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Lipid Nanoparticles with a Solid Matrix (SLN®, NLC®, LDC®) for Oral Drug Delivery

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Solid lipid nanoparticles (SLN®), nanostructured lipid carriers (NLC®), and lipid–drug conjugates (LDC®), commonly produced by high-pressure homogenization, are interesting vectors for oral delivery of lipophilic and, to a certain extent, hydrophilic substances. Their production can be done without the use of organic solvents. Techniques to make them a physically stable delivery system have been developed. Scaling up of the production process from lab-size to large-scale dimensions using high-pressure homogenization can be easily achieved by using a different type of homogenizer. The machines used for large-scale production often yield an even better product quality than the lab-scale types. This review article covers the methods of production, characterization, mechanisms of oral bioavailability enhancement, scale-up, final oral dosage forms, and regulatory aspects of lipid nanoparticles for oral drug delivery. It focuses mainly on high-pressure homogenization production methods.

Keywords SLN; NLC; LDC; drug nanocrystals; nanosuspensions; oral drug delivery; high-pressure homogenization

INTRODUCTION

Lipid-based drug delivery systems gained increased attention during the last years (AAPS Lipid-Based Drug Delivery Systems Focus Group: www.aaps.org/inside/focus_groups/Lipid). Lipids are known to promote oral absorption of drugs (Charman et al., 1992; Charman, 2000; Charman, Porter, Mithani, & Dressman, 1997; Holm, Porter, Mullertz, Kristensen, & Charman, 2002; Porter & Charmann, 2001a; Stuchlik & Zak, 2001). Vitamins such as vitamin A and E are better absorbed in the presence of fats/lipids (Kuksis, 1987). There are quite a number of drugs for which an increased oral bioavailability is reported when they are administered in the presence of fat-rich food. Examples are testosterone and halofantrine (Charman & Porter, 1996; Holm et al., 2002; Khoo, Porter, & Charman, 2000; Khoo, Shackleford, Porter, Edwards, & Charman, 2003; Porter, Charman, Humberstone, & Charman, 1996; Porter & Charmann, 2001b; Shackleford et al., 2003). An excellent model drug to demonstrate this—which is at the same time of high commercial interest—is cyclosporin A. Delivery of cyclosporin A in the form of a microemulsion (Sandimmun® Optoral/Neoral), the second generation product by Novartis, distinctly reduced the variation in bioavailability which was a major problem of the classic Sandimmun® emulsion. However, an undesired plasma peak occurred, which is being held responsible for side effects such as nephrotoxicity (Martindale, 1989).

In a comparative in vivo study, cyclosporin A was administered as a drug nanocrystal suspension (so called "nanosuspension") and incorporated into solid lipid nanoparticles (SLN®). Nanocrystals consist of 100% drug without any matrix material. The increases in oral bioavailability reported for drug nanocrystals are really impressive for certain drugs. An example is the increase in absolute bioavailability for the drug danazol from 5.1 ± 1.9% as a normal suspension to 82.3 ± 10.1% as a drug nanosuspension (Liversidge & Cundy, 1995). In contrast to this literature data, the results of the cyclosporin A

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nanosuspension study were disappointing; the oral bioavailability was very low and far away from the required pharmacological levels (Penkler, Müller, Runge, & Ravelli, 1999, 2003; Runge, 1998; Müller et al., 2006; Müller, Runge, & Ravelli, 1998).

However, this first in vivo animal study of cyclosporin A-loaded SLN® confirmed the theory of an absorption-enhancing effect of lipids. Instead of using an oil as in Sandimmun® Optoral/Neoral, the oil was replaced by a solid lipid. The lipid particles were already administered in the nanosize range (in contrast to in situ generation of a nanosized microemulsion by breaking in the stomach as in the commercial formulation). The cyclosporin A-loaded SLN® showed a sufficiently high oral bioavailability; they remained within the therapeutic window equally long as the Sandimmun® Optoral/Neoral microemulsion formulation. However, they avoided the nephrotoxic peak above 1000 ng/mL due to controlled release from the solid lipid (Müller et al., 2006; Runge, 1998). This in vivo study demonstrates that lipids can enhance oral bioavailability and that drug nanocrystals without lipids being present can potentially be of limited effect.

SLN® were developed in the beginning of the 1990s. Their characteristic property is that they are made from a solid lipid only (Gasco, 1993; Müller & Lucks, 1996; zur Mühlen, Schwarz, Mehnert, & Müller, 1993). At the turn of the millennium, the second generation, nanostructured lipid carriers (NLC®), was developed (Müller, Mäder, Lippacher, & Jenning, 2000a). Identical to SLN®, the particle matrix is still solid at body temperature, but is produced from a blend of a solid lipid with a liquid lipid (= oil) leading to certain advantages compared to SLN®.

SLN® and NLC® can incorporate only lipophilic molecules efficiently. Loading with hydrophilic molecules can only be achieved to a very low extent by solubilization in the lipid melt. This limits the use of SLN® and NLC® to very potent/low-dose lipophilic drugs such as erythropoietin (EPO) or other potent peptides. Lysozyme was successfully incorporated as "model peptide" (Almeida, Runge, & Müller, 1997). From this point, there was a definite need to create a lipid nano-delivery system for highly dosed hydrophilic actives having a substantial loading capacity (e.g., up to 30%) and exploiting the oral absorption enhancing effect of solid lipids.

An approach for improved oral drug delivery of hydrophilic molecules is the formation of lipid–drug conjugates (LDC®). Many hydrophilic drugs show a poor oral bioavailability due to their explicit hydrophilic character. The lipophilicity of the molecules is not high enough to sufficiently pass the gastrointestinal wall. Transferring them to a LDC® and forming a nanoparticle has three major effects:

1. In case of chemically labile drugs, transfer to an insoluble molecule/particle reduces distinctly the gastrointestinal enzymatic/nonenzymatic degradation.
2. Due to the increase in lipophilicity, the drug shows an improved permeation through the gastrointestinal wall.
3. Promotion of oral absorption takes place by the matrix lipids present in the LDC® nanoparticles.

The presence of lipids in the absorption process (SLN®, NLC®, LDC®) or transfer of hydrophilic molecules to more lipophilic molecules by conjugation (LDC®) can generally increase the oral absorption. The lipid absorption enhancing effect can even be more pronounced when combining lipid technology with nanotechnology, which is creating lipidic solid nanoparticles. This article deals with the production, characterization, and performance of various types of lipid nanoparticles with a solid matrix. Which are as follows:

1. SLN®,
2. NLC®, and the
3. LDC® nanoparticles.

DEFINITIONS

There are basically three different types of lipid nanoparticles with a solid matrix: SLN®, NLC®, and LDC® nanoparticles.

SLN

SLN® are particles made from a lipid being solid at room temperature and also at body temperature. The starting material is solely a solid lipid, e.g., Dynasan 112 (zur Mühlen, Schwarz, & Mehnert, 1998) or other lipids such as Compritol 888 ATO (Souto, Mehnert, & Müller, 2006) or Imwitor 900 (Müller et al., 2006). There are two main production methods for SLN®: the high-pressure homogenization method (Mehnert & Mäder, 2001; Mehnert, zur Mühlen, Dingler, Weyhers, & Müller, 1997; Müller, Dingler, Weyhers, zur Mühlen, & Mehnert, 1997a; Müller & Lucks, 1996; Müller, Mäder, & Gohla, 2000b; Müller et al., 1995; Müller, Schwarz, zur Mühlen, & Mehnert, 1994; Müller, Weyhers, zur Mühlen, Dingler, & Mehnert, 1997b; zur Mühlen et al., 1998;) and the mircoemulsion method (Cavalli, Caputo, & Gasco, 1993; Gasco, 1993). The homogenization method is described in detail in Section 4 of this article. The microemulsion method after Gasco utilizes a basic mechanism of microemulsions: They transform into an ultrafine emulsion by breaking (i.e., after addition of, e.g., water). During production of the microemulsion the solid lipid is melted, the drug is dissolved in the melted solid lipid. Surfactant, cosurfactant and water are then added until a hot microemulsion is formed. This hot microemulsion is subsequently poured into cold water. The microemulsion breaks and ultrafine emulsion droplets develop, which immediately crystallize to form SLN® (Gasco, 1993). Disadvantages of the microemulsion method are the partially required relatively high concentration of surfactants, the organic solvents used to form the microemulsion, and the strong dilution of the particle suspension by pouring the microemulsion into water. This is not very production friendly concerning the subsequent processing to solid oral

dosage forms (e.g., tablets, pellets) as a lot of water needs to be removed to end up with a dry dosage form. However, both production processes—homogenization and microemulsion method—yield an identical product: solid lipid particles dispersed in an aqueous dispersion medium. As described in the patent (Müller & Lucks, 1996), SLN® prepared by high-pressure homogenization can also be produced in nonaqueous dispersion media as long as the dispersion medium does not dissolve the lipid, e.g., liquid polyethylene glycol (PEG) or oils (e.g., mineral oil).

SLN® are an interesting delivery system. However, there are some potential limitations: the relatively low loading capacity for a number of drugs and potential expulsion of the drug during storage. Especially SLN® prepared from one highly purified lipid can crystallize in a more or less perfect crystalline lattice. Such a perfect crystalline structure leaves little room for the incorporation of drugs. Usually, drugs are incorporated between the fatty acid chains, alternatively in between lipid layers or in amorphous clusters in crystal imperfections. The more perfect densely packed the crystal is, the fewer drug can be incorporated.

After production, SLN® crystallize in high-energetic lipid modifications such as α and β'. During storage, the lipid molecules undergo a time-dependent restructuring process leading to formation of the low-energetic modifications βi and β. Consequently, the more perfect lipid crystalline structure leads to expulsion of drug (Westesen, Bunjes, & Koch, 1997). This phenomenon has been well known for a long time from suppositories (Müller, 1986).

NLC

To overcome these limitations, NLC® have been developed (Müller et al., 2000a). Characteristic feature of the NLC® is a controlled nanostructuring of the lipid particle matrix, i.e., creation of a lipid particle matrix as imperfect as possible. To achieve this, spatially very different molecules are mixed. In general a solid lipid is mixed with a liquid lipid (oil). This blend is used to produce the lipid particles that are still solid at temperatures up to about 40°C.

It can be summarized that SLN® are particles produced from a solid lipid only and NLC® are particles produced from a blend of solid lipid with a liquid lipid (oil). This results in differences of the particle matrix structure. NLC® posses many imperfections increasing drug loading capacity and minimizing or avoiding drug expulsion during storage.

LDC

Based on their lipophilic character, SLN® and NLC® have only a limited loading capacity for hydrophilic drugs. This is not a problem in case of highly potent hydrophilic drugs such as EPO: they can be solubilized in the melted lipid matrix using surfactant mixtures or just simply by the mono- and diglycerides present in the lipid anyway (e.g., Imwitor 900). However, a higher loading capacity was not achievable with this kind of lipid nanoparticles. To solve this problem, LDC® particles were developed. The hydrophilic drug is transformed to a more lipophilic, insoluble molecule by conjugation with a lipidic compound. The conjugation can be performed by covalent linkage or simply by formation of a salt with a fatty acid (in case of drugs having for example protonizable functional groups).

The LDC® are poorly water soluble; they typically have a melting range of approximately 50–100°C and can be transformed to nanoparticles using a high-pressure homogenization method similar to the one described for SLN® and NLC®. Considering the molecular weight of the two fractions in the conjugate molecule, i.e., of the drug itself and the lipid part, a drug loading of approximately 30–50% is achievable (e.g., as reported for diminazene of about 33% formulated as diminazene diaceturate–acid conjugate with palmitic acid/stearic acid (Olbrich, Geßner, Kayser, & Müller, 2002a)). LDC® nanoparticles can be made from the conjugated drug only or solid lipids can be additionally admixed to form a mixed matrix of LDC® and lipid.

PRODUCTION ON LAB SCALE

The production method of first choice for the three types of lipid nanoparticles—SLN®, NLC®, and LDC®—is high-pressure homogenization. In case of SLN®, the drug is dissolved or dispersed in the melt of a solid lipid, typically approximately 5–10°C above the lipid's melting point. In case of NLC®, the drug is dissolved in a melted blend of a solid lipid with a liquid lipid (oil), again slightly above the melting point of the lipid blend. LDC® nanoparticles are commonly produced at room temperature. In the next production step, the drug-containing lipid melt or LDC® is dispersed in a surfactant/stabilizer solution of identical temperature by high-speed stirring. This yields an aqueous oil-in-water "pre-emulsion" (SLN® and NLC®) or suspension (LDC®). To achieve a high dispersity in the subsequent homogenization process, it is recommended to use dispersion-efficient, fast diffusing, electrostatically stabilizing, low molecular weight surfactants (e.g., sodium dodecylsulfate [SDS]). They diffuse very fast into newly formed interfacial layers and stabilize the formed small droplets efficiently minimizing subsequent coalescence phenomena. To achieve highest physical stability in gastrointestinal medium, the combination with a steric stabilizer is recommended (e.g., Tween 80 or Poloxamer 188). Steric stabilization is little or less impaired by the presence of electrolytes compared with electrostatic stabilization (high zeta potential). The pre-emulsion/suspension is passed through a temperature-controlled high-pressure homogenizer, either from the piston-gap type (APV Gaulin, APV Deutschland GmbH, Unna, Germany; Avestin Europe, Mannheim, Germany) or from the jet-stream type (microfluidization principle, Microfluidizer®, Microfluidics Inc., Newton, MA, USA). Homogenization

temperature for SLN® and NLC® is again typically about 5°C above the melting point of the lipid phase and room temperature for LDC®. In principle for SLN® and NLC® production, one homogenization cycle at 500 bar is sufficient to yield a hot oil/water emulsion with a particle size of approximately 250–300 nm. Cooling of the nanoemulsion during their production leads to crystallization of the lipid and formation of solid nanoparticles.

Typical homogenization parameters reported in the literature are 500 bar and up to three homogenization cycles. The two additional cycles lead to a further slight reduction in the particle size to approximately 220 nm and a narrowing of the width of the distribution (reduction in the polydispersity index of photon correlation spectroscopy, PCS). However, for oral drug delivery, such a small difference as 30 or 40 nm does not matter. The same is valid for the homogeneity in the particle size distribution. Hence, one cycle at 500 bar is considered as being sufficient for particles intended for oral administration.

LOADING CAPACITY AND DRUG INCORPORATION MECHANISMS

It has to be differentiated between *loading capacity* and *entrapment efficiency*. *Entrapment efficiency* is defined as the percentage of drug incorporated into the lipid nanoparticles relative to the total drug added. It specifies how many percent of drug are included in the particles and how many percent of free drug are still present in the dispersion medium.

Loading capacity refers to the percentage of drug incorporated into the lipid nanoparticles relative to the total weight of the lipidic phase (i.e., lipid + drug). To give an example, we assume a 10% lipid nanoparticle dispersion (i.e., 10% lipid, about 1% surfactant, and 89% water). In this case, the particle mass is equivalent to 10%, the particle mass is defined as the sum of lipid and drug (= 100%). If the drug had a *loading capacity* of 10% in the lipid phase, this would correspond to 1% in the total aqueous lipid nanoparticle dispersion.

Entrapment efficiencies in the literature are relatively high. They range from 80% for the drug tetracaine to about 99% for example for prednisolone (Schwarz, 1995). Of course, *entrapment efficiency* figures might look nice in case only very little drug is added. The more important point is the *loading capacity* of the lipid particles themselves. How many percent of drug can be incorporated into the total lipidic mass of the particles? The loading capacity depends on the solubility of the drug in the solid lipid (in case of SLN®) or the lipid blend (in case of NLC®). In case of LDC® nanoparticles, the *loading capacity* is determined from the ratio of the molecular fractions in the conjugate (e.g., drug and conjugated fatty acid). Examples of loading *capacities* reported for SLN® are 1% for prednisolone (Schwarz, 1995), 10% for tetracaine (Schwarz, 1995), and 20% for cyclosporin A by Runge (Runge, 1998), and even 25% for cyclosporin A by Radtke (Radtke, 2003). In case of very lipophilic compounds, a good solubility in the lipid loading capacities up to 50% can be achieved (e.g., vitamin E) (Dingler, Blum, Niehus, Müller, & Gohla, 1999).

What are the mechanisms of drug incorporation? There are different models described in the literature. The major work has been done by the research group of Mehnert and coworkers (Mehnert & Mäder, 2001; Mehnert et al., 1997; Müller et al., 1995, 1997a; Schwarz, 1995; zur Mühlen, 1996; zur Mühlen & Mehnert, 1998). Basically, there are three incorporation models (Figure 1):

1. Homogenous matrix of solid solution
2. Core-shell model with drug enriched in the shell (= drug-enriched shell)
3. Core-shell model with drug enriched in the core (= drug-enriched core)

In case of a homogenous matrix (model 1, Figure 1, left), the drug is molecularly dispersed evenly in the particle matrix. Drug release takes place by diffusion from the solid lipid matrix and additionally by lipid nanoparticle degradation in the gut.

In model 2 (Figure 1, middle), the drug is enriched in the shell. This can be explained by a lipid precipitation mechanism occurring during particle production. After homogenization, there is a mixture of drug and lipid in each droplet. It is then being cooled. Depending on the TX solubility diagram, the lipid can precipitate earlier than the drug to form a drug-free core or at least a core with reduced drug content (TX diagram: a two-dimensional graphical representation, with temperature and concentration coordinates, of the isobaric phase relationship in a binary system). Reaching the eutectic temperature and composition, lipid and drug precipitate simultaneously in the outer shell of the particles. Examples are coenzyme Q10 SLN. They possess a soft Q10-rich outer shell as proven by atomic force microscopy (AFM) (Dingler, Lukowski, Gohla, & Müller, 1997; Lukowski, Hoell, Dingler, Kranold, & Pflegel, 1998; Lukowski, Hoell, Kranold, Gehrke, & Pflegel, 1997). Drug enrichment in the shell is also a function of the solubility of the drug in the water-surfactant mixture at increased temperature during the production process. The drug partially leaves the lipid particle and dissolves in the aqueous phase during hot homogenization. Reason for this is the increased solubility for many drugs in the outer phase (surfactant solution) at elevated temperatures. Cooling of the oil/water

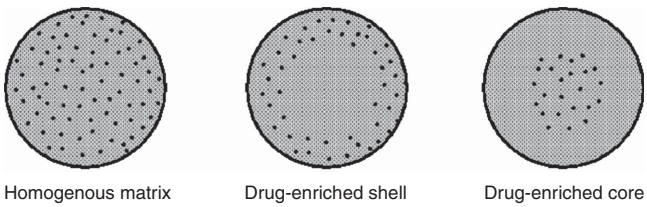

FIGURE 1. Incorporation models for the three types of SLN® (Modified after Müller et al., 2000b).

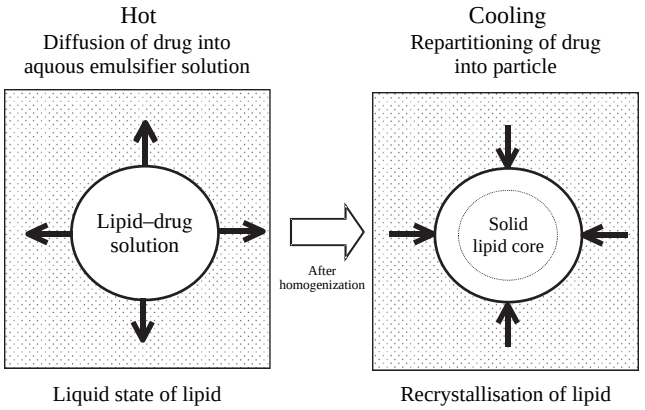

FIGURE 2. Forming of SLN® with drug-enriched shell: during production drug leaves the liquid particle and diffuses into the outer phase, having an increased solubility for the drug Because Of the high temperature. During cooling, drug repartitions into the particle shell. The core of the particle is less/ not accessible because lipid crystallization has started. (Modified after Müller et al., 2000b).

nanoemulsion reduces the drug solubility in the aqueous phase, drug tries to repartition into the lipid particles leading to enrichment in the particle shell in case the particle core already started to solidify (Figure 2). Such particles are known to lead to a burst release (Müller et al., 1995). In the case of the oral cyclosporin A formulation, this was a desired effect. The dissolution rate needed to be sufficiently high to reach the therapeutic drug level but not too high to avoid nephrotoxic levels. In case of other applications, it might be necessary to have a prolonged release formulation which can be achieved by varying the production conditions during the particle production (zur Mühlen & Mehnert, 1998). Such conditions are low production temperature (preferably "cold homogenization") and low surfactant concentration.

Enrichment in the shell (model 2) takes place when particles are produced by hot homogenization method and the drug used shows a distinctly increased solubility in the aqueous surfactant phase at production temperature. By contrast, the drug-enriched core (model 3, Figure 1, right) is formed in case cooling of the hot oil/water emulsion leads to precipitation of the drug first. This takes place preferentially in lipid solutions with drug dissolved at its saturation solubility in the lipid at production temperature. During cooling, a super saturation and subsequent drug precipitation are achieved. The prolonged release of prednisolone is explained by this model (zur Mühlen, 1996; zur Mühlen & Mehnert, 1998).

ORAL DELIVERY OF DRUGS USING LIPID NANOPARTICLES

The first drug intensively investigated to be formulated as SLN® dispersion was cyclosporin A (Müller et al., 1998, 2006; Müller, Runge, Ravelli, Thünemann, Mehnert, & Souto, 2007b; Penkler et al., 1999, 2003; Runge, 1998). As mentioned above, it was known from the literature that the "old" Sandimmun® emulsion showed variations in the oral bioavailability ranging from 10 to 60%. The second generation product, the microemulsion Sandimmun® Optoral/Neoral, avoids this strong variation in bioavailability but possesses potential nephrotoxicity as an undesired side effect due to plasma peaks well above 1000 ng/mL (Meinzer, Müller, & Vonderscher, 1998). The aim of formulating a cyclosporin A-loaded SLN® formulation was to avoid the undesired plasma peak and to achieve a similarly high reproducible oral bioavailability in the therapeutic window. Hence, combination of the advantages of the "old" Sandimmun® (no nephrotoxic plasma peak) and the "new" Sandimmun® Optoral/Neoral (little variation in bioavailability) should be achieved by the SLN® formulation. Three formulations were used in the study: cyclosporin A-loaded SLN® suspension, cyclosporin A drug nanocrystals, and Sandimmun® Optoral/Neoral microemulsion as a reference. The SLN® blood profile did not exhibit the undesired plasma peak and remained over a similar time period time in the therapeutic window as the microemulsion (Figure 3).

At the first glance, it was surprising that the cyclosporin A nanosuspension had such a low bioavailability. This is in contrast to increases in bioavailability reported for a number of drugs by the scientists of the company Nanosystems (nowadays Élan): as discussed above, the bioavailability of danazol could be increased from 5 to 82% by transferring the drug to a nanosuspension (Liversidge & Cundy, 1995). The reasons for the low bioavailability of cyclosporin A nanocrystals might be multifactorial. Cyclosporin A is a substrate of *p*-glycoprotein; thus the absorption is limited a priori. In addition, it cannot be excluded that the drug nanocrystals aggregated in the gastrointestinal fluid. It is known that nanocrystal aggregation can

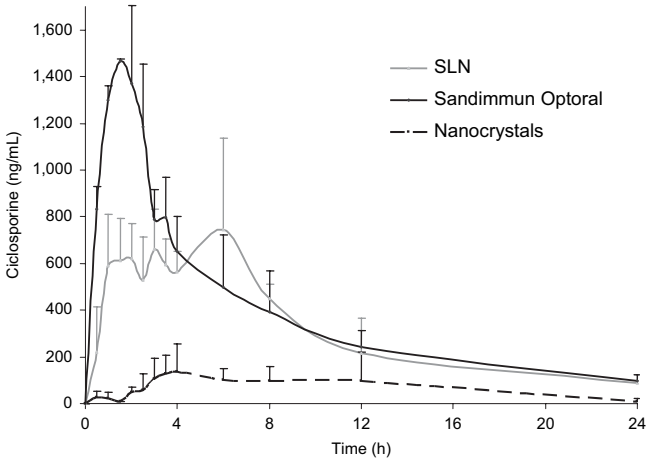

FIGURE 3. Blood profiles of cyclosporin-loaded SLN® suspension, drug nanocrystals, and sandimmun® microemulsion lipids. (Reprinted from Müller, R. H., Keck, C. M. (2004). Challenges and solutions for the delivery of biotech drugs – a review of drug nanocrystal technology and lipid nanoparticles. *J. Biotech., 113,* 151–170, Copyright (2004), with Permission from Elsevier).

reduce dissolution rate (Keck, Fichtinger, Viernstein, & Müller, 2004; Keck, 2006) and subsequently oral bioavailability. Furthermore, it might be that the dissolution rate of cyclosporin A from the crystals was not as high as from the lipid nanoparticles. The cyclosporin A was located—at least to a certain extent—in the outer shell of the SLN® as indicated by the fast release observed in in vitro release studies (Runge, 1998).

In sum, drug nanocrystals, often seen as the ultimate, universal formulation approach for poorly soluble drugs, do not work in any case. For a number of drugs, it seems to be beneficial to have lipids present during the absorption process. This is well in agreement with publications describing the positive effect of lipids on oral drug absorption (Charman, 2000; Charman et al., 1997; Holm et al., 2002; Porter et al., 1996; Porter & Charmann, 2001a). Therefore, the first choice in screening might be drug nanocrystals, but lipid nanoparticles are the essential back up formulation.

MECHANISM OF ORAL ABSORPTION ENHANCEMENT

There are different mechanisms discussed that lead to an absorption enhancement and also to a reproducible bioavailability (little variation in bioavailability). These mechanisms are

1. a general adhesiveness of nanoparticles;
2. reproducibility of adhesion; and
3. an absorption enhancing effect of lipids.

It is a general property of nanoparticles that they are adhesive (Tarr & Yalkowsky, 1989). The adhesiveness of particles to a surface increases with the surface area of the particles; the interaction forces can be calculated (Stieß, 1995). This is a general behavior of all nanoparticles, not specific for lipid nanoparticles. After adhesion to the gut wall, the drug is exactly released at its place of absorption (Liversidge & Cundy, 1995).

This adhesion process proved to be very reproducible. In vivo data obtained with drug nanocrystals show that there is little variability between the fasted and fed state of rats (Liversidge & Conzentino, 1995). The same is considered as valid for the lipid nanoparticles. The low variation in bioavailability observed with cyclosporin A-loaded SLN® suspension was similar to the Sandimmun® Optoral/Neoral microemulsion (Runge, 1998).

It is known that lipids can promote the absorption of active compounds; examples are lipid soluble vitamins such as vitamin A, D, E, and K (Kuksis, 1987). The body absorbs the fat and simultaneously the drug is taken up; it can be considered as a kind of "Trojan Horse" effect.

The lipid absorption enhancing effect can be explained more specifically by the studies performed by W. Charman and coworkers (Charman, 2000; Charman et al., 1992, 1997; Porter & Charmann, 2001a). In sum, the lipids are degraded by enzymes in the gut leading to the formation of surface active mono- and diglycerides on the surface of the lipid droplets or solid lipid particles. These molecules detach and form micelles. During the detachment and micelle forming process, the drug dissolved in the lipid is taken up in the micelle (solubilized). Solubilization is a well-known principle for solubility enhancement in pharmaceutical technology. The formed micelles interact with surface-active bile salts (e.g., sodium cholate) leading to the formation of so-called "mixed micelles." In the subsequent absorption process of the lipid degradation product, the drug is simultaneously absorbed (Figure 4). Charman and co-workers could show that the absorption enhancing effect differs from one lipid to the other. For example, it was observed that long-chain triglycerides (LCTs) are more effective in promoting absorption of the drug halofantrine compared with medium-chain triglycerides (MCTs) (Khoo et al., 2003).

Additionally, the length of the fatty acid chains affects the primary place of absorption. Fatty acids with C-14 chains to C-18 chains promote lymphatic absorption (Porter & Charmann, 2001a). This is of interest for drugs such as testosterone, which undergo a strong first-pass metabolism when they are absorbed through the gut wall and passing the liver.

Lymphatic absorption avoids this first-pass metabolism to a certain degree and can be used to increase the oral absorption of testosterone or testosterone undecanoate. A commercial formulation exploiting this concept is Andriol® capsules with testosterone undecanoate dissolved in oleic acid (Shackleford et al., 2003). Oleic acid as a C-18 fatty acid is ideally suited as oil compound in capsules. The bioavailability of testosterone undecanoate is reported to be $3.25 \pm 0.48\%$ in dogs (Shackleford et al., 2003). As a kind of "second generation formulation" with improved chemical stability, Andriol Testocaps® were launched by the company Organon. The capsules can be stored at room temperature, whereas the "first generation" needs to be stored below 8°C. Andriol Testocaps® contain a blend of oleic acid and castor oil (which major component is the C-18 fatty acid ricinoleic acid) as oil component and additionally a surfactant lauroglycol/FCC (=propylene glycol monolaurate) (Shackleford et al., 2003). The bioavailability reported is about $2.88 \pm 0.88\%$ (Shackleford et al., 2003). It can be imagined that the surfactant being present in this formulation will contribute or promote the formation of mixed micelles. Adding the surfactant moves this formulation to the direction of the self-emulsifying drug delivery systems (SEDDS) (Charman et al., 1992; Pouton, 2000).

Another important point is that for reaching the maximum absorption enhancing effect, the drug needs to be closely associated with the lipid. It is less efficient when drug and lipid are given separately (as it happens during normal food intake and simultaneous drug administration). If a separate administration of lipid and drug worked with similar efficiency, the easiest way to exploit the absorption enhancing effect would be simultaneous administration of a lipid-filled capsule with drug intake (Olbrich, Mehnert, & Müller, 1998). This required very close association of lipid and drug has been realized with the three

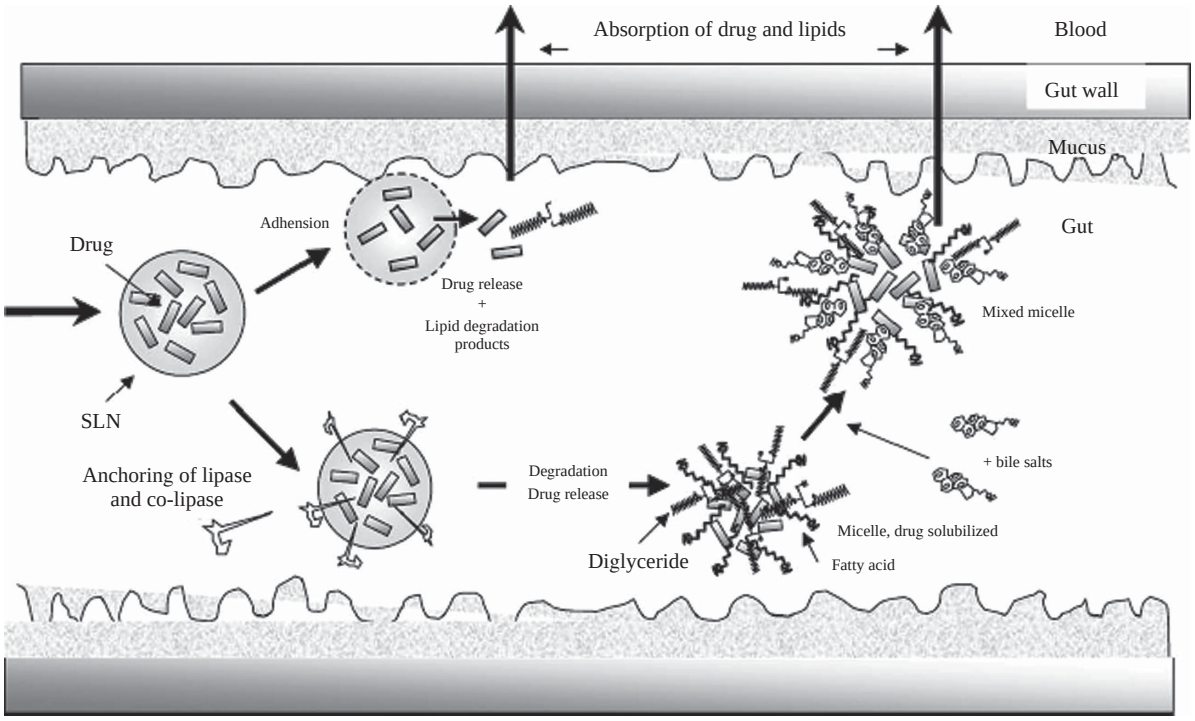

FIGURE 4. Mechanisms of absorption promoting effect of lipids being formulated as a lipid nanoparticle (reprinted from Müller and Keck (2004), Copyright (2004), with Permission from Elsevier).

types of lipid nanoparticles. The drug can be incorporated in between the fatty acid chains or the lipid lamella (Runge, 1998) (Figure 5) or alternatively be present as amorphous clusters in imperfections of the lipid crystal lattice. The surface active compounds in the gut disperse lipids from food to relatively fine droplets. Their diameter is typically in the range of 1–50 µm (Armand et al., 1996; Patton & Carey, 1979). Lipid nanoparticles represent an ultrafine dispersion with typical diameters of about 250 nm (= 0.25µm). Solubilization can take place very fast and efficiently due to the large surface area of the particles. In case a slower degradation is required (e.g., for prolonged release), a fraction of the lipid nanoparticles can be stabilized with a high molecular weight steric stabilizer (e.g., Poloxamer). It could be shown that to some extent the nature of the lipid matrix but dominantly the type of stabilizer affects the enzymatic degradation velocity (Olbrich, 2002; Olbrich, Mehnert, & Müller, 1998; Olbrich, Kayser, & Müller, 2002b).

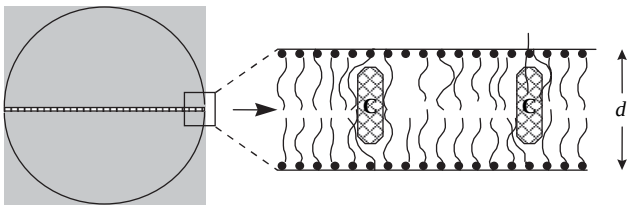

FIGURE 5. Proposed incorporation of cyclosporin A (c) in the lipid matrix of inwitor 900. (Reprinted from Müller et al., 2008, Copyright (2007), with permission from Elsevier).

Stabilization of the lipid particles with a mixture of sodium cholate and lecithin promotes the anchoring of the lipase/colipase complex and thus accelerates the degradation (Olbrich et al., 2002b; Olbrich & Müller, 1999). The use of high molecular weight stabilizers such as Poloxamer 407 leads to a steric hindrance of the anchoring of the complex, thus delaying the lipid degradation. It is also possible to adjust the degradation time between “fast” and “slow” by using mixtures of degradation accelerating and degradation delaying stabilizers (Olbrich & Müller, 1999).

LARGE-SCALE PRODUCTION

In most cases, scaling up of a process encounters problems. This is different for the production of lipid nanoparticles based on high-pressure homogenization. On the contrary, using larger scale machines leads to an even better quality of the product with regard to a smaller mean particle size and its homogeneity (width of the size distribution). Basic advantage is that high-pressure homogenization is a production technique widely used, e.g., in pharmaceutical industry for the production of emulsions for parenteral nutrition (Klang, Parnas, & Benita, 1998). High-pressure homogenization is also used in nonpharmaceutical areas, e.g., in food production, where homogenizers

are running with a capacity of up to several tons of homogenization volume per hour (e.g., for homogenized milk).

Typical machines for lab-scale production are the Micron LAB 40 (batch size: 20–40 mL, APV Deutschland GmbH) and the Avestin C5 (batch size: 7 mL to 1 L, capacity: 5 L/h, Avestin Europe). In case of very expensive drugs or if there is a limited supply (e.g., new chemical entities), it is favorable to reduce the batch size (scaling down capability). Batch size reduction can be done by using the Avestin B3 (Avestin Europe). The batch volume is 0.5–3.2 mL.

A first scaling up step to 500 mL to 1 L can be realized by using the continuous version of the Micron LAB 40. It uses the same homogenization tower as the discontinuous version with only slight modifications. Essential parts such as cylinder, piston, and geometry of the homogenization valve are identical. The continuous version is equipped with product containers. The product is pumped through the valve by a number of piston movements (e.g., for 400 mL homogenization volume, 10 piston movements with 40 mL each are required). In case of the continuous LAB 40 version, the homogenization tower and the two product containers are equipped with temperature control jackets. In theory, one could enlarge the product containers (e.g., to 2 kg); however, the machine is originally not designed to run larger volumes in continuous mode.

The next scaling up step with a minimum batch size of 2 kg and a maximum of 10 kg was achieved using the LAB 60 (APV Deutschland GmbH). The LAB 60 has a homogenization capacity of 60 L/h.

The two product containers of the LAB 60 are equipped with powerful high-speed stirring units. The feeding container has a dissolver disc built in to prepare the pre-emulsion in it. For the production of a 2-kg batch, the pre-emulsion is passed through the homogenization unit, and then it circulates back to the feeding container. It is a continuous homogenization process in a loop. It can be calculated that it takes 15 min to ensure that—statistically—99.9% of the droplets have passed the homogenization valve at least once (Leviton & Pallansch, 1959; Müller, Wissing, & Radomska, 2001). For such a relatively small production volume of 2 kg, it is not sensible to run the homogenization with the LAB 60 in a discontinuous process. The void volume is relatively large (~250 mL). Running discontinuous cycles would leave about 10–15% of the batch each time in the void volume being nonhomogenized. Running a 10-kg batch in continuous circulating required more than 90 min to ensure that 99.9% of the droplets have passed the homogenization gap at least once (Leviton & Pallansch, 1959). Therefore, a discontinuous production is recommended. The 10 kg pre-emulsion is prepared in the feeding container, passed through the homogenizing unit, and then collected in the second product container. When the pre-emulsion has passed the homogenizer, it will be fed back from the second container by gravity via a temperature-controlled tube to the first container. Then the second homogenization cycle starts. In general, two homogenization cycles at 500 bar are fully sufficient to yield a relatively monodisperse product; the total homogenization time for 10 kg is only 20 min (10 min per cycle). As already mentioned, for orally administered lipid nanoparticles, even one single homogenization cycle is considered to be sufficient. In that case, it does not make any difference if the particle size is 260 or 230 nm.

The LAB 60 unit was designed for a pharmaceutical company; that is why it can be used in a GMP area for clinical batch production. As pointed out above, the homogenization results obtained with large-scale machines is typically better than with the lab-scale LAB 40. There are several reasons for this: First of all, transfer of the process from a smaller capacity machine to a larger capacity machine is relatively easy because of the identical or similar geometry of the homogenization valves. Secondly, the larger machines have two or three plungers leading to less fluctuation in the actual homogenization pressure. The LAB 40 has only one plunger; therefore, the pressure needs to build up; the first small fraction of the pre-emulsion is less efficiently homogenized than the rest. This effect is distinctly reduced when a homogenizer is running with three plungers smoothing the pressure fluctuation profile. Furthermore, the temperature control of the larger machines is much more effective. Machines can be ordered equipped with a temperature control unit for the homogenization unit itself. Also, all required tubes and containers can be ordered double-walled for temperature control with a temperature controlling liquid. In addition, these homogenizers are equipped with two homogenization valves in series, main (first) valve and a second valve. When the homogenized droplets leave the first homogenization valve, they possess a relatively high kinetic energy. Their surface might not yet be fully covered with stabilizer. Therefore, limited coalescence can occur. A part of the coalesced or flocculated droplets is immediately re-dispersed when they pass the subsequent homogenization valve. Typically, the second valve operates at one tenth of the pressure of the first valve, e.g., 500 bar for the first valve and 50 bar for the second valve.

The next step in scale-up was the use of a Gaulin 5.5 (APV Deutschland GmbH) with a homogenization capacity of 150 L/h (~150 kg). The pre-emulsion is prepared in larger product containers. Product containers and homogenizer are made from material of pharmaceutical grade. The product containers can be sterilized by autoclaving; preparation of the pre-emulsion under protective gas is possible. They also have the features of cleaning-in-place (CIP) and sterilization-in-place (SIP). For the production, the lipid can be melted in the feeding container. Surfactant and sterile water from a sterile water supply system are then added. Advantageous is a hot storage supply system providing sterile water of 80°C, which is ideal for the production process. This way of production leads also to a very low microbiological load. For oral administration, one homogenization cycle with the Gaulin 5.5 is sufficient. The product is collected in the second product container and cooled in a controlled way under stirring. A batch size of about half a ton

can be produced in approximately 3-h homogenization time with the Gaulin 5.5. For many products, this is already a typical batch size. This way of production was used by the company "Chemisches Laboratorium Dr. Richter GmbH (CLR)" in Berlin to produce the first large-scale batch of NLC® for cosmetic industry to realize the first cosmetic NLC® products (Müller, Rimpler, Petersen, Hommoss and Schwabe, 2007a).

For even larger scales, an Avestin EmulsiFlex C1000 (Avestin Europe) or a Rannie 118 (APV Deutschland GmbH) can be employed. Their capacity is 1, 000/2, 000 L/h at the low pressure required for lipid nanoparticle production.

In this case, it is not sensible any more to prepare a 2-ton batch in a discontinuous way, i.e., preparing the pre-emulsion in a container. The heat exchange times are too long giving a too high temperature burden on the active (cosmetic active or drug). For production lines of this size, static blenders are recommended. The drug containing melted lipid is admixed in a static blender to the hot surfactant/stabilizer solution (e.g., blenders from Sulzer Chemtech, Winterthur, Switzerland). The homogenized product needs to be cooled; the temperature-controlled container will then be replaced by a heat exchanger to remove at least most of the heat. Figure 6 shows a design for such an arrangement.

To sum up, the larger machines are more effective in dispersing and more sophisticated regarding their features to control the process. As a result, the product quality is in general better when moving to larger scale machines.

PRODUCTION OF FINAL ORAL DOSAGE FORMS

In principle, the aqueous lipid nanoparticle suspensions can be used as an oral dosage form. Using optimal stabilizer formulations, a physical stability of up to 3 years has been reported (Müller et al., 1995). Liquid dosage forms might be convenient for certain groups of patients (e.g., children and elderly patients). However, the dosage forms of first choice for delivery are dry forms: tablets, capsules, or fast-dissolving drug delivery systems (FDDS) for the oral cavity. In some countries, sachets are also accepted. Pellets with incorporated SLN® have been produced by Pinto and Müller (1996). The aqueous SLN® dispersion was used as wetting agent in the production process of the pellets. It could be shown that the pellets released the SLN® completely and without or very little aggregation. Such pellets can be filled into hard gelatine capsules or can be compressed to tablets (similar to pellet-containing tablets such as Beloc ZOK®).

Alternatively, the aqueous SLN® dispersion can be used as granulation fluid in the production process of tablets or the SLN® can be spray-dried (Freitas, 1998; Freitas & Müller, 1998) and the obtained powder added to the tabletting mixture (Direct-Compress® technology) (Müller, 1997). As mentioned above, when producing oral dosage forms, the loading capacity of each single dose needs to be considered. In case too much lipid mass is required to dissolve the required drug dose, the excipients necessary to produce pellets or tablets might lead to an unacceptable large volume. In such cases, it might be considered to produce a spray-dried powder for oral administration.

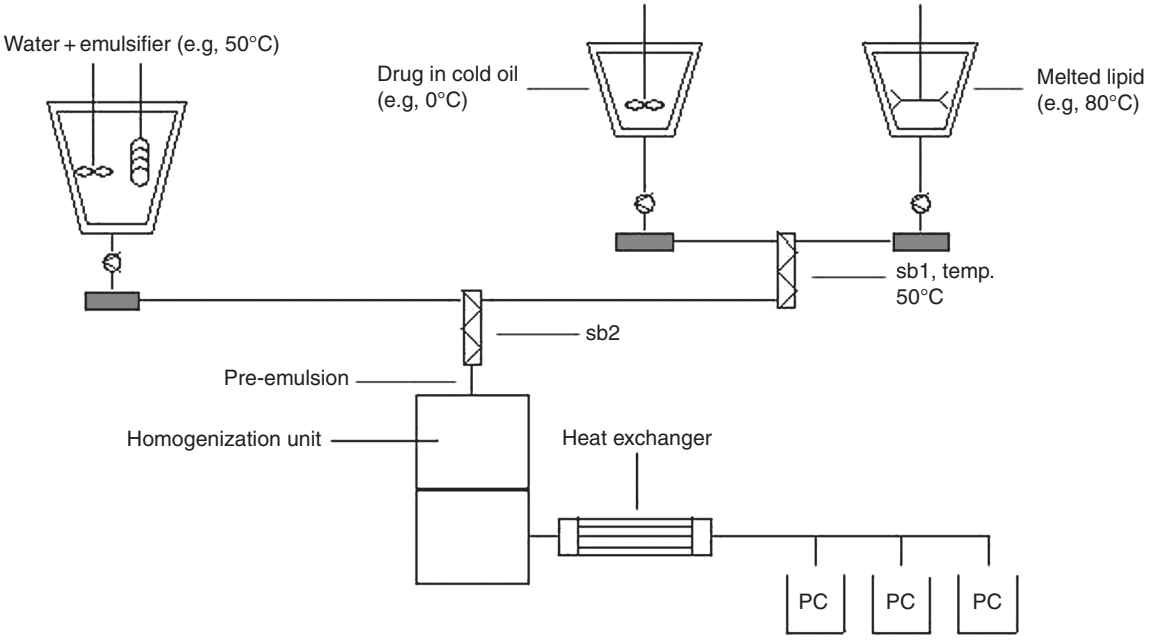

FIGURE 6. Production flow chart for a production capacity of 1–2 tons per hour. First cold drug solution (drug dissolved in oil) and "overheated" melted lipid (e.g., 80°C) are mixed in the first static blender (sb1). Mixing leads to a temperature of the melt of, for example, 50°C, which is still above the melting point of the solid–liquid lipid blend. Then the hot surfactant solution and melted drug-containing lipid are mixed in static blender 2 (sb2) the pre-emulsion passes the homogenization unit, the nanoemulsion is cooled by passing a heat exchanger, and final cooling is performed in the product container (PC).

Another interesting approach is the production of FDDS. The adhesive properties of lipid nanoparticles could be exploited for delivery to the mouth cavity. Incorporation into such an FDDS would be relatively easy by simply lyophilizing the lipid nanoparticle suspension under addition of cryoprotective agents such as mannitol or trehalose.

REGULATORY ASPECTS

One of the key pre-requisites for introducing a new technology and new products to the market is meeting regulatory requirements, not only with regard to excipients but also qualification and validation of production lines. In general, for the production of lipid nanoparticles, only excipients accepted by the regulatory authorities are used, which means excipients with a GRAS status (FDA Summary of GRAS Notices: http://vm. cfsan.fda.gov/~rdb/opagras.html [retrieved 10/07]) or excipients which are already used in products on the pharmaceutical market. In the latter case, these excipients need to be used in their commonly applied, regulatorily accepted concentrations. If distinctly higher concentrations are used, a limited toxicity study might be necessary to prove the safety of the excipient in this concentration. All lipids, surfactants, and stabilizers used in the production of capsules, pellets, and tablets can be fully exploited for the production of oral lipid suspensions. There is definitely no lack of accepted excipients. In addition, all lipids, surfactants, and polymeric stabilizers used in food industry can be employed. However, they need a registration for pharmaceutical purposes. One can refer to the toxicity data collected for registration as food or food additive. To sum up, a rich variety of excipients is available for oral lipid nanoparticles.

Another important point is the qualification of production lines within the general quality management (QM) (Müller, Dingler, Schneppe, & Gohla, 2000). The production lines need to be made out of materials and compounds allowing a qualification of the production line. In addition, other QM steps such as validation need to be performed. Also each compound of the line needs to be capable of validation. Unfortunately, many experimental production lines developed in academic research labs cannot meet these criteria. The production lines for lipid nanoparticles can be validated; for example, a clinical batch production unit is available (Müller, Dingler, Schneppe, & Gohla, 2000). A very important point is that the lipid nanoparticle technology uses production lines already established and existing in the pharmaceutical industry. The regulatory accepted production lines for emulsions can be used for lipid nanoparticle production because many are temperature-controlled anyway. Therefore, it is possible to use the lines of parenteral emulsions for the production of lipid particle suspensions. The only "disadvantage" is that a certain minimum batch size will be required, which is approximately half a ton.

CONCLUSION AND PERSPECTIVES

The three different types of lipid nanoparticles—SLN®, NLC®, and LDC®—represent a promising tool box for the oral delivery of lipophilic but also hydrophilic drugs, especially for hydrophilic drugs showing a reduced stability in the gut and a limited bioavailability. Lipid nanoparticles exploit the absorption enhancing properties of lipids, which are now commonly used for new improved delivery systems and oral dosage forms. Lipid nanoparticles fulfill essential prerequisites for entering the market with a new formulation. Such prerequisites are low cost production, clinical and large-scale production facilities, and accepted status of excipients. In contrast to liposomes, they are not only a low cost system but also physically more stable. Interesting for commercial exploitation is the exclusivity of the various lipid particle technologies; they are protected by issued patens or by patent applications in the major countries. By acquisition of the SLN® technology by SkyePharma PLC in 1999 the technology of SLN® has meanwhile entered pharmaceutical industry. The second generation technology of NLC® is meanwhile present on the cosmetic market in more than 20 products world wide (Müller et al., 2007). The cosmetic products proved the feasibility of NLC® products in general. Also large-scale production lines were established for these products in industry (e.g., Rimpler GmbH, www.rimpler.de), which can also be used for pharmaceutical products. In November 2007, the US pharmaceutical company Brookwood acquired the worldwide exclusive rights for the NLC® technology from Pharmasol to turn NLC® into pharmaceutical products. The future will show if this is successful.

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