold infections and concern about emerging clinical and microbiologic resistance to azoles. Due to the increasing prevalence of disseminated fungal infections associated with the acquired immune deficiency syndrome (AIDS) epidemic, increased utilization of organ transplantation and immunosuppression, and the increased number of invasive fungal nosocomial infections, antifungal agents are more widely used than ever before. Consequently, there is a need for alternative drugs that are both efficacious and well-tolerated. Recently, lipid formulations have been developed to improve the therapeutic index of AmB (Hoesley and Dismukes, 2005).

4. Lipid formulations of AmB

Over the past 2 decades, researchers have investigated the utility of incorporating AmB into phospholipid vesicles and/or cholesterol esters in order to provide larger amounts of parent drug and concomitantly, less nephrotoxicity. To date, three lipid formulations of AmB are commercially available; amphotericin lipid complex (ABLC, Abelcet); AmB cholesteryl sulfate complex, also called AmB colloidal dispersion (ABCD, Amphotec); and liposomal AmB (L-AmB, Ambisome) that shown in Table 4 (Hoesley and Dismukes, 2005)

General Features

1. AmB lipid complex (ABLC)

ABLC is composed of AmB complexed with dymyristoyl phosphatidylcholine and dimyristoyl phosphatidylglycerol. The configuration of this complex is ribbon-like. ABLC is now being manufactured by Enzon Pharmaceuticals (Fairfield, New Jersey, USA) and its trade name is Abelcet®.

AmB concentrations achieved in blood after administration of ABLC are lower compared to that with AmB deoxycholate. On the other hand, ABLC produces higher concentrations in liver, spleen, and lungs. The renal concentration, on the other hand, is similar between the two formulations. Importantly, when ABLC is administered at higher doses, its concentration in kidneys increases only slightly and that in plasma remains the same (Lewis, 2005).

Table 4 Commercial available AmB formulations

Generic Name	Trade Name	Manufacturer/ Maketer	FDA
AmB deoxycholate (AmBD)	Fungizone®	Bristol-Myers Squibb	1958
AmB lipid complex (ABLC)	Abelcet®	The Liposome/ Enzon Company	1995
AmB Cholesteryl sulfate complex, AmB colloidal dispersion (ABCD)	Amphotec®	SEQUUS Pharmaceuticals	1996
Liposomal AmB (L-AmB)	AmBisome®	Fugisawa USA and NeXstar Pharmaceuticals	1997

2. AmB Colloidal Dispersion (ABCD)

AmB colloidal dispersion (ABCD) is a lipid formulation of AmB. As with the other lipid formulations, the major goal of developing ABCD has been to attain a compound with lower toxicity and with at least similar efficacy compared to the parent compound, AmB deoxycholate. ABCD is composed of AmB complexed with cholesteryl sulfate. It is a disk-like structure. ABCD is being manufactured by Sequus Pharmaceuticals (Menlo Park, CA, USA) under two trade names, Amphocil® and Amphotec®. ABCD displays two-compartment pharmacokinetics. After infusion, it is rapidly removed from the blood by the reticuloendothelial system and then re-released to the circulation. Plasma clearance and volume of distribution increase with escalating doses of ABCD. Compared to the conventional AmB deoxycholate preparation, peak serum levels are lower with ABCD. The amount of AmB bound to the LDL fraction is also lower after administration of ABCD in comparison to AmB deoxycholate. ABCD achieves high concentrations in liver. However, its concentrations in kidneys, spleen, brain and lungs are lower than that of AmB (Lewis, 2005).

3. Liposomal AmB (L-AmB)

L-AmB is composed of AmB complexed with hydrogenated soy phosphatidylcholine, distearoylphosphatidylglycerol, and cholesterol. Unlike the other lipid formulations of AmB, it is a true liposome composed of unilamellar lipid vesicles. L-AmB is being manufactured by Nexstar Pharmaceuticals. Its trade name is Ambisome®. Compared to the other lipid formulations of AmB, L-AmB reaches higher concentrations in plasma and remains in the circulation longer. Similar to the other lipid formulations, L-AmB concentrates in reticuloendothelial system. However, its uptake to the reticuloendothelial system cells is slower. This feature is presumably due to the smaller size, higher transition (melting) temperature and more rigid bilayer of the liposome. This finally provides a persistent pool of L-AmB in plasma and a sustained delivery to the site of infection. L-AmB attains high concentrations in brain tissue. Elimination of L-AmB from serum is biphasic. This pattern suggests that L-

AmB is first concentrated in reticuloendothelial system cells and then is redistributed (Lewis, 2005).

Chemical Structure

Lipid formulations of AmB were developed to improve the amphipathic nature of positive surface electrical charge, and higher cholesterol concentration in the phospholipid bilayers. The chemical properties and physical characteristics of available AmB products are outlined in Table 5.

For reference purpose, AmB, the parent drug, consists structurally of micelles (< 25 nm) with deoxycholate as the main lipid. By contrast, L-AmB is configured in small unilamellar vesicles (liposomes), with a lipid layer enclosing an aqueous core. The average size of the liposomes is 90 nm. L-AmB contains about 10 mol% AmB. ABLC is configured in large ribbon-like sheets, which range in size from 500-5000 nm. ABLC contains 30-35% mol% AmB. The third lipid-based formulation is ABCD which is configured in uniform disc-like structures, 125 nm in size, with a 1:1 admixture of AmB (50 mol%) and lipids, primarily cholesteryl sulfate (Dismukes, 2001).

Table 5 Chemical and physical properties of AmB formulations

	Lipid Configuration	Size (nanometers)	Lipid Component	AmB Content (mol%)
AmBD	Micelle	<25	Sodium deoxycholate	-
ABLC	Ribbon-like	500-5000	Dimyristoylphosphatidylcholine Dimyristoylphosphatidylglycerol	33%
ABCD	Disc-like	125	Cholesteryl sulfate	50%
L-AmB	Unilamellar vesicle (spherical)	90	Hydrogenated phosphatidylcholine Cholesterol Distearoylphosphatidylglycerol	10%

How lipid based carriers can reduce the toxicity

There are major differences in the physical and pharmaceutical properties of these different liposomal preparations and there seems to be no single feature, even surface charge, which distinguishes the effective from the ineffective. None of these preparations really conform to the popular conception of liposomal delivery systems as encapsulations of drug. Rather these formulations are aggregations of drug and lipid with little, if any, AmB entrapped as a solution inside. Some, e.g. ABCD, do not even contain closed vesicles. The reason for the success of these systems must involve the favourable disposition and release of drug. The heterogeneity of the physical states and structures suggests that there is more than one way to accomplish this.

In vitro studies have shown that, most of the time, AmB in lipid formulations retains all or part of its antifungal activity, whereas its toxicity is greatly reduced or abolished. Two hypotheses have been formulated for the origin of this increased *in vitro* selectivity. According to the first hypothesis, selective transfer of the drug occurs to fungal but not to mammalian cells. The second hypothesis is based on the notion that only free (unbound to the lipid carrier) AmB damage cells and that AmB is gradually released from a liposomal formulation with increased dilution (Hartsel and Bolard 1996).

By taking into consideration the effects of the physicochemical properties of the vesicles on the fate of liposomes *in vivo*, some general conclusions which presented by Hillery (1997) can be drawn as to why association with a lipid-carrier can attenuate the toxicity of AmB:

1. The composition of the lipid-carrier ensures that AmB remains associated with the carrier and is thus unavailable to interact with mammalian cells and exert its toxic effects. This requires that the lipid carriers remain stable in the circulation and uncontrolled drug leakage from the carriers does not occur. In fact, a complex balance of affinities is required, between the AmB molecules and the cholesterol in the human cell membrane, the lipids of the lipid carrier, and the ergosterol in the fungal membrane.

2. Association with the lipid-carrier facilitates uptake of the system by the RES. Passive targeting in this manner to the RES organs of the liver, spleen and bone marrow results in high local concentrations of the antibiotic in these organs, the main sites of systemic fungal infections. Engulfment of liposomes by circulating monocytes, and migration of the latter to sites of infection, represents a further mechanism of increasing AmB concentration at infection sites.

3. Altering the pharmacokinetic profile may also serve to divert the drug from other tissues such as the kidney, an organ particularly susceptible to the toxic effects of AmB

4. Fungi such as Aspergillus and Candida can invade blood vessel linings, thus creating a potentially "leaky" capillary. This suggests that in some cases the lipid-carrier-drug complex may be able to extravasate and localize at the site of the fungal infection.

5. Finally, after achieving the selective transfer of AmB from the lipid carrier to the fungal infection, involving minimal interaction with the human cell membrane, the AmB can preferentially accumulate in the fungus due to the high affinity of the drug for ergosterol.

Susceptibility Patterns

The significance of susceptibility testing for ABCD, ABLC, and L-AmB, as for that of any of the lipid formulations of AmB, is not known. This is mainly due to the fact that the enhanced activity of lipid formulations of AmB follows from the lesser toxicity of the lipid preparation. This permits administration of increased doses of AmB and, presumably, increased delivery of active drug to sites of infection. Nevertheless, comparative results have been reported. For *Aspergillus spp.*, the ranking of the in vitro activity of AmB and its lipid formulations has been reported as AmB deoxycholate = ABCD > L-AMB > ~ ABLC. As for the *Candida* spp., while ABCD MICs have been found to be higher than those of AmB deoxycholate for *C. glabrata*, *C. krusei*, *C. kefyr*, *C. tropicalis*, *C. lusitaniae*, and

Cryptococcus neoformans, those for *C. albicans* have been found to be similar with both formulations (Clinical Pharmacology, 2000).

The Pharmacokinetic

The pharmacokinetics and pharmacodynamics of the lipid formulations of AmB differ greatly from those of AmBD and each lipid formulation has distinct pharmacologic properties, including clearance, area under the curve (AUC), maximum plasma concentration (C_{max}), and volume of distribution. When differences in units of clearance are considered, the clearance of all lipid-based formulations is much lower than that of AmBD. Infusion of L-AmB results in strikingly higher AUC and C_{max} values than are achieved with AmBD or the other lipid formulations. Although the 3 lipid agents are distributed extensively into tissues, especially the primary reticuloendothelial organs (liver, spleen, and lungs), the apparent volume of distribution for ABCD and ABLC after single-dose infusion is substantially greater than that of L-AmB. For all 3 lipid-based drugs (ABCD, ABLC, and L-AmB) kidney tissue concentrations are substantially lower than those of AmBD. These disparities in pharmacologic properties may be linked to the binding affinity and subsequent release of AmB by the individual lipid vehicles. The liposomal component of L-AmB has the tightest bond to AmB, explaining in part its high plasma concentrations and lower clearance rates relative to the other lipid formulations. As a general principle, the lipid formulations of AmB at dosages 5- to 10-fold higher than dosages of AmBD can be administered safely. Animal studies have demonstrated lipid-based AmB preparations are clearly less toxic to mammalian cells than AmBD (Hoesley and Dismukes, 2005).

Clinical indications

The majority of clinical efficacy data related to the lipid-based AmB drugs are derived from compassionate use studies and small case series. Few randomized studies have been performed comparing the lipid formulations to AmBD or to each other. Table 6 provides the Food and Drug Administration (FDA) current approved indications for ABLC, ABCD, and L-AmB (Hoesley and Dismukes, 2005).

Table 6 FDA-Approved indications for lipid formulations of AmB

FDA-Approved indication	ABLC	ABCD	L-AmB
1. Treatment of the following conditions in patients who are refractory to AmBD or in whom renal impairment or unacceptable toxicity precludes use of AmBD:			
- Invasive fungal infection	√[1]		
- *Aspergillus* species		√[2]	√[3]
- *Candida* species			√[3]
- *Cryptococcus* species			√[3]
2. Empiric therapy for presumed fungal infection in fibrile, neutropenic patients			√[4]
3. Treatment of visceral leishmaniasis			√[5]

Recommended dosing

[1]ABLC 5mg/kg/d; [2]ABCD 3 to 4 mg/kg/d; [3]L-AmB 3 to 5 mg/kg/d; [4]L-AmB 3 mg/kg/d;

[5]L-AmB 3 mg/kg/d on days 1 through 5, 14, 21 (immunocompromised patients will require higher and more frequent dosing)

Adverse Events

The adverse effects of AmB formulations are shown in Table 7. The primary adverse events are related to infusion (e.g., fever, chills, and nausea and vomiting) and nephrotoxicity. Infusion-related events connected with ABCD and ABLC appear to be considerably more common than those associated with L-AmB and similar in frequency to infusion-related events associated with AmBD. In a large comparative trial, during initial infusion without premedication, L-AmB recipients experienced significantly less fever and chills than AmBD recipients (fever, 16.9% versus 43.6%, respectively; and chills, 18.4% versus 54.4%, respectively). Fever and chills associated with the lipid-based drugs tend to occur mainly after the first 2 infusions and are less frequent with subsequent infusions. Infusion-related hypoxia

Table 7 Adverse effects of AmB formulations

Infusion-related adverse events	AmBD	ABLC	ABCD	L-AmB
Chills/rigors	++++	+++	++++	++
Fever	++++	+++	++++	++
Nausea	+++	+	++	++
Vomiting	++	+	+	+
Headache	+	+	+	+
Dyspnea	++	+	+	+
Hypotension	+	NA	++	+
Tachycardia	++	++	+	+
Hypertension	++	++	NA	+
Hypoxia	+	++	++	+
Nephrotoxicity (2 x baseline serum creatinine)	~30%to50%	~25%	~15%	~20%

Note +,++,+++,++++: ≤ 10%, 11% to 25%, 26% to 50%, > 50%

has been documented in as many as 25% of ABCD and ABLC recipients but is usually reversible and without long-term sequelae. Infusion-related adverse events associated with the lipid-based drugs, just as with AmBD, can be attenuated or prevented by premedicating with acetaminophen, antihistamines, corticosteroids, and meperidine. Clinical experience has shown a patient intolerant of a AmB lipid formulation may tolerate another well.

Nephrotoxicity is less common with all 3 lipid formulations of AmB than with AmBD. In comparative studies the respective rates of nephrotoxicity (defined as twice baseline serum creatinine) are: ABLC ~25%, ABCD ~15%, L-AmB ~20%, and AmBD ~30% to 50%. Importantly, in open label studies, administration of lipid-based AmB drugs are reported to stabilize or even improve renal function in patients with preexisting renal insufficiency. In addition, individuals receiving concurrent nephrotoxic agents (e.g., cyclosporine, aminoglycosides) are less likely to

have renal dysfunction when receiving lipid formulations compared to AmBD. Hydration and sodium repletion have been utilized to reduce the risk of nephrotoxicity with AmBD but the utility of these measures is unclear with lipid formulations. Other adverse events associated with lipid-based products have included elevations in liver transaminases, alkaline phosphatase, and serum bilirubin levels. Liver function test abnormalities have been noted in 25% to 50% of patients treated with L-AmB but these findings are reversible without drug discontinuation in the majority of patients (Dismukes, 2001).

AmB therapy cost analysis

The costs of lipid formulations of AmB are considerable. The daily treatment costs based on the 1999 average wholesale price for AmB products are as follows: AmBD 1 mg/kg $25, ABLC 5 mg/kg $776, ABCD 4 mg/kg $480, and L-AmB 3 mg/kg $942. In the same way, the other reference from the 2004 Drug Topics Redbook (Medical Econimics, Inc., 2004) present all average wholesale price (AWP) pricing data in Table 8. The AWP does not necessarily reflect contract prices paid by individual institutions, which can vary widely based on antifungal usage and healthcare setting. The costs are acquisition costs, only one component of the price of using any drug. If side effects or monitoring costs are significant, then these secondary cost considerations may outweigh the acquisition cost. Thus, they separately provide an analysis of the pharmacoeconomics of antifungal therapy, with a special emphasis on secondary costs associated with AmB nephrotoxicity (Lewis, 2005).

Hillery (1997) also reviewed the cost of treatment per day for Fungizone® and the commercial lipid-based preparations as shown in Table 9. It was also difficult to directly compare the cost of the respective treatment regimens. Data was presently insufficient to define total dosage requirements and duration of treatment necessary for resolution of mycoses, and although guidelines were available, it was necessary to treat each case individually. However, calculating the cost per day of each formulation at its recommended daily adult dose provided a rough guide for comparison, and showed that all three lipid preparations were

somewhat similar in daily cost and that this cost is considerably higher than the daily cost of Fungizone® therapy.

Table 8 AmB formulations commonly used in the treatment of invasive fungal Infections

Drug	AWP Product Cost	Typical Doses	Estimated Daily Cost for 70 kg Patient
AmBD	$12 per 50 mg (10 ml vial)	0.5 mg/kg/day 1.0 mg/kg/day	$8 $17
ABLC	$135 per 50 mg (10 ml vial) $230 per 100 mg (20 ml vial)	5.0 mg/kg/day	$805
ABCD	$93 per 50 mg (20 ml vial) $160 per 100 mg (50 ml vial)	4.0 mg/kg/day	$448
L-AmB	$188 per 50 mg vial	3.0 mg/kg/day 5.0 mg/kg/day 10 mg/kg/day	$790 $1,316 $2,632

The cost-benefit of the treatments represented a more important consideration than the daily cost of the respective regimens. It was determined that in organ or bone marrow transplant recipients with systemic mycoses, the higher cost of AmBisome® treatment could be offset by the increase in survival and by comparing the cost-benefit derived from the additional life-years gained without alternative use of resources in health care. AmBisome® was also shown to be cost-effective as a short-term prophylaxis in liver transplant prophylaxis.

Table 9 Cost of treatment per day, at the recommended daily adult dose, for Fungizone® and the commercial lipid-based preparations

Treatment	1 mg/kg daily	3 mg/kg daily	5 mg/kg daily
Fungizone	£5.15	NI[a]	NI[a]
AmBisome	£203	£609	NI
Abelcet	£60	NI[a]	£301
Amphocil	£139	£418	NI[a]

[a]Not indicated as this dose