Lipid Nanoparticles: Effect on Bioavailability and Pharmacokinetic Changes

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Contents

Abstract The main aim of pharmaceutical technology research is the design of successful formulations for effective therapy, taking into account several issues including therapeutic requirements and patient compliance. In this regard, several achievements have been reported with colloidal carriers, in particular with lipid nanoparticles, due to their unique physicochemical properties. For several years these carriers have been showing potential success for several administration routes, namely oral, dermal, parenteral, and, more recently, for pulmonary and brain targeting. The present chapter provides a review of the use of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) to modify the release profile and the pharmacokinetic parameters of active pharmaceutical ingredients (APIs) incorporated in these lipid matrices, aiming to modify the API

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bioavailability, either upwards or downwards depending on the therapeutic requirement. Definitions of the morphological characteristics, surface properties, and polymorphic structures will also be given, emphasizing their influence on the incorporation parameters of the API, such as yield of production, loading capacity, and encapsulation efficiency.

Keywords Lipid nanoparticles · Lipid polymorphism · Pharmacokinetics · Bioavailability · API release · Orals · Dermalics · Parenterals · Pulmonary delivery · Brain delivery

Abbreviations

AFM	Atomic force microscopy
API	Active pharmaceutical ingredient
AUC	Area under the curve
BBB	Blood–brain barrier
BSC	Biopharmaceutical classification system
CNS	Central nervous system
DSC	Differential scanning calorimetry
EE	Encapsulation efficiency
ESR	Electron spin resonance
FFF	Field flow fractionation
GIT	Gastrointestinal tract
HLB	Hydrophilic–lipophilic balance
HPH	High pressure homogenization
IES	Inter-endothelial cell slits
LC	Loading capacity
LD	Laser diffractometry
LDL	Low density lipoproteins
LHRH	Luteinizing hormone releasing hormone
MPS	Mononuclear phagocytic system
NLC	Nanostructured lipid carriers
NMR	Nuclear magnetic resonance
PCS	Photon correlation spectroscopy
PEG	Polyethylene glycol
RES	Reticulo-endothelial system
SAXS	Small angle X-ray scattering
SEM	Scanning electron microscopy
SFEE	Supercritical fluid extraction of emulsion
SLN	Solid lipid nanoparticles
TEM	Transmission electron microscopy

TPGS D-α-Tocopheryl polyethylene glycol 1,000 succinate
WAXS Wide angle X-ray scattering
YP Yield of production

1 Introduction

The success of drug therapy with is highly dependent on the design of active pharmaceutical ingredients (APIs) delivery. A properly designed delivery system aims to achieve an optimized concentration of the API at the site of action in order to produce a therapeutic response with minimum adverse effects. Nevertheless, individual variations in the pharmacokinetic and pharmacodynamic parameters makes the dosage regimens somewhat difficult to establish. Therefore, novel approaches are being developed e.g. within the field of lipid-based colloidal carriers in order to achieve proper clinical response.

Most conventional formulations are designed to release the API immediately to obtain its rapid and complete systemic absorption. Recently, however, various modified API delivery systems have been developed to release the API at a controlled/well-defined rate. Within those novel delivery systems, the lipid-based colloidal carriers, such as solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC), receive particular attention. A variety of modified-release SLN and NLC designed for different administration routes have been formulated for several APIs, based on their physicochemical and pharmacokinetic properties as well as the effect induced.

Lipid nanoparticles (SLN and NLC) combine advantages of other colloidal carriers, e.g., polymeric nanoparticles, liposomes, and conventional oil-in-water (o/w) emulsions. It has been reported (Kaur et al. 2008; Müller et al. 2000) that: (1) small particles ranging between 120 and 200 nm only rarely undergo blood clearance by the cells of the reticulo-endothelial system (RES), therefore liver and spleen filtration is avoided (Chen et al. 2004); (2) modified release profiles can be obtained when the API is incorporated within the lipid matrix (Hu et al. 2006; Manjunath et al. 2005; Pople and Singh 2006; Saupe et al. 2006; Schwarz and Mehnert 1999; Schwarz et al. 1994); and (3) API targeting can be achieved by means of ligands placed onto the surface of lipid nanoparticles (Lockman et al. 2003). Furthermore, high loadings (for both hydrophilic and lipophilic APIs) (Chen et al. 2001; Fundaro et al. 2000; Reddy and Venkateswarlu 2004), long-term shelf stability (Freitas and Müller 1998, 1999a, b), and the possibility of sterilization and large-scale production (in particular avoiding organic solvents) (Gohla and Dingler 2001; Kuntsche and Bunjes 2007; Manjunath et al. 2005), have also been pointed out. To improve handling and stability, lipid nanoparticle dispersions can be spray-dried, maintaining their colloidal size after reconstitution, and exhibiting good redispersibility (Varia et al. 2008). Other advantages include the lipid composition of SLN and NLC, making them biocompatible, biodegradable, and safe.

2 Definition of Lipid Nanoparticles (SLN vs. NLC)

SLN and NLC are composed of pure lipids or a mixture of lipid compounds (triacylglycerols, fatty acids, steroids, waxes, and oils), and a single surfactant (or in association with a co-surfactant) surrounding the particles. Lipid composition, as well as the production method, will define several nanoparticle characteristics, including the type of surfactant to be selected for SLN/NLC stabilization (anionic, cationic, or non-ionic), the particle size and size distribution, the yield of production (YP), the loading capacity (LC), and the encapsulation efficiency (EE). Obviously, the amount of API that lipid nanoparticles can carry and deliver will also be dependent on its lipophilicity, i.e., the ability of the API to be dissolved in the lipid matrix.

The YP can be measured in terms of nanoparticles produced per dispersion, or as a function of the EE and LC, which are determined as follows:

$$\mathrm{YP} = \frac{W_\mathrm{L}}{V_\mathrm{D}} \times 100 \tag{1}$$

$$\mathrm{EE} = \frac{W_\mathrm{a} - W_\mathrm{s}}{W_\mathrm{a}} \times 100 \tag{2}$$

$$\mathrm{LC} = \frac{W_\mathrm{a} - W_\mathrm{s}}{W_\mathrm{a} - W_\mathrm{s} + W_\mathrm{L}} \times 100 \tag{3}$$

where W_L is the weight of lipid added in the formulation, V_D is the volume of the aqueous phase, W_a is the weight of API added in the formulation, and W_s is the weight of API analyzed in the supernatant (after separation of lipid and aqueous phases by centrifugation). EE is thus defined as the ratio between the mass of entrapped API and the total mass of API, whereas LC is the ratio between the mass of entrapped API and the total mass of lipid. Factors determining LC and EE are: (1) the solubility and miscibility of the API in the melted lipid phase, (2) the physico-chemical structure of the solid lipid matrix, and (3) the polymorphic state of the lipid material (Kaur et al. 2008).

High encapsulation parameters are obviously desirable, since they can reduce the number of particles required to achieve therapeutic levels. Depending on their lipophilicity and hydrophilicity, APIs will be located in the lipid nanoparticles in a particular way. To achieve a high EE and LC for a particular API, its sufficiently high solubility in the melted lipid is the main requisite (Wissing et al. 2004). Therefore, hydrophilic molecules are hardly incorporated due to their low affinity with the lipid matrix. Moreover, API solubility should in general be higher in the melted lipid state that in the solid state, since the solubility usually decreases when the melt cools down, and it might even be lower in the solid lipid. However, biotechnological APIs have successfully been loaded into SLN (Almeida et al. 1997; Müller and Keck 2004a). To enhance solubility in the melted lipid,

solubilizers can be added. Examples of these are non-ionic surfactants such as polysorbates and polyoxyls, covering a hydrophilic–lipophilic balance (HLB) range between 2 and 18, which can be used in combination with lipids to promote self-emulsification (Gibson 2007). Furthermore, when using mono- and di-acylglycerols as lipid matrix composition, API solubility might increase in comparison to very pure lipids, such as monoacid triacylglycerols. Naturally occurring oils and fats comprise mixtures of mono-, di- and tri-acylglycerols, containing fatty acids of varying chain length and degree of unsaturation (Hauss 2007). The melting point of these lipids increases with the length of the fatty acid chain, and decreases with the degree of unsaturation. The chemical nature of the lipid is also important because lipids which form highly crystalline particles with a perfect lattice (e.g., monoacid triacylglycerols) lead to API expulsion during storage time. Mixtures of lipids containing fatty acids of different chain length form less perfect crystals with many imperfections offering space to accommodate guest molecules. Therefore, an important issue to be addressed in the lipid nanoparticle formulation is the selection of the lipid excipients. Although a systematic procedure to select an appropriate lipid composition has not been published yet, there are a number of criteria to be kept in mind. These are the API lipophilicity (Log *P*), in particular solubility in pharmaceutically acceptable lipids, which should be sufficient to allow the required therapeutic dose of API to be administered.

Physicochemically stable lipid nanoparticles will be obtained only when the right surfactant and adjusted concentration have been employed. For a particular lipid matrix, the surfactant composition is usually chosen according to its HLB, which is based on packing parameter theory (P) (Israelachvili et al. 1980).

SLN/NLC dispersions have been stabilized with surfactants having HLB values below 12. Nevertheless, one needs to keep in mind that lipid molecular characteristics, bulk, and surface properties strongly affect physicochemical stability and suitability of SLN/NLC as nanoscaled API delivery systems (Bummer 2004; Wissing et al. 2004).

Another critical situation is the risk of peroxidation of the materials used to produce SLN/NLC. It is well known that a number of lipids and surfactants are susceptible to oxidation, and may create highly-reactive peroxide species (Mead et al. 1986). Lipid peroxidation can be deleterious to the physicochemical stability of both the API and the SLN/NLC dispersion. Nevertheless, such phenomena can be limited and rationally controlled using anti-oxidants.

Polymorphism is also an important issue determining both EE and LC (2 and 3). To create a solid matrix, crystallization of the lipid occurs differently in SLN/NLC than in bulk material, i.e., the lipid matrix recrystallizes at least partially in the α-form (unstable polymorphic form) or in the β′-form (metastable polymorphic form), while the lipid as a bulk tends to recrystallize preferentially in the β′-form, which transforms quickly into the β-form (Westesen et al. 1993). During organization into more stable polymorphic forms, the number of imperfections in the lipid lattice decreases, i.e., formation of β′/β-modification promoting API leakage. Generally, the transformation is slower for long-chain than for short-chain triacylglycerols. An optimized SLN/NLC formulation can be generated in a controlled

way when a certain fraction of the β′-form is created and preserved during the storage time. Within this concept, SLN/NLC can be considered intelligent API delivery systems achieving a built-in triggering mechanism to initiate transformation from β′- to β-forms and consequently controlled API release (Jenning and Gohla 2001). The connection between the physical properties of SLN/NLC and their in vitro and in vivo performance should always be addressed (Kristl et al. 2003; Westesen et al. 1997), and therefore studies on the inner structure should always be carried out, since their lack can cause misinterpretation of the in vivo results (Westesen and Bunjes 1995).

Finally, the production procedures critically influence the bioavailability of loaded APIs since they affect the design and the structure of the system itself.

To produce SLN and NLC, the high pressure homogenization (HPH) procedure is typically applied, either the hot or the cold technique (Souto et al. 2007). For the hot HPH the lipid phase is previously heated 5–10°C above its melting point, followed by API dissolution or fine dispersion in the melted phase. Stirring this melted phase in a hot surfactant solution, a pre-emulsion will be produced. The pre-emulsion is homogenized under high pressure producing a hot nanoemulsion, which is further cooled, recrystallizing the lipid and forming SLN or NLC. The cold HPH technique requires a previous step of melting the solid lipid so that the API can be dissolved and/or admixed in this phase. By applying liquid nitrogen or dry ice, the lipid phase cools down rapidly, solidifying, and then by means of mortar milling it is ground to obtain microparticles. These microparticles are further dispersed in a cold aqueous surfactant solution producing a pre-suspension that is homogenized at or below room temperature using the HPH.

Other methods reported in the literature include those that require also the melting of lipid phase: i.e., the microemulsion (Bondi et al. 2007, 2003; Brioschi et al. 2008; Cavalli et al. 1997, 1998, 2001; Fontana et al. 2005; Mandawgade and Patravale 2008; Miglietta et al. 2000; Ugazio et al. 2002), the phase-inversion (Anton et al. 2008, 2007; Jayagopal et al. 2008; Malzert-Freon et al. 2006), and the extrusion techniques (El-Harati et al. 2006; Joshi and Patravale 2008), and those based on the previous dissolution of the lipid in an organic solvent (non-polar, semi-polar, or polar) (Cortesi et al. 2002; Hu et al. 2002, 2005, 2006, 2008; Trickler et al. 2008). In comparison to the HPH technique, these methods are reported to achieve low lipid nanoparticle YP (1) (Mehnert and Mäder 2001; Müller et al. 2000).

More recently, supercritical fluid technology has also been adapted to produce lipid nanoparticles (Chattopadhyay et al. 2007; de Sousa et al. 2006, 2007; Young et al. 2004). In particular, supercritical fluid extraction of emulsions (SFEE) has been reported to show high YP (Chattopadhyay et al. 2007). The method allowed the production of stable SLN of a narrow size distribution, with a mean diameter below 30 nm. Thus, the particle size obtained was significantly smaller than that reported by other techniques. The residual solvent content in the final suspension was shown to be below 20 ppm. When the o/w emulsion containing the lipid and the API is introduced into the supercritical CO_2 phase, parallel processes of solvent extraction into the supercritical CO_2 phase and inverse flux of CO_2 into the emulsion droplets occur, leading to expansion of the organic phase of the emulsion.

This leads to precipitation of lipid-API material dissolved in the organic phase producing the solid matrix. The solvent extraction efficiency using supercritical CO_2 is much higher than for the conventional methods such as evaporation, liquid extraction, and dilution, providing a more uniform particle size distribution, because of the fast removal of the organic solvent. Supercritical CO_2 also tends to extract other low-molecular weight impurities, purifying the lipids. In addition, supercritical CO_2 typically results in a depression of the lipid melting point and plasticization of the amorphous lipid structures. This plasticization can be beneficial in establishing a thermodynamically stable lipid form, such as β-polymorph of the triacylglycerol, facilitating as well a more uniform distribution of the API within the lipid phase. The size of SLN obtained in the SFEE process is directly related to the emulsion droplet size and is therefore dependent upon the method of formulation and the stability of the emulsions employed for precipitation.

With regard to the design and structure of the systems, basically the structure of both SLN and NLC is composed of a solid core covered by a layer of surfactant molecules. In the following sections the different types of each will be described.

2.1 Solid Lipid Nanoparticles (SLN)

The SLN Type I is defined as the homogeneous matrix model, because the API is molecularly dispersed in the lipid core or is present in form of amorphous clusters (Mehnert and Mäder 2001; Müller et al. 2000; Souto et al. 2007; Souto and Müller 2007). This model is obtained when using optimized ratios of API and lipid passing through the HPH at above the melting point of the lipid, or when using the cold HPH technique. As consequence of their structure, SLN Type I can show controlled release properties. The SLN Type II, or API-enriched shell model (Lukowski and Werner 1998), is obtained when the API concentration in the melted lipid is low. After applying the hot HPH technique, during the cooling of the homogenized nanoemulsion, the lipid phase precipitates first, leading to a steadily increasing concentration of API in the remaining lipid melt with increased fraction of solidified lipid. An API-free (or API-reduced) lipid core is formed; when the API reaches its saturation solubility in the remaining melt, an outer shell containing both API and lipid will solidify around this core which contains low amount of API. This model is not suitable for prolonged API release; nevertheless, it may be used to obtain a burst release of API, in addition to the occlusive properties of the lipid core. The SLN Type III, or API-enriched core model (Souto et al. 2004b; Westesen et al. 1997), is formed when the API concentration is relatively close to or at its saturation solubility in the lipid melt. On cooling the nanoemulsion, the solubility of the API will decrease; when the saturation solubility is exceeded the API precipitates, and is covered by a shell of lipid almost free of API. This SLN type is useful for achieving a prolonged release of API since it is immobilized within the lipid core.

2.2 Nanostructured Lipid Carriers (NLC)

NLC are also composed of a solid core covered by the surfactant used during the production procedure. For these carriers also, three incorporation models have been proposed, mainly differing in the type of lipid compounds used for their production.

The NLC Type I is termed the imperfect crystal model, and consists of a matrix with many voids and vacancies that are able to accommodate the API. These particles are obtained when mixing solid lipids with a sufficient amount of liquid lipids (oils). Due to the different chain length of the fatty acids and the mixture of mono-, di- and triacylglycerols, the matrix of NLC is not able to form a highly ordered structure (Müller et al. 2002), thus creating available spaces (structural imperfections). The NLC Type II, or the amorphous model, is obtained when mixing special lipids (e.g., hydroxyoctacosanylhydroxystearate, isopropylmyristate, dibutyl adipate) that do not recrystallize after homogenization and cooling of the nanoemulsion. These lipids create amorphous matrices, which avoid/delay the recrystallization phenomenon of lipids on cooling and during shelf life, thus minimizing API expulsion during storage time. The NLC Type III is defined as the multiple model because it is composed of very small oily nanocompartments created inside the solid lipid matrix of the nanoparticles by a phase separation process (Müller et al. 2002). It results when mixing solid lipids with oils (e.g., medium (Hu et al. 2006) and long-chain triacylglycerols (Souto et al. 2004a), oleic acid (Hu et al. 2005) in such a ratio that the solubility of the oil molecules in the solid lipid is exceeded. During the cooling of the nanoemulsion the lipid droplets reach the miscibility gap (40°C), and the oil precipitates forming tiny oil droplets. Subsequent solidification of the solid lipid surrounding these droplets leads to fixation of the oily nanocompartments. The advantage of this model is the increase of LC for APIs of higher solubility in liquid lipids than in solid lipids (Jenning et al. 2000). The structure of NLC Type III defined by the presence of nanocompartments or nanostructures within the matrix is still a controversial subject (Castelli et al. 2005; Jores et al. 2003, 2004, 2005; Müller et al. 2002). The precise structure may be intrinsically dependent on the composition of the formulation (i.e., lipid, surfactant, and API), as well as on the production procedure (Schäfer-Korting et al. 2007). These theoretical NLC models have been established based on analytical data, which can be used to physicochemically characterize NLC matrices.

Several techniques have been applied to outline the physical and chemical inner organization of SLN/NLC, such as differential scanning calorimetry (DSC), nuclear magnetic resonance (NMR), electron spin resonance (ESR), and small angle and wide angle X-ray scattering techniques (SAXS, WAXS) (Castelli et al. 2005; Jores et al. 2003; Mayer and Lukowski 2000; Zimmermann et al. 2005). DSC, WAXS and SAXS are useful for characterizing the polymorphic forms of lipid molecules of the nanoparticle matrix, which are dependent on the lipid and surfactant composition. NMR and ESR are useful for evaluating the dynamic phenomena and the presence of oily nanocompartments, which are characteristic of NLC Type III (Müller et al. 2000). Other analytical procedures for assessing morphology, surface

characteristics, and particle size include microscopic analysis, e.g., scanning (SEM) and transmission (TEM) electron microscopy, and atomic force microscopy (AFM) (Mehnert and Mäder 2001; zur Mühlen et al. 1996), as well as photon correlation spectroscopy (PCS), laser diffractometry (LD), and field flow fractionation (FFF).

3 Effects of Lipid Polymorphism on API Bioavailability

When the lipid bulk material is formulated as nanoparticles (solid lipid core surrounded by surfactant molecules) the formulation will show altered properties (Bummer 2004). These properties are due to (1) the changes involved in the physical state of lipid molecules, (2) the level of molecular interaction within the lipid core and with the aqueous surfactant environment, and (3) the energies involved. When decreasing the particle size below a submicrometer range, a relative increase of the surface area will occur, with a high curvature radius followed by higher energy of interaction between the lipid/surfactant/API molecules. This will clearly influence the bioavailability of API-loaded SLN/NLC, since the nanoparticle dose administered is proportional to the loading capacity (2) as well as to the number of particles per volume.

The inner structure is another important parameter that dramatically changes when decreasing the particle size (Bunjes et al. 2000; Lippacher et al. 2002). Since SLN/NLC are composed of pure lipids or mixtures of short, medium or long mono-, di- and triacylglycerols, their inner structure will be very different in comparison to the bulk material.

As mentioned previously, to transform the bulk lipid into nanoparticles, the lipid has to be either melted or solubilized in an organic solvent, followed by cooling down or solvent removal, respectively, so that the lipid recrystallizes, becoming solid again. Generally, recrystallization of melted lipid molecules creates an unstable hexagonal α-form which is converted, via a metastable orthorhombic β′-form, into a more stable triclinic β-form upon reheating and storage (Bunjes et al. 1996; Freitas and Müller 1999a; Westesen and Siekmann 1997). The particle size is the main factor affecting the transition rate from α to β′ to β, which is much faster in colloidal lipid particles that in the bulk lipid. Furthermore, the occurrence of such transitions is higher when using lipids of lower melting points. The LC and EE (2 and 3) are intrinsically dependent on these transition rates. The changes in the physical structure of the lipid matrix also influence both the particle shape and morphology. In general, a platelet-like shape is observed when the content of the β-form is higher. Depending on the particle size, different shapes will be observed with the increase of the α-form. Larger nanoparticles (>200 nm) are usually more spherical, while smaller nanoparticles (<100 nm) are characterized by a blocky isometric layered shape (Bunjes et al. 2003). Polymorphic transitions followed by changes in the particle surface area will obviously influence the physical stability of the lipid nanoparticle dispersions (Westesen and Siekmann 1997; Lukowski et al. 2000).

According to the lipid chain length, the melting and crystallization temperatures of the SLN/NLC dispersions are very different from the bulk materials (Bunjes et al. 1996). Depending on the lipid structure, crystallization does not always occur, creating so-called supercooled melts (Westesen et al. 1997). Since it is difficult to predict and to characterize the actual physical state of the lipid matrix, in vitro or in vivo performance of the systems might be easily misunderstood. In fact, supercooled melts behave mainly as emulsions. A comparison study has been run between SLN composed of different triacylglycerols varying in their chain length. Due to their higher melting point, tristearin and tripalmitin SLN were crystalline at room temperature, whereas trimyristin and trilaurin nanoparticles maintained their liquid status, behaving as emulsions for several months upon storage under the same conditions. By DSC analysis it was observed that trimyristin SLN started recrystallizing at 10°C while trilaurin SLN were still liquid at 4°C (Bunjes et al. 1996). Such a phenomenon was attributed to the small size of the particles, which can reduce their melting point by several degrees in comparison to the bulk material. The same effect may also happen for the crystallization temperature. Thus supercooled melts may often occur, especially for lipid mixtures, short-chain lipids or less pure ones. The possibility of polymorph coexistence strongly influences lipid nanoparticle stability. Trilaurin exhibits four different polymorphs, i.e., α, β', β_1, and β_2 (Lippacher et al. 2000). Upon fast cooling, trilaurin SLN recrystallized directly into the metastable α-form. Other than with NLC, this factor strongly affects API loading in SLN. Although high EE (3) have been reported, especially for lipophilic APIs, the LC (2) in SLN is limited by their small size. Lipid polymorphic structures often undergo modification upon API loading as a result of the intercalation of the API between lipid layers (Westesen et al. 1997). An acylglycerol behenate SLN formulation showed small amounts of the unstable α-form that disappeared upon heating or when loading the system with the API (Hou et al. 2003).

Generally, the presence of guest molecules in the lipid matrix also influences its crystallization degree, decreasing the lipid layer organization. In fact, depending on the lipid chain length, a depression of melting and crystallization temperatures is usually reported, indicating a strong tendency towards supercooling (Westesen et al. 1997; Bunjes et al. 1996). Moreover, the LC and EE (2 and 3) are generally higher in the case of mixtures of acylglycerols as a result of their lower crystallinity in comparison to pure lipids (Westesen et al. 1997). Such a characteristic influences API distribution and motility and also the pharmacokinetics and biodistribution. An increase of LC from 1% up to 50% caused dramatic changes in the lipid structure; API leakage from the lipid matrix occurred upon storage. The rate of API expulsion was dependent on the lipid matrices' composition and this feature was correlated to the rate of polymorphic transformation (Westesen et al. 1997). Nevertheless, stable mifepristone-loaded SLN formulations could be produced with less ordered crystalline organizations (Siekmann and Westesen 1994). This has been attributed to the less rigid and unordered structures which can provide vacancies to guest molecules, and their expulsion is less likely to occur upon storage. Furthermore, lipid nanoparticles of spherical shape are usually of lower crystalline status. If the formulation

is not intended for controlled/prolonged API release, supercooled melts may be a suitable alternative, since in some cases they can enhance the solubility of poorly soluble APIs and increase both LC and EE. Nevertheless, these melts are not thermodynamically stable, having the risk of long-term recrystallization.

4 Lipid Nanoparticles Applications

SLN and NLC have been proposed as alternative carriers to well-known liposomes and polymeric nanoparticles in order to overcome some of their common problems, achieving API bioavailability enhancement, controlled release, and API targeting. Due to the high lipid biocompatibility, virtually all the existing administration routes are possible and many of them have been investigated, namely the oral, ocular, topical, dermal and transdermal, pulmonary, and parenteral delivery. Several examples will be given in the following sections.

4.1 Oral Delivery

Oral delivery of poorly soluble APIs remains a significant challenge in pharmaceutical technology. Nevertheless, the ability of lipid-based formulations to facilitate absorption from the gastrointestinal tract (GIT) is well documented, and the pharmacological activity of API is not impaired.

Lipids are considered to be safe materials in the development of API delivery systems (Müller et al. 1997a; Schwarz et al. 1994; Wissing et al. 2004). This is easily exemplified by emulsions and microemulsions, which have widely been used to enhance the absorption and bioavailability of APIs belonging, respectively, to class III and class II of the Biopharmaceutical Classification System (BCS, Table 1) (Bummer 2004). The stability of such systems is strictly related to particle size distribution, lipid content, and presence of a surfactant able to stabilize the dispersion. The molecular properties of the phases involved deeply influence the lipid organization and its assembly.

Clinical applications of very potent agents are in general difficult to assess because of the high risk of API toxicity, poor oral bioavailability, insolubility,

Table 1 Biopharmaceutical classification system (BCS)

Class	Solubility	Permeability	In vitro/In vivo correlations
I	High	High	Easy to establish bioequivalence
II	Low	High	In vitro dissolution is similar to in vivo dissolution
III	High	Low	Absorption is the limiting factor
IV	Low	Low	Difficult to establish bioequivalence

and poor physicochemical stability. One possibility to overcome such limitations is the incorporation of those APIs in lipid nanoparticles. Micro- and nanoencapsulation in lipid-based colloidal delivery systems is usually applied to enhance API stability, increase oral bioavailability, reduce adverse side effects and/or API toxicity, and also has the possibility to modify the API release profile.

Cyclosporine A is an example of a hydrophobic cyclic peptide that shows low oral bioavailability, about 30% (Fahr 1993; Noble and Markham 1995). In addition, the absorption rate and extent is limited by several factors, such as food intake, bile production, and GIT motility. Many attempts have been made to enhance cyclosporine bioavailability using different dosage forms. The commercial microemulsion Sandimmun Neoral/Optoral®, commonly administered in many therapies, consists of oil, propylene glycol and, as surfactant, polyoxyl-40 hydrogenated castor oil; the amount of cyclosporine in this microemulsion is about 10%. With the purpose of the development of an improved oral cyclosporine delivery system to treat autoimmune diseases and to prevent transplant rejection, this immunosuppressive API has been formulated into SLN using several production procedures, e.g., HPH (Müller et al. 2006, 2008; Varia et al. 2008), via the microemulsion method (Ugazio et al. 2002), or by means of organic solvent diffusion (Hu et al. 2004b). The effect of lipid composition and particle size on the oral cyclosporine bioavailability has been assessed. The formulations composed of API, acylglycerol monostearate as solid lipid and a combination of surfactant/cosurfactant (Tagat/sodium cholate), resulted in physicochemically stable SLN of approx. 160 nm (PCS mean diameter) (Müller et al. 2006). The oral bioavailability of the peptide was determined in pigs following the cyclosporine blood levels after oral administration of the SLN formulation, in comparison to the commercial Sandimmun Neoral/Optoral®. Administration of cyclosporine-loaded SLN led to a mean plasma profile with almost similarly low variations in comparison to the commercial formulation, however, no initial blood peak was observed with the Sandimmun Neoral/Optoral®.

SLN composed of stearylamine as solid matrix and produced by a solvent diffusion method showed a burst release of 18% cyclosporine over the first 12 h, followed by a sustained release over 16 days when about 4% of the peptide was released per day (Hu et al. 2004b). The release kinetics were dependent on the composition of the lipid matrix (Varia et al. 2008).

Despite of the high EE (3) achieved for cyclosporine in SLN, e.g., 100% with the optimized lipid and surfactant composition (Varia et al. 2008), the bioavailability ranged from 20 to 60%. Concerning colloidal carriers, a correlation between the particle size and the oral bioavailability of cyclosporine formulations has been reported. Nanoparticles composed mainly of solid triacylglycerols (e.g., tricaprin, trilaurin, tristearin) and a certain amount of hydrogenated vegetable oil, stabilized by egg or soybean phosphatidylcholine, revealed higher cyclosporine bioavailability when the particle size was below 60 nm (Bekerman et al. 2004). In fact, several examples emphasize that the GIT uptake of APIs loaded on nanoparticles is greater when compared to microparticles (Bekerman et al. 2004; Desai et al. 1996, 1997; Pescovitz et al. 1992).

Attempts have also been made to incorporate hydrophilic peptides/proteins within lipid matrices. Successful examples in SLN are gonadorelin (Hu et al. 2004a), insulin (Battaglia et al. 2007; Gallarate et al. 2008; Sarmento et al. 2007; Zhang et al. 2006a, b), and salmon calcitonin (Garcia-Fuentes et al. 2003; Martins et al. 2009).

An EE of 70% was achieved for gonadorelin in SLN, and the peptide-loaded SLN revealed a PCS diameter of about 420 nm with a zeta potential of −22 mV (dispersed in distilled water) (Hu et al. 2004a). The in vitro release assay was performed in simulated GIT conditions revealing a biphasic profile, i.e., after a burst release of 24.4% of loaded gonadorelin within the first 6 h, a distinctly prolonged release over a monitored period of 12 days was observed and nearly 3.81% gonadorelin was released per day. Insulin was incorporated in SLN by a modified double-emulsion procedure, achieving an EE of approx. 40% (Gallarate et al. 2008; Sarmento et al. 2007). Cetylpalmitate-based SLN were orally administered to diabetic rats and a considerable hypoglycemic effect over 24 h was observed (Sarmento et al. 2007). Trimyristin-based SLN showed a mean diameter of 200 nm with a calcitonin EE of approx. 86% (Martins et al. 2009). This protein was released at a rate up to 8 h, under both gastric and intestinal simulated pH conditions. Being hydrophilic in nature, salmon calcitonin is not soluble in SLN matrix, therefore a novel production procedure based on a double w/o/w emulsion technique has been developed (Martins et al. 2009).

The pharmacological activity of calcitonin was evaluated following oral dosage of protein-loaded SLN in rats. When loaded into SLN, calcitonin decreased the basal blood calcium levels by up to 20% with 500 IU/kg for at least 8 h (Martins et al. 2009). The minimum calcium serum level was obtained 1 h after administration. In contrast, the serum calcium levels increased due to the stress induced in the rats during administration following calcitonin solution testing for reference (Martins et al. 2009). The efficacy of calcitonin-loaded SLN was attributed to SLN uptake through Peyer's patches. In fact, the ileum is an ideal site for nanoparticle uptake, where abundant Peyer's patches exist with proteolytic enzyme activity (des Rieux et al. 2006). The paracellular pathway has also been shown to contribute to protein absorption; most protein and polypeptide APIs diffuse through the aqueous-filled tight junctional pathway due to their hydrophilic nature (Salamat-Miller and Johnston 2005). Thus salmon calcitonin released from SLN within GIT might be immediately absorbed. However, due to the tightness of the junctions of the intercellular spaces, the calcitonin absorption rate might be somewhat reduced (Salamat-Miller and Johnston 2005).

Another example of enhanced API uptake from GIT is tobramycin, which is not absorbed following oral administration. Loaded into SLN and administered duodenally, tobramicin was targeted to the lymph, showing a high availability and a sustained release profile (Bargoni et al. 1998, 2001; Cavalli et al. 2000b).

The poorly soluble fenofibrate formulated in SLN and as API nanocrystals (so-called DissoCubes®) was investigated in rats following oral administration; two nanosuspensions of micronized fenofibrate were used as reference (Hanafy et al. 2007). Both colloidal delivery systems showed approximately two-fold bioavailability enhancement in terms of rate and extent compared to the reference

formulations. Between SLN and nanocrystals no significant differences were found in AUC, C_{max} and t_{max}.

Factors increasing solubility of APIs in GIT are solubilising agents, bile salts, and lecithin from intestinal fluid making contact with the lipid nanoparticles (Dressman and Reppas 2000). Moreover, the surfactant vitamin E TPGS figuring in the SLN composition can enhance the solubility as reported for spironolactone (Langguth et al. 2005). A 5.7-fold bioavailability enhancement was observed for the spironolactone-loaded SLN composed of of 9.5% vitamin E TPGS and 10% vitamin E6-100. The small particle size was not the major factor for bioavailability improvement, but the type of surfactant used in the formulation. The greater improvement in bioavailability for spironolactone formulated with vitamin E TPGS could be explained by an additional P-glycoprotein inhibition (Dintaman and Silverman 1999). Since spironolactone has affinity to the P-glycoprotein efflux pump (Wu and Benet 2005), combining the P-glycoprotein substrate with an inhibitor may improve and enhance absorption and API bioavailability. Developing SLN/NLC with vitamin E TPGS may be a very interesting approach to increase oral uptake for other poorly soluble drugs and also those which are P-glycoprotein substrates.

Liquid dosage forms are extremely important, in particular for elderly people and children, due to their difficulties in swallowing solid dosage forms. API-loaded SLN/NLC dispersions show multiple advantages to overcome such limitations, since they can be added to fruit juices or yogurts, to syrups simplex, and can even be loaded into soft gelatine capsules which are easy to swallow. Furthermore, the latter approach can also take advantage of using phospholipids as surfactants surrounding the particles. After oral administration of soft capsules, their content is released to gastric juices and the phospholipid molecules may adhere onto the GIT membrane enhancing oral API absorption. Although the small particle size seems to significantly improve bioavailability of APIs, the composition, and particularly the surface properties of the nanoparticles, may also affect the oral bioavailability (Andrysek 2003, 2006).

4.2 Pulmonary Delivery

Increasing attention has also been given to the potential of the pulmonary route as an alternative for non-invasive systemic delivery of therapeutic agents for both local and systemic API delivery (Scheuch et al. 2006). Advantages of pulmonary delivery using lipid nanoparticles rely on the possibility of site-specific application and controlled release to the lung. Since several advantages can be pointed out for this route (Hussain et al. 2004; Patton et al. 2004), e.g., large absorption area, extensive vasculature, easily permeable membrane, low extracellular and intracellular enzyme activity, pulmonary delivery of APIs becomes an opened and relatively unexplored field, in particular for peptides and proteins (Hussain et al. 2004; Malik et al. 2007). Nevertheless, for successful development of pulmonary delivery systems several challenges still remain, a major issue being the formulation of APIs into inhalable forms with sufficient stability and appropriate size (Abu-Dahab

et al. 2001; Dailey et al. 2003). Inhalation devices as well as the physicochemical characteristics of the formulation may influence aerodynamic particle size and thereafter affect the localization of aerosolized nanoparticles.

The pharmaceutical industry provides several inhalation devices, including metered-dose inhalers and API powder inhalers. Aqueous dispersions of lipid nanoparticles can be lyophilized to obtain powders, which may then be administered by means of these inhalers. Nevertheless, the particle size obtained after passing the sample through these devices is usually very large and thus might not be suitable for efficient deposition due to inertial impaction in the upper respiratory tract. More appropriate inhalers would be those generating a mist of small particles, which could penetrate the lung regions readily, and are better fitted for pulmonary delivery of APIs (Roche and Huchon 2000).

Colloidal carriers have also been pointed out as a suitable alternative for effectiveness of pulmonary API delivery. Examples include liposomes (Huang and Wang 2006; Karathanasis et al. 2005) and nanoparticles (Kawashima et al. 1999; Zhang et al. 2001), which exhibit some well-defined characteristics, especially for proteins. Higher bioavailability, controlled release properties, and enzymatic tolerance may be obtained (Chattopadhyay et al. 2007). SLN have also been recently proposed as a non-toxic API delivery system for pulmonary administration due to their unique physicochemical characteristics (e.g., small size, long-term physicochemical stability, biocompatibility and biotolerability, deep-lung deposition). By controlling the aerosolized particle size populations (mist of small particles versus larger particles) a dual effect of prolonged API release and rapid API transport could be achieved by means of SLN (Pandey and Khuller 2005; Videira et al. 2002). However lung targeting using nanoparticles has not been fully accepted yet. Most published data are limited to in vitro characterization of the nanoparticles for pulmonary delivery, and most of the reports address the treatment of local diseases, instead of systemic treatment by means of proteins or gene delivery (Almeida and Souto 2007; Rudolph et al. 2004).

To develop SLN-based formulations for such purposes, one needs to make sure the physicochemical stability of the aerosolized nanoparticles can be guaranteed. Chattopadhyay et al. have loaded triacylglycerols-based SLN with ketoprofen and indomethacin using the SFEE technique (Chattopadhyay et al. 2007). They successfully aerosolized the API-loaded SLN formulations using micron-sized nozzle devices. The particle size of aerosolized SLN dispersion was assessed by cascade impactor and by laser diffractometry, and it was shown to be similar to the size of aerosolized droplets usually obtained when administering API solution formulations using these devices. When using the micron-sized nozzles, the emitted dose was shown to be relatively higher and superior to those using the larger size API suspensions (Yim et al. 2005). Such results were easily attributed to the fact that smaller particles are less likely to clog the nozzle holes, and therefore aerosolization was close to the typical emitted dose of 65–70% observed with solution formulations (Boyd et al. 2004). The authors reported that aerosolized indomethacin-loaded SLN revealed more narrow size distribution and smaller mean particle size in comparison to ketoprofen-loaded SLN (Chattopadhyay et al. 2007). SLN

formulations were very stable during the SFEE with small emulsion droplet size leading to very uniform particles.

In another report, insulin-loaded SLN for pulmonary delivery were developed by a reverse micelle-double emulsion method, using a mixture of stearic and palmitic acids as solid lipid matrix, stabilized by sodium cholate and soybean phosphatidylcholine in aqueous dispersion (Liu et al. 2007, 2008). SLN remained stable under aerosolization achieving approx. 97% of EE, with the respirable fraction and nebulization efficiency of 82% and 63%, respectively. Pulmonary administration of 20 IU/kg SLN formulation reduced fasting plasma glucose within the first 4 h by about 40%, with an increased insulin level of approx. 170 μIU/ml. Pharmacological bioavailability was 24% and relative bioavailability 22% relative to subcutaneous injection as a reference. Aerosolized SLN were effectively and homogeneously distributed in the lung alveoli, with improved in vitro and in vivo stability, and prolonged hypoglycemic effect.

4.3 Parenteral Delivery and Drug Distribution

The major limiting factor for the parenteral delivery of lipid nanoparticles is their rapid clearance from the systemic circulation by the RES, which is dependent on the particle size, surface charge, and hydrophilic/lipophilic surface characteristics (Borm et al. 2006; Hoet et al. 2004). Colloidal API carriers usually depict a lipophilic surface, being therefore recognized as foreign elements by specific plasma components (opsonins), such as immunoglobulins (IgG), albumin, the elements of the complement system, fibronectin, and others, and then cleared from the blood stream by the phagocytic cells within minutes (Furumoto et al. 2004; Moghimi et al. 2001, 2005). Following intravenous (i.v.) injection, approx. 60–90% of the particles are distributed to the liver, and the remaining ones into spleen (2–10%), lungs (3–20% and more), and bone marrow (> 1%) (Kreuter 1994). The distribution in the body is also affected by the extravasation of nanoparticles from the peripheral capillary walls of these organs due to their large interendothelial gaps of about 150 nm. Thus, the passive targeting strongly limits the use of nanoparticles in API delivery to sites other than those belonging to the RES (Wolburg and Lippoldt 2002). To overcome such limitations, nanoparticles are usually surface-modified by hydrophilic molecules (e.g., surfactants and hydrophilic polymers or proteins) to avoid recognition by the mononuclear phagocytic system (MPS). Furthermore, it is also generally accepted that negative surfaces activate the complement system and coagulation factors (Moghimi et al. 2001). In addition to particle size reduction, changes in API biodistribution will occur, enhancing the systemic time circulation of the carriers and their deposition in non-RES organs (Kreuter 2001). In fact, one of the current approaches to achieve site-specific delivery is to bypass the normal physiological defense processes by reducing the particle size, thereby remaining for a prolonged period of time in the systemic circulation.

It is also known that the size and deformability of nanoparticles are of major importance in their clearance by the sinusoidal spleens of humans and rats, i.e., to avoid the splenic filtration at the inter-endothelial cell slits (IES) in the walls of venous sinuses, nanoparticles must be sufficiently small or deformable (Moghimi et al. 1993, 1991). It has been reported that ideally the size of an engineered long-circulatory particle should not exceed 200 nm (Groom 1987). Otherwise, the nanoparticle must be deformable enough to bypass IES filtration. Alternatively, long-circulating rigid particles of greater than 200 nm may act as splenotropic agents and be removed later on, if they are not rigid (Moghimi et al. 1991). If SLN are below 200 nm they will show an increased systemic circulation and thus an increase in the time for which the API remains in contact with the target site.

SLN have been proposed as a suitable system for parenteral delivery of hydrophilic APIs, such as diminazine, as well as of other BCS class IV APIs, e.g., paclitaxel, vinblastine, camptothecin, etoposide, and cyclosporine (Cavalli et al. 2000a; Chen et al. 2001; Yang et al. 1999a, b). Due to their lipophilic nature, SLN can be rapidly taken up by the RES, which may result in therapeutic failure due to insufficient API concentration in the plasma.

Steric stabilization is also an option because it creates a dense conformational cloud surrounding the particles, reducing opsonization and phagocytosis as well as the uptake by neutrophilic granulocytes. The result will be an increase in the systemic half-life of the API. An example of steric stabilization is the lipid nanoparticle stability provided by polyethylene glycol (PEG) molecules. PEG is a hydrophilic and electrically neutral polymer with a high chain flexibility. Its lack of functional groups prevents it from physicochemical interaction with the biological surroundings. PEG molecules with a molecular weight between 2,000 and 5,000 kDa are usually required to suppress plasma protein adsorption, and those creating thicker hydrophilic layers surrounding the particles will also contribute to the reduction of liver clearance (Chen et al. 2001).

To increase selectivity of SLN to a particular target, ligands or homing devices (which specifically bind to surface epitopes or receptors on the target sites) could be coupled onto their surface. It is known that cancer cells over-express specific receptors, such as folic acid receptors (over-expressed in cells of cancers with epithelial origin), low density lipoproteins (LDL) receptors (i.e., B16 melanoma cell line shows higher expression of LDL receptors), and peptide receptors (e.g., for somatostatin, vasoactive intestinal peptide, gastrin related peptides, cholecystokinin, gonadotropin releasing hormone). Therefore, attaching suitable ligands for these particular receptors onto the SLN surface may increase selectivity (Pardridge 2007b).

4.4 Brain Targeting

In the last decade, there has been emerging interest in API targeting to the brain (Blasi et al. 2007; Göppert and Müller 2005; Kreuter 2001; Pardridge 2005, 2007b, c, d, e). The lack of knowledge regarding the physiology of the central

nervous system (CNS) is one of the limiting factors in the development of effective APIs and appropriate API delivery systems for brain targeting and delivery (Pardridge 2003, 2007a,c,d). The specific blood–brain barrier (BBB) tightly regulates the exchange between the peripheral blood circulation and the cerebrospinal fluid circulatory system. Thus, these physiological features of the brain microvasculature restrict enormously the number of APIs that can enter the brain upon systemic administration. In fact, more than 98% of the new potential CNS active drugs are unable to cross the BBB (Pardridge 2007a). A drug molecule with a high lipophilicity and a molecular weight below 500 Da can pass through the BBB. Several strategies have been tried to effectively achieve API delivery and deposition to the CNS (Badruddoja and Black 2006; Johanson et al. 2005; Vyas et al. 2005), in particular the use of API carrier systems (Tiwari and Amiji 2006).

One possibility for access to the brain is receptor-mediated transport, because the BBB at the luminal side expresses receptors for endogenous large molecules (e.g., insulin, transferrin, leptin, ApoE, thiamin). The receptor-mediated transport of these molecules can be used for specific delivery into the brain (Cornford and Hyman 1999). The binding of the drug or the carrier (e.g., liposomes and nanoparticles) to specific ligands (peptides) or peptidomimetic monoclonal antibodies will shuttle the API directly into the brain (Pardridge 2003). These monoclonal antibodies act as Trojan horses for delivery of nanoparticles to the brain. The use of peptidomimetic antibodies which can bind to BBB transcytosis receptor, brain-targeted pegylated immuno-nanoparticles, has also been proposed. The delivery of entrapped APIs into the brain parenchyma can be achieved without inducing alteration in BBB permeability (Harris and Chess 2003). Yet some transporters such as P-glycoprotein existing in the BBB may also limit brain API delivery and can prevent the accumulation of various agents including APIs in the brain (Stouch and Gudmundsson 2002). To overcome this limitation, P-glycoprotein inhibition has been proposed using the generally accepted pharmaceutical surfactants (Batrakova et al. 1999; Miller et al. 1999).

Polymeric nanoparticles have been considered particularly useful to overcome the BBB (Garcia-Garcia et al. 2005; Müller and Keck 2004b), which seems to be high if nanoparticles are coated with polysorbate 80 (Tween 80) (Göppert and Müller 2005; Koziara et al. 2003). SLN have also been tested for brain targeting (Garcia-Garcia et al. 2005; Göppert and Müller 2005; Müller and Keck 2004a, b). The potential advantages of SLN over polymeric nanoparticles for brain targeting are based on their lower cytotoxicity, higher API loading capacity, and better production scalability. The surfactant-coated technology designed for brain targeting has been transferred to SLN and related carriers with relatively high success.

Göppert and Müller developed polysorbate-surfaced SLN to deliver several APIs to the brain. These studies demonstrated in addition that ApoC and ApoCII adsorbed onto SLN surface inhibit the receptor-mediated binding of β-VLDl expressing ApoE at the particle surface to the LDL receptor (Goppert and Müller 2005). The authors have emphasized the advantage of having a high ApoE/ApoCII ratio absorbed on the particles to achieve brain targeting. Furthermore, they found

that stealth SLN with polysorbate 80 adsorbed the lowest amount of ApoCII onto the particle surface. The pathfinder technology, i.e., differential protein adsorption, exploits plasma proteins which adsorb onto the surface of intravenously injected SLN for targeting. ApoE is such a moiety for SLN targeting to the endothelial cells of the BBB (Müller and Keck 2004a).

Zara and colleagues developed SLN and PEG-coated SLN containing increasing amounts of this stealth agent, for brain delivery of doxorubicin following i.v. administration (Kaur et al. 2008). The brain concentration of doxorubicin increased when increasing the stealth agent. The amount of doxorubicin in the rabbit brain ranged from 27.5 ng g^{-1} for non-stealth SLN to 242.0 ng g^{-1} for stealth SLN (surfaced with PEG molecules).

Thole et al. reported improved interaction with brain endothelial cells and higher intracellular accumulation of sterically stabilized liposomes coupled to cationized albumin in comparison to bovine serum albumin nanoparticles (Thole et al. 2002). Positively charged albumin nanoparticles were taken up into the brain endothelia via a caveolae-mediated endocytic pathway.

The effect of the surface charge of SLN on brain delivery was also assessed following administration of etoposide-loaded tripalmitin SLN. Brain levels were compared to the etoposide solution. Positively charged etoposide-loaded SLN achieved the highest brain concentration (0.07% of injected dose/g) clearly exceeding the uptake compared to negatively charged etoposide-loaded SLN (0.02%) and etoposide solution (0.01%) (Reddy and Venkateswarlu 2004).

Moreover, nitrendipine-loaded SLN composed of different acylglycerols (tripalmitin, trimyristin, tristearin), surfactants (soy lecithin, poloxamer 188), and charge modifiers (dicetyl phosphate, stearylamine) were produced aiming to compare the systemic half-life of API upon i.v. administration, in comparison to a conventional nitrendipine suspension (Manjunath and Venkateswarlu 2006). SLN formulation was found to be taken up to a greater extent by the brain and maintained high API levels for 6 h, whereas nitrendipine suspension achieved such levels only for 3 h. A 3.2-, 7.3- and 9.1-fold enhancement in C_{max} was shown when using SLN composed of tripalmitin, tripalmitin dicetyl phosphate, or tripalmitin stearylamine, respectively, in comparison to the API suspension. Similar findings were reported with 3′,5′-dioctanoyl-5-fluoro-2′-deoxyuridine-loaded SLN (Wang et al. 2002).

Stearic acid-based SLN loaded with camptothecin were administered i.v. to mice (1.3 mg kg^{-1}) resulting in a significantly prolonged drug residence time in the body in comparison to the camptothecin solution (Yang et al. 1999a). A fivefold increase in plasma AUC and a tenfold increase in brain AUC was observed on increasing the dose of camptothecin from 1.3 to 3.3 mg kg^{-1}.

In addition to the advantages of SLN for enhancing drug uptake by the brain, the very low brain cytotoxicity of SLN makes these carriers very attractive candidates for brain delivery (Müller et al. 1997b). It is important to underline that the toxicity of SLN is not only related to the lipid type, but also to the surfactant employed to stabilize the particle in aqueous dispersion. The most common surfactant exploited for nanoparticle brain targeting is polysorbate 80. Interestingly, free polysorbate 80 was more toxic than when bound (Koziara et al. 2006), which has been attributed to

the fact that this surfactant is more likely to be incorporated into SLN matrix rather than adsorbed, and thus its minimal release will also decrease toxicity.

5 Conclusions and Perspectives

The present chapter reviews current achievements in modifying the API pharmacokinetic parameters and bioavailability by means of lipid nanoparticles (SLN and NLC). These carriers are composed of materials compatible with the biological environment. SLN and NLC have been exploited for oral, dermal, pulmonary, and parenteral administration. Obviously, the in vivo behavior and consequently therapeutic potential of these nanoparticles are defined by their physicochemical properties as well as by the administration route. The type of lipid nanoparticle system (SLN versus NLC) should be critically selected according to the administration route, e.g., NLC are less likely to be used for brain delivery. Nevertheless, both systems can be used to decrease API toxicity.

The pharmaceutical industry is interested in the development of a delivery system that could be sufficiently versatile to be exploited for several administration routes. Changes in the carrier surface properties (electric charge, hydrophilicity) and matrix composition may be required to minimize or overcome limitations associated with more conventional colloidal carriers (e.g., liposomes, polymeric nanoparticles, nanoemulsions). SLN and NLC can be designed according to the physicochemical properties of API molecules, as well as to the administration route and target/delivery purposes.

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