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Review Article

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Recent Advances in Lipid Nanoparticle Formulations with Solid Matrix for Oral Drug Delivery

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Abstract. Lipid nanoparticles based on solid matrix have emerged as potential drug carriers to improve gastrointestinal (GI) absorption and oral bioavailability of several drugs, especially lipophilic compounds. These formulations may also be used for sustained drug release. Solid lipid nanoparticle (SLN) and the newer generation lipid nanoparticle, nanostructured lipid carrier (NLC), have been studied for their capability as oral drug carriers. Biodegradable, biocompatible, and physiological lipids are generally used to prepare these nanoparticles. Hence, toxicity problems related with the polymeric nanoparticles can be minimized. Furthermore, stability of the formulations might increase than other liquid nano-carriers due to the solid matrix of these lipid nanoparticles. These nanoparticles can be produced by different formulation techniques. Scaling up of the production process from lab scale to industrial scale can be easily achieved. Reasonably high drug encapsulation efficiency of the nanoparticles was documented. Oral absorption and bioavailability of several drugs were improved after oral administration of the drug-loaded SLNs or NLCs. In this review, pros and cons, different formulation and characterization techniques, drug incorporation models, GI absorption and oral bioavailability enhancement mechanisms, stability and storage condition of the formulations, and recent advances in oral delivery of the lipid nanoparticles based on solid matrix will be discussed.

KEY WORDS: bioavailability; gastrointestinal absorption; lipid nanoparticle; oral delivery.

INTRODUCTION

Oral route is the most preferred route for drug administration due to greater convenience, less pain, high patient compliance, reduced risk of cross-infection, and needle stick injuries. Major portion of the drug delivery market is occupied by oral drug delivery systems. However, oral drug delivery is continuously looking into newer avenues due to realization of the factors like low drug solubility, poor gastrointestinal (GI) absorption, rapid metabolism, high fluctuation in the drug plasma level, and variability due to food effects. These factors may cause disappointing *in vivo* results leading to failure of the conventional delivery systems (1). Colloidal drug carriers, such as, micelles, nanoemulsions, nanosuspensions, polymeric nanoparticles, and liposomes might overcome many of the solubility related problems. For the past few years, these drug delivery systems gained more attention. However, these systems are associated with several drawbacks, such as limited physical stability, aggregation, drug leakage on storage, lack of a suitable low cost large-scale production method yielding a product of a quality accepted by the regulatory authorities, presence of organic solvent residues in the final product, cytotoxicity, etc. (2,3).

From the last decade, oral drug delivery has taken a new dimension with the increasing application of lipids as carriers for the delivery of poorly water-soluble drugs (4). These systems minimize the above mentioned problems associated with other colloidal systems. Due to the increasing attention towards lipid-based drug delivery systems, American Association of Pharmaceutical Scientists has formed a "Lipid-Based Drug Delivery Systems Focus Group" (www.aaps.org/inside/focus_groups/Lipid). The lipids employed to prepare lipid nanoparticles are usually physiological lipids (biocompatible and biodegradable) with low acute and chronic toxicity (5). In case of polymeric nanoparticles, the *in vivo* degradation of the polymer might cause toxic effects (5,6). Lipid nanoparticles adopted the best features of other colloidal carriers, such as polymeric nanoparticles, liposomes, conventional oil-in-water emulsions, and nanoemulsions (7). The physiochemical diversity and biocompatibility of lipids and their ability to enhance oral bioavailability of drugs have made lipid nanoparticles very attractive carriers for oral drug delivery. Furthermore, lipid nanoparticles with solid matrix demonstrated high drug loading (both hydrophilic and lipophilic drugs) (8,9), long-term shelf stability (1,2,10–12), and hassle-free large-scale production (1,2,13–15). Lipids are able to

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promote oral absorption of the encapsulated drugs via selective lymphatic uptake (16–20). Moreover, small particles ranging between 120 and 200 nm rarely undergo blood clearance by the reticuloendothelial system (i.e., liver and spleen filtrations are avoided; 2,21,22). Altogether, lipid nanoparticles based on solid matrix exhibited strong potential as oral drug delivery systems. Although lipid nanoparticles have also been extensively studied for topical and parenteral purpose, they are beyond the scope of this review. Reviews of topical and parenteral lipid nanoparticles can be found elsewhere (23–25). The following sections will discuss about two types of lipid nanoparticles with solid matrix, their pros and cons, different formulation and characterization procedures, drug incorporation models, impact on GI absorption and oral bioavailability, stability and storage condition of the formulations, and recent advances as oral drug carriers.

LIPID NANOPARTICLES

Lipid nanoparticles with a solid matrix are generally 2 types: solid lipid nanoparticle (SLN) and nanostructured lipid carrier (NLC).

Solid Lipid Nanoparticle

SLNs are prepared from lipids which are solid at room temperature as well as at body temperature. Different solid lipids are exploited to produce SLNs, such as, tripalmitin/Dynasan® 116 (26–28), cetyl alcohol (29,30), cetyl palmitate (31,32), Compritol® 888 ATO (33–38), Glyceryl monostearate (39), Precirol® ATO5 (34,40), trimyristin/Dynasan® 114 (27,41), tristearin/Dynasan® 118 (27), stearic acid (30), Imwitor® 900 (42,43). There are several advantages of SLN formulations (2,7,44), such as: (a) photosensitive, moisture sensitive, and chemically labile drug molecules can be protected from degradation in external environment (during storage) and in the gut (following oral administration), (b) bioavailability of highly lipophilic molecules can be improved, (c) biodegradable and physiological lipids are used to prepare SLNs, (d) scaling up of the formulation technique to industrial production level is feasible at low cost and in a relatively simple way, (e) use of organic solvents can be avoided to produce SLNs. In contrary, several disadvantages are also associated with SLNs (45,46), such as: (a) SLN dispersions contain high amount of water, (b) drug-loading capacity of SLNs are limited due to crystalline structure of solid lipid, (c) expulsion of encapsulated drug may take place during storage due to formation of a perfect crystalline lattice especially when SLNs are prepared from one highly purified lipid, (d) drug release profile may change with storage time, (e) polymorphic transitions are possible, (f) particle growth is possible during storage, and (g) gelation of the dispersion may take place during storage.

Nanostructured Lipid Carrier

Generally, drugs are incorporated between the fatty acid chains or in between lipid layers or in amorphous clusters in crystal imperfections within SLN matrix. However, SLNs prepared from one highly purified lipid can crystallize in a perfect crystalline lattice that allows very small space for the incorporation of drugs (Fig. 1a). Lipids crystallize in high-energetic lipid modifications, α and β', immediately after preparation of SLN. However, the lipid molecules undergo a time-dependent restructuring process leading to formation of the low-energetic modifications, βi and β, during storage. Formation of this perfect lipid crystalline structure leads to expulsion of drug (47). Therefore, despite SLNs being interesting delivery systems, relatively low drug-loading capacity and potential expulsion of the drug during storage led scientists to think about new strategies. As a result, NLCs have been developed, which in some extent can avoid the aforementioned limitations. In case of NLCs, spatially very different lipid molecules are mixed to create a lipid particle matrix as imperfect as possible (Fig. 1b). Generally, solid and liquid (oil) lipids are mixed to produce NLCs that are still solid at room temperature as well as at body temperature (40). Due to many imperfections in NLCs, drug-loading capacity is enhanced and drug expulsion during storage is minimized. NLCs have several advantages (3,45), such as: (a) NLC dispersions with higher solid content can be produced, (b) drug-loading capacity is better than SLNs, (c) drug release profile can be easily modulated, (d) drug leakage during storage is lower than SLNs, and (e) production of final dosage forms (e.g., tablets, capsules) is feasible.

FORMULATION TECHNIQUES

Various formulation techniques exist for the production of SLNs and NLCs (Table I). Among them, high-pressure homogenization (HPH) and microemulsion techniques have demonstrated strong potential for scaling up to industrial production scale (48,49). The following sections describe different existing approaches for SLN and NLC formulations. However, in some instances combination of different methods has been utilized to prepare the nanoparticles (Table I).

High-Pressure Homogenization

HPH is a reliable and suitable technique for the preparation of lipid nanoparticles. There are two types of HPH, hot HPH and cold HPH (50).

Hot high-pressure homogenization. In this technique, first the lipid(s) is/are melted at 5–10°C above its/their melting point(s)

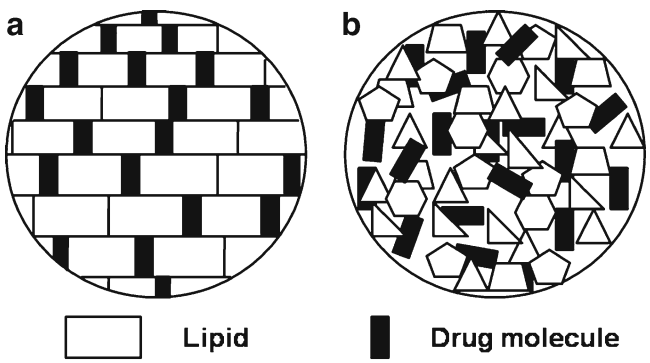

Fig. 1. Schematic structure of solid lipid nanoparticle (**a**) and nanostructured lipid carrier (**b**). The figure was reproduced from (119) with permission

and the drug is dissolved or homogeneously dispersed in the melted lipid(s). Then a hot aqueous surfactant solution (pre-heated at the same temperature) is added to the drug–lipid melt and homogeneously dispersed (pre-emulsion) by a high shear mixing device. Subsequently, this hot pre-emulsion is subjected to a high-pressure homogenizer at the same temperature. This homogenization process is repeated till the nanoemulsion of desired average particle size is obtained. The obtained nanoemulsion is then cooled down to room temperature. During this cooling down, lipid droplets of the nanoemulsion re-crystallize and form lipid nanoparticles with solid matrix.

Cold high-pressure homogenization. Similar to hot HPH, the lipid(s) is/are melted at 5–10°C above its/their melting points and the drug is dissolved or homogeneously dispersed in the melted lipid(s) in the cold HPH technique. Then the drug-lipid melt is rapidly cooled down by means of liquid nitrogen or dry ice and subsequently milled to microparticles by means of a ball mill or mortar. These microparticles are suspended in a cold aqueous surfactant solution and then homogenized at or below room temperature forming lipid nanoparticles. This cold HPH technique is suitable for hydrophilic or thermo-labile drugs as this method is expected to avoid temperature-induced drug degradation and drug distribution into aqueous phase during homogenization. However, complete avoidance of drug exposure to high temperature is impossible as the drug needs to dissolve or disperse in the molten lipid and some heat is generated during the homogenization process.

Generally, scaling up of a process encounters several problems. Nevertheless, usage of the larger scale machines during HPH leads to an even better quality of the product with regard to a smaller particle size and its homogeneity (51). Additionally, HPH technique is widely used and well-established technique in pharmaceutical and food industry. SLN prepared by HPH can also be produced in non-aqueous dispersion media as long as the dispersion medium does not dissolve the lipid, e.g., liquid polyethylene glycol or oils (e.g., mineral oil) (52).

Emulsification-Sonification

The first part of this method is similar to HPH. Briefly, the lipid(s) is/are melted at a temperature of 5–10°C above its/their melting point(s) and the drug is dissolved/dispersed in the melted lipid(s). Then a hot aqueous surfactant solution (preheated at the same temperature) is added to the drug-lipid melt and homogeneously dispersed by a high shear mixing device. Coarse hot oil-in-water emulsion obtained is ultrasonicated using probe sonicator till the desired sized nanoemulsion is formed. Finally, lipid nanoparticles are obtained by allowing hot nanoemulsion to cool to room temperature. However, metallic contamination of the product may happen during sonication by probe sonicator.

Microemulsion

Microemulsion method for the preparation of SLNs was developed by Gasco *et al.* (53), which has been adapted and/or modified by other researchers (54–56). In this method, first the solid lipid(s) is/are melted and the drug is dissolved/dispersed in the molten lipid(s). After that, aqueous surfactant–cosurfactant solution (pre-heated above the melting temperature of solid lipid) is added to the lipid melt with mild agitation to obtain transparent microemulsion. Subsequently, the microemulsion is dispersed in cold water (2–10°C) with mild agitation, where the microemulsion breaks into ultrafine nanoemulsion droplets which immediately crystallize to form SLNs. Strong dilution of the particle suspension due to usage of large volume of water (ratio of hot microemulsion to cold water=1:25–1:50) is the main concern of this technique. Thus, the excess water needs to remove either by ultra-filtration or by lyophilization to obtain a concentrated dispersion. Another disadvantage of this method is the necessity of high concentrations of surfactants and co-surfactants, which is not desirable. Industrial scale production of lipid nanoparticles by the microemulsion technique is possible. In the large-scale production, a large temperature-controlled tank is used to prepare the microemulsion. Subsequently, the microemulsion is pumped into a cold water tank for the precipitation step. The temperature of the microemulsion and water, temperature flow in the aqueous medium, and hydrodynamics of mixing are the critical process parameters in the large-scale production.

Solvent Emulsification-Evaporation

In this technique, first the lipid(s) is/are dissolved in a water-immiscible organic solvent (e.g., cyclohexane, chloroform) and then emulsified in an aqueous phase containing surfactants under continuous stirring (57,58). The organic solvent evaporates during emulsification, which results in lipid precipitation. As the whole formulation procedure can be conducted in room temperature, this technique is highly suitable for thermo-labile drugs. However, the major concern is the production of very dilute dispersion that needs to be concentrated by means of ultra-filtration or evaporation. Another concern is the use of organic solvent, some of which may remain in the final preparation.

Solvent Diffusion

In contrary to solvent emulsification–evaporation technique, partially water-miscible organic solvents (e.g., benzyl alcohol, ethyl formate) are used in solvent-diffusion technique (59,60). In this case, organic solvents are mutually saturated with water to ensure initial thermodynamic equilibrium of both liquids. The transient oil-in-water emulsion is passed into water under continuous stirring, which leads to solidification of dispersed phase forming lipid nanoparticles due to diffusion of the organic solvent. However, similar to microemulsion technique, dilute nanoparticle dispersion is produced, which needs to be concentrated by ultra-filtration or lyophilization. Usage of organic solvent is also a concern as some of it may remain in the final preparation.

Solvent Injection

The basic principle of the solvent injection method is similar to the solvent diffusion method. In case of solvent injection method, lipids are dissolved in a water-miscible solvent (e.g., acetone, isopropanol, and methanol) or water-

miscible solvent mixture and quickly injected into an aqueous solution of surfactants through an injection needle (61). The advantages of this method are the easy handling and fast production process without technically sophisticated equipment (e.g., high-pressure homogenizer). However, the main disadvantage is the use of organic solvents.

Double Emulsion

The double emulsion (*w*/*o*/*w*) method is based on solvent emulsification–evaporation method (54). This method is mainly for the production of lipid nanoparticles loaded with hydrophilic drugs. In this case, the drug and stabilizer are encapsulated in the inner aqueous phase of the *w*/*o*/*w* double emulsion. A stabilizer is necessary to prevent drug partitioning to the outer aqueous phase during solvent evaporation. This type of formulations is usually named as 'lipospheres' due to their comparatively larger particle size than SLNs.

CHARACTERIZATION

Characterization of the lipid nanoparticles is critical due to complexity of the system and colloidal size of the particles (1). Nevertheless, proper characterization of the formulations is necessary to control the product quality, stability, and release kinetics. Hence, accurate and sensitive characterization methods should be used. There are several important characterization techniques as follows.

Particle Size

Particle size plays a crucial role in the gastrointestinal uptake and their clearance by the reticuloendothelial system. Hence, the precise determination of the particle size is very important. Particle size less than 300 nm are advisable for the intestinal transport (62). Photon correlation spectroscopy (PCS; 28,31,38,58,63,64) and laser diffraction (LD; 33,43,63) are the most powerful and widely used techniques for the particle size measurement of lipid nanoparticles. PCS is also known as dynamic light scattering. The fluctuation of the intensity of the scattered light, caused by particles movement, is measured by this technique. PCS is relatively accurate and sensitive method. However, only size range from few nanometers to about 3 μ can be measured by PCS (42,43). This size range is enough to characterize lipid nanoparticles. On the other hand, LD can measure bigger particle sizes (>3 μ; 42,43). LD covers a broad size range from the nanometer to the lower millimeter range. This method is based on the dependence of the diffraction angle on the particle radius. Smaller particles lead to more intense scattering at high angles than the larger particles. However, it is always recommended to use both PCS and LD method simultaneously as both methods do not directly measure particle sizes, rather particle sizes are calculated from their light scattering effects. This is because particles are non-spherical in many instances (65).

Polydispersity Index

As SLNs/NLCs are usually polydisperse in nature, measurement of polydispersity index (PI) is important to know the size distribution of the nanoparticles. The lower the PI value, the more monodispered the nanoparticle dispersion is. Most of the researchers accept PI value less than 0.3 as optimum value (66,67). PI can be measured by PCS (38,64,68).

Zeta Potential

The zeta potential (ZP) indicates the overall charge a particle acquires in a specific medium. Stability of the nanodispersion during storage can be predicted form the ZP value. The ZP indicates the degree of repulsion between close and similarly charged particles in the dispersion. High ZP indicates highly charged particles. Generally, high ZP (negative or positive) prevents aggregation of the particles due to electric repulsion and electrically stabilizes the nanoparticle dispersion. On the other hand, in case of low ZP, attraction exceeds repulsion and the dispersion coagulates or flocculates. However, this assumption is not applicable for all colloidal dispersion, especially the dispersion which contain steric stabilizers. The ZP value of −30 mV is enough for good stabilization of a nanodispersion (69). The ZP of the nanodispersions can be determined by PCS (28,31,38,63,64,70).

Shape and Morphology

Scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) are very useful techniques to determine the shape and morphology of lipid nanoparticles. These techniques can also determine the particle size and size distribution. SEM utilizes electron transmission from the sample surface, whereas TEM utilizes electron transmission through the sample. In contrast to PCS and LD, SEM and TEM provide direct information on the particle shape and size. Several SEM (29) and TEM (28,33,38,65,68) study showed spherical shape of the lipid nanoparticles. Although normal SEM is not very sensitive to the nanometer size range, field emission SEM (FESEM) can detect nanometer size range (71). However, sample preparation (e.g., solvent removal) may influence the particle shape. Cryogenic FESEM might be helpful in this case, where liquid dispersion is frozen by liquid nitrogen and micrographs are taken at the frozen condition. AFM technique is also gaining popularity for nanoparticle characterization (58). AFM provides a three-dimensional surface profile unlike electron microscopy which provides two-dimensional image of a sample. AFM directly provides structural, mechanical, functional, and topographical information about surfaces with nanometer- to angstrom-scale resolution. In this technique, the force acting between a surface and a probing tip results in a spatial resolution of up to 0.01 nm for imaging. Direct analysis of the originally hydrated, solvent-containing samples is possible as no vacuum is needed during operation and the sample does not need to be conductive. zur Muhlen compared AFM with SEM and reported same particle size of the nanoparticles by both methods (72).

Crystallinity and Polymorphism

Determination of the crystallinity of the components of SLN/NLC formulations is crucial as the lipid matrix as well as

the incorporated drug may undergo a polymorphic transition leading to a possible undesirable drug expulsion during storage (73). Lipid crystallinity is also strongly correlated with drug incorporation and release rates. Thermodynamic stability and lipid packing density increase, whereas drug incorporation rates decrease in the following order: Supercooled melt, α-modification, β′-modification, and β-modification. However, lipid crystallization and modification changes might be highly retarded due to the small size of the particles and the presence of emulsifiers. Differential scanning calorimetry (DSC; 63,68) and X-Ray diffractometry (XRD; 68) are two widely used techniques to determine the crystallinity and polymorphic behavior of the components of the SLNs/NLCs. DSC provides information on the melting and crystallization behavior of all solid and liquid constituents of the particles, whereas XRD can identify specific crystalline compounds based on their crystal structure (74). DSC utilizes the fact that different lipid modifications possess different melting points and melting enthalpies. In XRD, the monochromatic beam of X-ray is diffracted at angles determined by the spacing of the planes in the crystals and the type and arrangement of the atoms, which is recorded by a detector as a pattern. The intensity and position of the diffractions are unique to each type of crystalline material. XRD pattern can predict the manner of arrangement of lipid molecules, phase behavior, and characterize and identify the structure of lipid and drug molecules (75,76). However, best results are observed when SLN dispersions are investigated directly as solvent removal may change the modification. Another two techniques, infrared and Raman spectroscopy are also useful to investigate structural properties of lipids (2). However, they have not been extensively used to characterize SLNs/NLCs.

Assessment of Alternative Colloidal Structures

In several cases, lipid nanoparticles coexist with other colloidal structures (e.g., micelles, liposomes, mixed micelles, supercooled melts, and drug nanoparticles). However, characterization and quantification of these colloidal structures are difficult due to the similarities in size, low resolution of PCS to detect multimodal distributions, modification of the equilibrium of the complex colloidal system during sample preparation. Furthermore, dilution of the original nanoparticle dispersion with water might cause the removal of surfactant molecules from the particle surface and induce further changes. Hence, the methods which are sensitive to the simultaneous detection of different colloidal species and which do not require preparatory steps should be used. Nuclear magnetic resonance (NMR;77) and electron spin resonance (ESR; 78) techniques are suitable for this purpose. These techniques are useful for investigating dynamic phenomena and the characteristics of the nanocompartments in colloidal lipid dispersions. Detection of supercooled melts due to the low line widths of the lipid protons is possible by ^{1}H-NMR spectroscopy (79). This technique is based on the different proton relaxation times in the liquid and semisolid/solid state. NMR also can characterize liquid nanocompartments in NLCs. ESR requires a paramagnetic spin probes to investigate SLN dispersions. Direct, repeatable, and noninvasive characterization of the distribution of the spin probe between the aqueous and the lipid phase can be performed by ESR (78,80). However, despite the great potential, NMR and ESR have been rarely applied to characterize SLNs and NLCs.

DRUG INCORPORATION MODELS

In general, there are three models for drug incorporation within the lipid nanoparticles (1,2): (a) homogenous matrix of solid solution, (b) drug-enriched shell, and (c) drug-enriched core. In case of the first model, the drug is molecularly dispersed homogeneously in the lipid matrix of the particles. Hence, drug release occurs via diffusion from the solid lipid matrix and/or by degradation of lipid matrix in the gut. In case of the second model (drug enriched shell), the drug is concentrated on the outer shell of the nanoparticles. This model can be explained as follows. During HPH process, each nanoemulsion droplet contains a mixture of drug and lipid. However, during cooling the lipid may precipitate faster than the drug, which forms a drug-free core or a core with less drug content. Subsequently, lipid and drug precipitate simultaneously in the outer shell of the particles after reaching the eutectic temperature and composition. Furthermore, solubility of many drugs in surfactant solution increases at elevated temperatures. Hence, during hot homogenization, drug may partially leave the lipid matrix and dissolves in the aqueous phase. However, drug solubility in outer phase (surfactant solution) decreases during cooling of nanoemulsion. Then the drug shows tendency to repartition into the lipid matrix, which leads to the drug-enriched shell as the particle core has already started to solidify. Several researchers have shown drug-enriched shell SLNs (81). This type of nanoparticles exhibit burst release of the drug (82), which is desirable for some drugs (e.g., oral cyclosporin A formulation). However, this initial burst release can be modified by varying the formulation conditions such as, production temperature (preferably cold homogenization) and surfactant concentration (83). In contrary to drug-enriched shell model, drug-enriched core model is formed when precipitation of the drug is faster than lipid during cooling of the nanoemulsion. This phenomenon is observed when the drug is dissolved at its saturation solubility in the lipid at production temperature. A super saturation and subsequent drug precipitation occur during cooling. Generally, prolonged drug release is observed from these SLNs (83).

GASTROINTESTINAL ABSORPTION

High solubility and permeability are considered prerequisites for GI absorption. Many drugs have been identified to exhibit poor and variable bioavailability due to their poor aqueous solubility (84). Co-administration of high-fat meals may enhance the bioavailability of such drugs (17,85,86). Intake of a high-fat meal leads to prolongation of GI tract residence time, stimulation of biliary and pancreatic secretions, stimulation of lymphatic transport, enhancement of intestinal wall permeability, reduction of metabolism and efflux activity, and alteration in mesenteric and liver blood flow, which significantly contribute to improve oral bioavailability of drug (87,88). Therefore, design of lipid-based formulations may reduce the inherent limitations of slow and incomplete

dissolution of poorly soluble drugs and facilitate the formation of solubilized phases from which absorption may occur. Absorption-enhancing effect and adhesiveness of lipid nanoparticles lead to GI absorption enhancement of many drugs, especially hydrophobic drugs. After adhesion to the gut wall, the drug is released at its place of absorption (89). Drug is absorbed through GI tract together with fat (lipid). Charman *et al.* (16,17,20,90) described the absorption-enhancing effect of lipid as follows: 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. Drug dissolved in the lipid is taken up to the micelle (solubilized) during the detachment and micelle-forming process. These micelles then interact with surface-active bile salts and form mixed micelles. Subsequently, drug is absorbed together with the micelles.

Materials absorbed across the small intestine epithelial cells can enter either lymphatic or blood capillaries. The majority of orally administered drugs reach to the systemic circulation by absorption into the portal blood. However, some extremely lipophilic compounds (log P >5) reach to the systemic circulation via lymphatic route. Hence, lipid can augment lymphatic uptake of several drugs, especially lipophilic drugs or large molecular weight macromolecules (91,92). Furthermore, lymphatic capillaries are significantly more permeable to nanoparticles than the blood capillaries (68). Drugs that are absorbed via the intestinal lymphatic system are protected from hepatic first-pass metabolism due to the unique anatomy and physiology. The oral bioavailability of the drugs, which undergo high hepatic first-pass metabolism, can therefore be significantly enhanced by transport via the lymphatic system (93). However, lymphatic absorption depends on the length of the fatty acid chains. Khoo *et al.* found that long-chain triglycerides are more effective in promoting absorption in compared with medium-chain triglycerides (94). Researchers (20) have shown that fatty acids with C-14 chains to C-18 chains promote lymphatic absorption.

ORAL ADMINISTRATION

Several drugs (hydrophobic and hydrophilic) have been incorporated in the SLN and/or NLC formulations for oral administration (Table I). In most cases, the aim was to improve oral bioavailability either by increasing GI absorption or by bypassing the first-pass metabolism (Table I). Different formulation techniques were adopted to prepare the formulations (Table I). The following sections discuss about the studies performed on different drugs for oral administration via SLNs/NLCs.

All-trans retinoic acid. In a study, SLNs loaded with all-*trans* retinoic acid (ATRA) were prepared by HPH method using Compritol® 888 ATO as lipid matrix (37). The aim of this work was to improve the oral bioavailability of poorly soluble drug (ATRA) by incorporation into SLNs. The pharmacokinetic study was conducted in male rats following oral administration of 8 mg kg^{-1} ATRA in different formulations. It was found that the relative bioavailability of ATRA was significantly higher in case of SLNs than the ATRA solution. The amount of surfactant in SLN formulations also demonstrated a marked effect on the oral absorption of ATRA. The study indicated that ATRA absorption is markedly improved by SLNs. Although ATRA absorption was enhanced in case of an emulsion formulation, it was very unstable.

Apomorphine. Apomorphine is a dopamine receptor agonist which is used for the treatment of Parkinson's disease. However, apomorphine showed poor oral bioavailability due to first-pass effect. Tsai *et al.* prepared SLNs containing apomorphine to improve the oral bioavailability and brain regional distribution of apomorphine (28). SLNs were prepared using two emulsifiers, glyceryl monostearate (GMS) and polyethylene glycol monostearate (PMS), individually. The study showed significant effect of emulsifiers on the physicochemical characteristics of SLNs. Average diameter of the SLNs prepared using GMS was larger (155 nm) than the SLNs prepared using PMS (63 nm). Entrapment efficiency of the SLNs was >90%. The SLNs prepared using PMS was more stable in terms of particle size and encapsulation efficiency than the SLNs prepared using GMS when incubated in simulated intestinal medium. Nevertheless, both apomorphine-loaded SLNs showed 12- to 13-fold higher bioavailability than the apomorphine solution after oral administration of SLNs and solution formulations (26 mg kg^{-1} dose). Additionally, the drug distribution in the striatum (predominant site of therapeutic action) increased following administration of SLNs. The anti-Parkinsonian activity of apomorphine was evaluated in rat model with 6-hydroxydopamine-induced lesions. The contralateral rotation behavior suggested improvement of disease state following oral administration of both apomorphine-loaded SLNs. However, the SLNs prepared using PMS showed better effect than the SLNs prepared using GMS. The study indicated that SLNs might be a promising strategy for oral delivery of apomorphine.

α-Asarone. The α-asarone-loaded SLNs were prepared by ultrasonic-homogenization method (95). This work investigated the potential of SLNs to improve oral bioavailability and tissue uptake of α-asarone (lipophilic drug). The pharmacokinetic study in male rats at an oral dose equivalent to 10 mg kg^{-1} α-asarone suggested that the relative bioavailability of α-asarone was significantly improved in α-asarone -SLN group compared to α-asarone solution group. Furthermore, the study also indicated an increased uptake of α-asarone in brain and lung in α-asarone-SLN group compared to α-asarone solution group. Hence, the absorption and tissue distribution of α-asarone was significantly enhanced by SLN formulation. The results indicated that SLNs are useful to improve the oral bioavailability of poorly soluble drugs.

Buspirone HCl. In another study, a water-soluble drug, buspirone HCl, was incorporated in SLNs (30). SLNs were produced by emulsification–evaporation followed by the ultrasonification. The formulation variables were optimized as follows: lipid=cetyl alcohol, surfactant=Tween® 20, lecithin: lipid=2:7 (*w*/*w*), sonication time=30 s. The optimized SLNs had particle size of 345.7 nm, loading efficiency of 32.8%, and zeta potential of −6.8 mV. The pharmacokinetic study was conducted

in male Wistar rats following oral administration of 15 mg kg^{-1} buspirone in the form of free drug or SLNs. The relative bioavailability of the drug was significantly increased for SLNs in comparison to the drug solution.

Camptothecin. In a separate study, camptothecin-loaded SLNs were prepared by HPH method (96). The prepared SLNs had an average diameter 196.8 nm, zeta potential of −69.3 mV, and drug encapsulation efficiency of 99.6%. The specific changes in body distribution of camptothecin were investigated following oral delivery of SLN and solution formulations of camptothecin in mice. In tested organs, the area under curve (AUC) and mean residence time (MRT) of SLN formulation increased significantly as compared with solution formulation. The increase of brain AUC was the highest among all tested organs. The study suggested that SLNs could be a promising sustained release and targeting system for camptothecin or other lipophilic antitumor drugs after oral administration.

Carvedilol. Another work (68) aimed to investigate the effect of different concentrations of Poloxamer® 188 (surfactant) on lymphatic uptake of carvedilol-loaded SLNs for oral bioavailability enhancement. Microemulsion technique was employed to produce SLNs with varying concentrations of Poloxamer® 188. Pharmacokinetic study indicated, the $AUC_{(0-t)}$ of SLN formulations were significantly higher than that of carvedilol suspension. However, on increasing the concentration of Poloxamer® 188, the bioavailability of carvedilol decreased from 4.91- to 2.84-fold following intraduodenal administration of carvedilol-loaded SLNs to male Wistar rats. This research indicated the possibility of enhancement of the oral bioavailability of drug via the lymphatic system bypassing hepatic first-pass metabolism.

Clozapine. Clozapine-loaded SLNs have been developed using various triglycerides (trimyristin, tripalmitin, and tristearin), soylecithin, Poloxamer® 188, and stearylamine (positive charge inducer) by hot homogenization followed by ultrasonication method (27). Average size and zeta potential of SLNs ranged from 96.7±3.8 to 163.3±0.7 nm and 21.3±1.3 to 33.2±0.6 mV, respectively. Clozapine (a lipophilic drug) has very poor oral bioavailability due to first-pass effect. The aim of this work was to improve the bioavailability of clozapine upon intraduodenal administration of clozapine-loaded SLNs in male Wister rats. Furthermore, tissue distribution studies of clozapine SLNs and suspension were carried out in Swiss albino mice. Bioavailability of clozapine-loaded SLNs were 2.45- to 4.51-fold higher after intraduodenal administration than that of clozapine suspension. In tested organs, the AUC and MRT of clozapine-loaded SLNs were higher than those of clozapine suspension. The study suggested that SLNs are suitable drug carriers to improve the bioavailability of lipophilic drugs.

Cryptotanshinone. Hu *et al*. prepared cryptotanshinone-loaded SLNs by ultrasonic and HPH method to improve the oral bioavailability of the poorly water-soluble drug cryptotanshinone (35). Upon oral administration of different cryptotanshinone-loaded SLNs in rats, the relative bioavailability of cryptotanshinone in the SLNs was significantly increased compared with that of a cryptotanshinone-suspension. In addition, incorporation of cryptotanshinone in SLNs also significantly changed the metabolism behavior of cryptotanshinone. The study indicated the improvement of the oral absorption of poorly soluble drugs by employing SLN formulations.

Cyclosporine A. In a study, lipid nanoparticles (lipospheres) containing cyclosporine A were prepared (97). Effect of composition and particle size of the lipospheres on the oral bioavailability of cyclosporine A was investigated. The particle size of the formulations was 25–400 nm. A correlation between the AUC and C_{max} (maximum plasma drug concentration) with the particle size of the formulations was observed in human after oral intake of the formulated cyclosporine A. In another study, Muller *et al.* (43) developed SLNs containing cyclosporine A (mean size 157 nm and encapsulation rate 96.1%) and assessed the pharmacokinetic parameters of the developed formulations after oral administration in the young pigs. The study indicated a lower variation in bioavailability and higher blood concentration of the drug than the cyclosporine nanocrystals. The study also demonstrated that the huge initial plasma peak of cyclosporine was absent in case of SLN formulation, which was associated with marketed microemulsion formulation (Sandimmun®).

Curcumin. A recent study aimed to improve oral bioavailability of curcumin by incorporating curcumin in SLNs (33). Microemulsification method was used to prepare the curcumin-loaded SLNs. Average particle size and total drug content of the SLNs were 134.6 nm and 92.33±1.63%, respectively. TEM study showed spherical particles. High drug entrapment of 81.92±2.91% was noticed at 10% drug loading. SLNs exhibited prolonged *in vitro* drug release (up to 7 days) predominantly by diffusion mechanism. SLNs were stable after their 12-month storage at 5±3°C as insignificant deviation from initial size and drug content of SLNs were observed. *In vivo* pharmacokinetics following oral administration of curcumin-loaded SLNs (50, 25, 12.5, and 1 mg kg^{-1} dose) and curcumin solution (50 mg kg^{-1}) demonstrated significant improvement in oral bioavailability (39, 32, 59, and 155 times at 50, 25, 12.5, and 1 mg kg^{-1} dose, respectively) after administration of SLNs in compare to curcumin solution.

Digoxin. Digoxin-loaded SLNs were prepared by an ultrasonic and HPH method by Hu *et al*. (36). The pharmacokinetic study in rabbits revealed that the relative bioavailability of digoxin in the SLNs significantly increased compared with that of a digoxin solution after oral administration of 0.25 mg digoxin in different SLNs. Additionally, oral absorption of digoxin was markedly increased by the addition of CMC-Na in SLNs. The study suggested the absorption of the poorly water-soluble drugs like digoxin can be enhanced by employing SLN formulations.

Fenofibrate. The bioavailability of the poorly soluble fenofibrate following oral administration of fenofibrate-loaded SLNs was investigated in rats (98). The SLN formulation demonstrated approximately twofold bioavailability enhancement in terms of rate and extent compared to the suspension formulations of fenofibrate. The work sug-

gested that nanosuspensions are potential carriers to improve the oral bioavailability of lipophilic drugs.

Insulin. In a study, lectin-modified SLNs containing insulin were prepared by three different methods (99). Furthermore, some insulin-loaded SLNs were modified with wheat germ agglutinin-*N*-glutaryl-phosphatidylethanolamine (WGA-*N*-glut-PE). Highest drug-entrapment efficiency (>60%) was found in case of the insulin-loaded SLNs prepared by an appropriate modification of the double dispersion method. SLNs and WGA-modified SLNs protected insulin against *in vitro* degradation by digestive enzymes. WGA-modified SLNs were found to be more stable than SLNs. In comparison to subcutaneous injection of insulin, oral administration of insulin-loaded SLNs or WGA-modified SLNs in rats showed the relative pharmacological bioavailabilities of 4.46% and 6.08%, and the relative bioavailabilities of 4.99% and 7.11%, respectively. In another study, SLNs loaded with insulin and a cell-penetrating peptide, R8 (R8-Ins-SLNs) were prepared using the emulsion solvent diffusion method (100). Particles were spherical and the mean particle size, zeta potential, encapsulation efficiency were150.8±23.4 nm, 32.65±2.02 mV, 62.29±0.52% (insulin), and 58.05±0.66% (R8), respectively. *In vivo* study showed that the relative pharmacological bioavailability of R8-Ins-SLN was 10.39±0.46%. The results demonstrated that SLNs loaded with cell-penetrating peptide could be a promising carrier for oral delivery of insulin. Sarmento *et al.* (32) prepared cetyl palmitate-based SLN containing insulin by a modified solvent emulsification evaporation method based on *w*/*o*/*w* double emulsion. The particle size and zeta potential of the SLNs were found to be ~350 nm and negatively charged, respectively. The insulin association efficiency was >43%. A marked hypoglycemic effect was observed after oral administration of insulin-loaded SLNs to diabetic rats. The study suggested that SLNs can promote oral absorption of insulin. In a separate study, *in situ* local intestinal perfusion experiment of WGA-modified liposomes and SLNs was performed in rats (101). The formulations containing 100 IU kg^{-1} insulin were administered to the duodenum, jejunum, and ileum of fasted rats. Serum insulin concentrations decreased for the various formulations in different absorption sites according to the following trends: duodenum>ileum>jejunum for WGA-modified liposomes; duodenum>jejunum>ileum for WGA-modified SLNs; ileum>jejunum>duodenum for liposomes; ileum>duodenum>jejunum for SLNs; and duodenum>or=ileum>jejunum for aqueous solution of insulin. The results suggested that the delivery sites were important factors with respect to increasing the bioavailability of orally administered insulin. In another work, the influence of the lipid's characteristics on the formulation, *in vitro* release, and *in vivo* absorption of SLNs was studied (34). SLNs were prepared by the double emulsion method. Several lipids, such as stearic acid, octadecyl alcohol, cetyl palmitate (CP), glyceryl monostearate, glyceryl palmitostearate (GP), glyceryl tripalmitate (GT), and glyceryl behenate were evaluated. Insulin or thymopentin were incorporated into SLNs as the model protein drugs. SLNs were successfully prepared using CP, GT, and GP, and their particle size was 447.5±50.8, 444.8±72.5, and 213.7±38.4 nm, respectively. Insulin solution, insulin-CP-SLNs, insulin-GP-SLNs, and insulin-GT-SLNs were administered to streptozotocin-induced male diabetic Sprague–Dawley rats by oral gavage at a dose of 50 IU kg^{-1} and plasma glucose level was determined. Blood glucose levels decreased following the administration of SLNs. The pharmacological availability of insulin-CP-SLNs, insulin-GT-SLNs, and insulin-GP-SLNs after oral administration to diabetic rats were 2.92%, 3.44%, and 4.53%, respectively. GP-SLNs demonstrated lower burst release, and a stable particle size, together with a relatively high pharmacological availability. This study suggested that GP is a promising lipid to prepare SLNs for oral delivery of proteins.

Lovastatin. Lovastatin-Loaded NLCs were prepared from the mixtures of Precirol® and squalene, and compared with SLNs and lipid emulsions prepared from pure Precirol® and squalene, respectively (40). The mean size range and zeta potential range of the nanoparticles were 180–290 nm and −3 to −35 mV, respectively. Entrapment efficiency of lovastatin in NLCs and lipid emulsions was significantly higher than SLNs. The *in vitro* drug release study depicted that lovastatin release could be reduced up to 60% with lipid nanoparticles containing Myverol® (lipophilic emulsifier) at a decreasing order of NLCs>lipid emulsions>SLNs. Drug release further decreased when soybean phosphatidylcholine was used (NLCs and SLNs demonstrated slowest delivery). The oral bioavailability of lovastatin increased from 4% to 24% and 13% after the oral administration of lovastatin-loaded NLCs containing Myverol® and soybean phosphatidylcholine, respectively. The formulations prepared with Myverol® were more stable in the gastric environment in compare to the formulations prepared with soybean phosphatidylcholine.

Melatonin. Pharmacokinetics of melatonin after oral and transdermal administration of melatonin-loaded SLNs in human were performed (102). The aim of this study was to make SLN as a drug reservoir, permitting a constant and prolonged release of the incorporated drug. Time to reach maximum plasma drug concentration (T_{max}) after melatonin–SLNs oral administration resulted delayed of about 20 min compared to melatonin solution, while mean AUC and mean half-life of elimination was significantly higher. Melatonin absorption and elimination after transdermal administration of SLNs were slow. The researchers concluded that by varying dosages and concentrations of the incorporated drugs, different plasma level profile could be obtained, so disclosing new possibilities for sustained delivery systems.

Methotrexate. Different SLNs were prepared using tristearin, glycerol monostearate, stearic acid, and Compritol® 888 ATO by solvent diffusion method (38). However, SLNs prepared with Compritol® 888 ATO demonstrated better drug-loading and release characteristics than other formulations. The *in vivo* absorption and bioavailability studies were performed on the selected formulations. In addition, periodic lymphatic concentration of drug following oral administration of respective formulations was also measured. The results indicated that the methotrexate-loaded SLNs based on Compritol® 888 ATO markedly enhanced the oral bioavail-

ability of methotrexate, presumably following SLNs constituting lipid digestion and co-absorption through lymphatic route.

N_3-O-toluyl-fluorouracil. Cationic SLNs containing N_3-O-toluyl-fluorouracil (TFu) were produced film dispersion–ultrasonication method using hexadecyltrimethylammonium bromide as cationic tenside (65). The aim of the study was to evaluate the potential of these SLNs to enhance the oral absorption of TFu. The morphology study indicated almost spherical shape of the SLNs. The mean particle size, zeta potential, entrapment efficiency, and drug loading were 178.8 ±9.99 nm, +19.54±0.32 mV, 71.03±1.19%, and 3.57±0.08%, respectively. The pharmacokinetic studies in mice revealed that the oral bioavailability of TFu was noticeably enhanced following oral administration of TFu-loaded SLNs when compare with that of the TFu suspensions. The absorption of TFu-SLNs through intestine was fitted to first-order kinetics with passive diffusion mechanism. This study also demonstrated that the main segments of TFu–SLNs absorption in intestine were duodenum and jejunum. The bio-adhesion mediated by electrostatic interaction between the positively charged SLNs and the negatively charged mucosal surface was responsible for the enhanced absorption. The study revealed that cationic SLNs are a promising delivery system for the enhancement of the oral bioavailability of the drugs with poor oral absorption. In another experiment, a comparative study was performed between liposomes and SLNs (anionic and cationic) containing TFu (103). In both studies of crossing Caco-2 cell monolayers and the single-pass intestinal perfusion in rat, SLNs exhibited more capability to enhance transport TFu than liposomes. The cell study showed that the cationic SLNs had the most effective capacity. The Ka and uptake percentage of these three formulations in the perfusion study were according to the following order: anionic SLN>cationic SLN>liposomes. In addition, all these formulations exhibited site-dependent absorption behavior. By comparing the *in vitro* and *in vivo* transmucosal behavior of these nanocarriers, the anionic SLNs were identified to be more effective for the transport of TFu.

Nitrendipine. To increase the oral bioavailability, different nitrendipine-loaded SLNs were prepared by hot homogenization–ultrasonication method using triglyceride (tripalmitin), monoglyceride (glyceryl monostearate), and wax (cetyl palmitate; 31). The results showed three- to fourfold augmentation of the bioavailability of nitrendipine following intraduodenal administration of SLN formulations than the nitrendipine suspension. The study recommended SLNs as potential carriers of lipophilic drugs for improving the bioavailability by minimizing first-pass metabolism.

Otcadecylamine. Yuan *et al*. incorporated otcadecylamine-fluorescein isothiocyanate (ODA-FITC) into stearic acid SLNs by solvent diffusion method (104). Entrapment efficiency of ODA-FITC in the SLNs was ~97.9%. The *in vivo* transport experiments revealed that the transport efficiency of the SLNs upon oral administration was 30%. The SLNs were extensively absorbed and showed a linear absorption mechanism in GI tract within certain range of concentrations. The study also revealed that ~77.9% of the absorbed SLNs was transported into systematic circulation via lymph and rest of the absorbed SLNs was transported directly into blood, which might be through capillary vessel or intestinal epithelial cell by paracellular pathway. Further, study revealed that the polyethylene glycol monostearate-modified SLNs were also absorbed through GI tract and showed a prolonged effect *in vivo*.

Pentoxifylline. Pentoxifylline, a highly water-soluble hemorheologic drug, has poor oral bioavailability (20%) due to high first-pass metabolism. Pentoxifylline-loaded SLNs were developed by homogenization followed by the sonification method (29). The aim of the study was to increase oral bioavailability of pentoxifylline. SLNs were prepared by varying different variables. The average particle size, zeta potential, and EE of the SLNs were at least 250 nm, −30.2 mV, and 70%, respectively. The optimized SLNs were prepared using 80 mg of cetyl alcohol, 10 mg of lecithin, acetone:DCM ratio of 1:2, 30-s sonication, 3% Tween® 20, and a mixing rate of 800 rpm. The pharmacokinetic study performed in male Wistar rats following oral administration of 10 mg kg^{-1} pentoxifylline in the form of SLNs or free drug showed that the relative bioavailability of pentoxifylline in SLNs was significantly increased in compare to that of the pentoxifylline solution. The study indicated that SLNs could be potential carrier of pentoxifylline to improve the oral bioavailability by avoiding high first-pass effect.

Praziquantel. Praziquantel-loaded SLNs were prepared by ultrasound technique to enhance the oral bioavailability of praziquantel (105). SLNs with an average diameter of 110 nm, zeta potential of −66.3 mV, and encapsulation efficiency of ~80% were produced. Two peaks in the praziquantel concentration–time curves in plasma were observed after oral administration of praziquantel-loaded SLNs in rats. The first peak might be due to free drug and that adsorbed onto the surface of the SLNs. The second peak was due to gut uptake of the SLNs. The $AUC_{(0-\infty)}$ and MRT values of praziquantel following oral administration of SLNs was 4.1- and twofold higher than that obtained with the praziquantel tablets, respectively. Hence, the oral bioavailability of praziquantel SLNs markedly increased in compare to praziquantel tablets suggesting SLNs as valuable drug delivery system of praziquantel. In another recent study (64), praziquantel-loaded hydrogenated castor oil SLNs were prepared to increase bioavailability and prolong systemic circulation of the drug. SLNs were prepared by hot homogenization and ultrasonication method. The particle size, polydispersity index, zeta potential, encapsulation efficiency, and loading capacity of the SLNs were 344.0±15.1 nm, 0.31±0.08, −16.7±0.5 mV, 62.17±6.53%, and 12.43±1.31%, respectively. An initial burst release followed by a sustained release was observed from *in vitro* drug release study of the SLNs. Pharmacokinetic study in mice following oral, subcutaneous, and intramuscular administration of the praziquantel-loaded SLNs indicated increase in bioavailability of praziquantel by 14.9-, 16.1-, and 2.6-fold, respectively. The mean residence times of the drug were also extended from 7.6, 6.6, and 8.2 to 95.9, 151.6, and 48.2 h after oral, subcutaneous, and intramuscular administration, respectively. The authors concluded that praziquantel-loaded hydrogenated castor oil SLNs could be a promising formulation to enhance the pharmacological activity of praziquantel.

Quercetin. SLNs containing quercetin (poorly aqueous soluble) were prepared by an emulsification and low-temperature

solidification method (106). Spherical particles with average diameter of 155.3 nm, drug entrapment efficiency of 91.1%, drug loading of 13.2%, and zeta potential of −32.2 mV were produced. The GI absorption experiment using an *in situ* perfusion method in rats revealed that the absorption in the stomach for 2 h was only 6.2% and the main absorptive segments were ileum and colon by passive diffusion. The pharmacokinetic study in rats following oral administration of quercetin (50 mg kg^{-1}) in the form of either SLNs or suspension demonstrated that the relative bioavailability of quercetin–SLNs to quercetin suspension was 571.4%. The T_{max} and MRT for quercetin in plasma were delayed. The study suggested that SLNs could be potential oral delivery carrier to enhance the absorption of a poorly water-soluble drug, quercetin.

Rifampicin, Isoniazid, and Pyrazinamide. Pandey *et al.* incorporated rifampicin, isoniazid, and pyrazinamide into SLNs prepared by emulsion solvent diffusion technique and evaluate their potential against experimental tuberculosis (107). Encapsulation efficiencies for rifampicin, isoniazid, and pyrazinamide were 51 ±5%, 45±4%, and 41±4%, respectively. Therapeutic drug concentrations were maintained in the plasma for 8 days and in the organs (lungs, liver, and spleen) for 10 days after a single oral administration of SLNs to mice, whereas free drugs were cleared by 1–2 days. No tubercle bacilli could be detected in the lungs/spleen after five oral doses of SLNs administered at every tenth day in *Mycobacterium tuberculosis* v H_{37}Rv-infected mice, whereas 46 daily doses of oral-free drugs were required to obtain an equivalent therapeutic benefit. The study suggested that SLN based antitubercular drug therapy may reduce dosing frequency and improve patient compliance for better management of tuberculosis.

Salmon calcitonin. In a study, potential of the surface-modified lipid nanoparticles as oral delivery systems for salmon calcitonin (hydrophilic macromolecule) was evaluated (26). The results showed that the PEG-coated nanoparticles did not affect the permeability of Caco-2 monolayer but chitosan-coated nanoparticles exhibited a dose-dependent reduction in the transepithelial electric resistance and increased Texas Red®-dextran transport. A significant and prolonged reduction in the serum calcium levels was observed after oral administration of calcitonin-loaded chitosan-coated nanoparticles in rats in comparison to those of calcitonin solution. On the other hand, the hypocalcemic response of calcitonin-loaded PEG-coated nanoparticles was not significantly different from calcitonin solution. The study reflected the importance of the surface composition of the particles for improvement of the efficiency of oral calcitonin-loaded nanoparticle formulations. The results also suggested that chitosan-coated nanoparticles have potential as carriers for oral protein and peptide delivery. In another work, salmon calcitonin-loaded SLNs composed of trimyristin (solid lipid) and poloxamer 407 (surfactant) were prepared by a *w/o/w* emulsion technique (41). SLNs were spherical in shape with a smooth surface. The mean particle size and association efficiency for calcitonin of the SLNs were ~200 nm and ~86%, respectively. Following 500 IU kg^{-1} oral dose of calcitonin-loaded SLNs in rats, the basal blood calcium level was reduced up to 20% and hypocalcaemia was sustained for >8 h. The study predicted that the incorporation of salmon calcitonin into trimyristin SLNs was responsible for the improvement of the efficiency of this protein.

Simvastatin. Simvastatin, a cholesterol-lowering agent, possesses poor oral bioavailability due to its low water solubility and extensive metabolism in the guts and liver. Zhang *et al.* (65) developed simvastatin-loaded SLNs to enhance its oral bioavailability. Spherical nano sized particles with >95% encapsulation efficiency were produced. The *in situ* intestinal absorption results demonstrated that the absorption of SLNs was significantly improved in comparison to that of free simvastatin. Furthermore, the absorption changed with the site of the intestinal segments. SLNs could be uptaken into the enterocytes through both clathrin- and caveolae-mediated endocytosis pathways. Following oral administration of simvastatin-loaded SLNs in rats, the oral bioavailability of simvastatin was enhanced by 2.55- to 3.37-fold in comparison with that from free simvastatin. Additionally, plasma concentration of simvastatin acid (active metabolite of simvastatin) also increased after oral administration of simvastatin-loaded SLNs than free simvastatin. The study suggested that SLNs could be a promising delivery system to enhance the oral bioavailability of simvastatin.

Spironolactone. Different formulations (SLNs and DissoCubes®) of spironolactone (poorly water soluble) with various particle sizes (micro and nano) were prepared and tested in rats (108). The bioavailability of spironolactone was determined on the basis of its metabolites canrenone and 7-α-thiomethylspirolactone. SLNs showed 5.7-fold increase in AUC for canrenone and a similar improvement of C_{max} (maximum plasma drug concentration). The results demonstrated that the particle size minimization was not the major determining factor in the bioavailability improvement. Rather, the type of surfactant used in the formulations showed huge influence on the bioavailability. The study predicted enhancement of drug solubility, dissolution rate, and permeability of spironolactone in the intestine were main mechanisms for bioavailability improvement.

Vinpocetine. To estimate the potential of NLCs as oral delivery system for poorly water-soluble drug, Zhuang *et al.* prepared vinpocetine-loaded NLCs by HPH method (109). Spherical particles with smooth surface and average encapsulation efficiency of 94.9±0.4% were produced. The powder X-ray diffraction and differential scanning calorimetric studies indicated that the drug was in an amorphous state in the NLC matrix. The pharmacokinetic study in Wistar rats showed that the relative bioavailability of vinpocetine-loaded NLCs was 322% in compare with vinpocetine suspension. The study demonstrated the NLC formulation as a promising perspective for oral delivery of poorly water-soluble drugs. In another study, vinpocetine-loaded glyceryl monostearate nanodispersions were prepared by ultrasonic-solvent emulsification technique (39). The pharmacokinetic study in male rats showed significant enhancement of the relative bioavailability of vinpocetine upon oral administration of 10 mg kg^{-1} vinpocetine-loaded SLNs in comparison with that of the vinpocetine solution. The surfactant concentration in the SLNs also showed a significant influence on the oral absorption of vinpocetine. The study indicated that SLNs can be used to improve the oral bioavailability of poorly soluble drugs.

Table I. Different Drug-Loaded Lipid Nanoparticles for Oral Administration, their *In vivo* Implications and Formulation Methods

Drug	Implications	Type	Formulation method	Ref.
All-*trans* retinoic acid	Enhancement of GI absorption Improvement of oral bioavailability	SLN	HPH	(37)
Apomorphine	Improvement of oral bioavailability Improvement of brain regional distribution	SLN	Dispersion-ultrasonication	(28)
α-Asarone	Enhancement of GI absorption Improvement of oral bioavailability Improvement of tissue uptake and distribution	SLN	Ultrasonic homogenization	(95)
Buspirone HCl	Improvement of oral bioavailability	SLN	Emulsification-evaporation followed by ultrasonification	(30)
Camptothecin	Body distribution Sustained release and tissue targeting	SLN	HPH	(96)
Carvedilol	Enhancement of oral bioavailability Lymphatic uptake Bypass hepatic first-pass metabolism	SLN	Microemulsion	(68)
Clozapine	Improvement of oral bioavailability Tissue distribution	SLN	Hot homogenization followed by ultrasonication	(27)
Cryptotanshinone	Enhancement of GI absorption Improvement of oral bioavailability Change in metabolism	SLN	Ultrasonic and HPH	(35)
Cyclosporine A	Changes in oral bioavailability	Liposphere	–	(97)
Cyclosporine A	Low variation in bioavailability Avoidance of the huge initial plasma concentration	SLN	HPH	(43)
Curcumin	Improvement of oral bioavailability Prolonged release	SLN	Microemulsion	(33)
Digoxin	Enhancement of GI absorption Improvement of oral bioavailability	SLN	Ultrasonic and HPH	(36)
Fenofibrate	Improvement of oral bioavailability	SLN	HPH	(98)
Insulin	Protection of insulin degradation by enzyme Enhancement of GI absorption Improvement of oral bioavailability	Lectin-modified SLN	Dispersion-ultrasonication	(99)
Insulin	Improvement of oral bioavailability	SLN	Emulsion solvent diffusion	(100)
Insulin	Hypoglycemic effect Enhancement of GI absorption	SLN	Solvent emulsification evaporation (based on *w*/*o*/*w* double emulsion)	(32)
Insulin	Enhancement of GI absorption Importance of delivery site	SLN	Dispersion–ultrasonication	(101)
Insulin	Reduction of blood glucose level	SLN	Double emulsion	(34)
Lovastatin	Enhancement of encapsulation efficiency Improvement of oral bioavailability Improvement of stability in GI environment	NLC	Hot homogenization–ultrasonication	(40)
Melatonin	Sustained delivery	SLN		(102)
Methotrexate	Improvement of oral bioavailability Enhancement of GI absorption Lymphatic transport	SLN	Solvent diffusion	(38)
N_3-O-toluyl-fluorouracil	Enhancement of GI absorption Improvement of oral bioavailability	Cationic SLN	Film dispersion–ultrasonication	(65)
N_3-O-toluyl-fluorouracil	Improvement of intestinal transport	SLN (cationic, anionic)	Film dispersion–ultrasonication	(103)
Nitrendipine	Improvement of oral bioavailability Reduction of first-pass metabolism	SLN	Hot homogenization–ultrasonication	(31)
Otcadecylamine	Enhancement of GI absorption Lymphatic transport Prolonged release	SLN	Solvent diffusion	(104)
Pentoxifylline	Improvement of oral bioavailability Reduction of first-pass metabolism	SLN	Homogenization followed by the ultrasonication	(29)
Praziquantel	Improvement of oral bioavailability	SLN	Ultrasonication	(105)
Praziquantel	Improvement of bioavailability Prolonged systemic circulation	SLN	Hot homogenization and ultrasonication	(64)

Table I. (Continued)

Drug	Implications	Type	Formulation method	Ref.
Quercetin	Enhancement of GI absorption Improvement of oral bioavailability	SLN	Emulsification and low-temperature solidification	(106)
Rifampicin, Isoniazid, and Pyrazinamide	Anti- tubercular activity Prolonged release	SLN	Emulsion solvent diffusion	(107)
Salmon calcitonin	Prolonged reduction of serum calcium level	Coated SLN	Double emulsion solvent evaporation	(26)
Salmon calcitonin	Prolonged reduction of blood calcium level	SLN	Double emulsion	(41)
Simvastatin	Improvement of oral bioavailability	SLN	Emulsification solvent evaporation	(65)
Spironolactone	Improvement of oral bioavailability	SLN	HPH	(108)
Vinpocetine	Improvement of oral bioavailability	NLC	HPH	(109)
Vinpocetine	Enhancement of GI absorption Improvement of oral bioavailability	SLN	Ultrasonic-solvent emulsification	(39)

STABILITY AND STORAGE CONDITION

SLNs/NLCs may contain additional colloidal structures, such as micelles, mixed micelles, liposomes, and nanoemulsions (25). Furthermore, SLNs/NLCs have additional features (supercooled melts, different modifications, non-spherical shapes) which contribute to their stability. There are several major stability issues during storage, such as particle size enhancement, gelation of the dispersion, and drug expulsion from the lipid matrix. Gelation takes place due to formation of the network and lipid bridges between the particles (69). The first product formed after hot homogenization is supercooled melt (spherical lipid droplets) which has high drug-loading capacity. However, transformation of the lipid melt to lipid crystals results decrease in drug loading capacity of the lipid, which results expulsion of drug from lipid matrix.

The physical stability of SLNs/NLCs dispersions is generally investigated by measurements of particle size (PCS, LD), zeta potential (PCS), and thermal analysis (DSC). Several studies indicated physical stability of SLNs dispersion more than 1 year (33,47,58,110). A study investigated the effect of light and temperature on the physical stability of SLNs dispersion (69). The study reported that light- and temperature-induced particle growth. Gelation of the dispersion occurred within 7 days and 3 months storage under artificial light and day light, respectively. In contrary, particle growth started after 4 months storage under dark condition. The ZPs were also decreased from −24.7 to below −18 mV upon storage under light. The study also reported increase in particle size at elevated temperatures, whereas insignificant change in particle size when stored in refrigerator for more than 3 months. Additionally, ZP decreased from −24.7 to −15 mV upon particle growth. Another study demonstrated that development of optimal storage conditions can improve the physical stability of SLN formulations (111). One recent study (33) revealed that SLNs were stable after 1 year of storage at 5±3°C. Insignificant increase in particle size was observed after 1 year. Although entrapment efficiency decreased about 9%, total drug content dropped only 3% indicating the stability of the prepared SLNs. However, stability of the formulation also depend on the formulation components, such as emulsifier (28), type of lipid (31). Another recent study showed that SLNs were more stable in terms of change in size and entrapment efficiency when stored at refrigerated temperature (4±1°C), in comparison to room temperature storage (38). Generally, the lipid in SLN is present in a mixture of β′, α and sub α polymorphs after hot HPH. However, kinetic energy (e.g., light, temperature) causes a transformation to β polymorph accompanied by gel formation. This transformation could be avoided/minimized by storing the formulations in refrigerator under dark condition (69).

The chemical stability of the lipid matrix of SLNs can be measured by gas chromatography in combination with a method for lipid extraction from aqueous SLN dispersions (63). Radomska-Soukharev (63) showed a negligible decomposition of the formulation structure during incubation at 25°C where lipids consisted of trigylicerides. Dynasan® 118 demonstrated the highest chemical stability (loss <4%) within 2 years.

In addition to the optimized storage conditions, SLNs/NLCs dispersions can also be spray dried to increase their stability. However, melting point of the lipid matrix should be more than 70°C for spray drying. Freitas *et al.* (112) have converted aqueous dispersions of SLNs by spray drying into dry, reconstitutable powders which could be stored over a long period. The study indicated that the particle size was influenced by the applied spraying parameters and by the chemical nature of the lipid phase, the type of carbohydrate and the spraying, and the redispersion medium. The resulting spray dried SLNs were reconstitutable to the identical particle size distribution of the original dispersion.

Another efficient way to increase stability is lyophilisation (113,114). However, when SLN are lyophilized without cryoprotectants, the final product commonly results in the aggregation of particles. The most widely used cryoprotectants in SLNs are trehalose, sorbitol, glucose, sucrose, mannose, and maltose. Schwarz and Mehnert reported trehalose as the most effective cryoprotectant in preventing particle growth (114). A study has investigated the effect of cryoprotective sugars on the size of SLNs after lyophilisation and reconstitution (115). Trehalose was found to be the most effective cryoprotectant. In addition, trehalose was most effective for preventing drug expulsion upon reconstitution. Another study has investigated the influence of different

parameters of lyophilization, such as the protective effect of cryoprotectants, freezing velocity, and thermal treatment (116). The study suggested that small particle size of the SLNs dispersions can be preserved by lyophilization. Lim *et al.* (117) showed only slight augmentation of the mean particle size and polydispersity index of SLNs after liyophilization. The study also revealed that there were insignificant changes drug concentration and zeta potential of SLNs after liyophilization. Another study compared glucose and trehalose as cryoprotectants for liophylization of SLNs and suggested trehalose as better cryoprotectant than glucose (118).

CONCLUSIONS

SLN and NLC formulations have shown huge potential for oral delivery to improve GI absorption and oral bioavailability of many drugs. These formulations are also useful for sustained/prolonged release or targeted drug delivery. The excipients approved by the regulatory authorities are used to prepare the lipid nanoparticles. Therefore, excipients used are of GRAS (Generally Recognized As Safe) status or are already used in on the pharmaceutical or food products. However, the excipients need to be used in their regulatorily accepted concentrations. If distinctly higher concentrations need to be used, a limited toxicity study should be performed to prove the safety of the excipients at that concentration. Easy scale up of the formulation technique is also an attractive feature of this formulation. Although several studies have been performed on SLNs for oral delivery, only few works focused on NLCs till now. In the future, more focus should be on NLCs as oral drug carrier due to their higher drug-loading capacity and lower drug expulsion during storage than SLNs. More pre-clinical and clinical studies should also be performed in near future to establish these formulations in the market.

REFERENCES

1. Mehnert W, Mader K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev. 2001;47 (2–3):165–96.
2. Muller RH, Mader K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery—a review of the state of the art. Eur J Pharm Biopharm. 2000;50(1):161–77.
3. Radtke M, Souto EB, Müller RH. Nanostructured Lipid Carriers: a novel generation of solid lipid drug carriers. Pharm Technol Eur. 2005;17(4):45–50.
4. Pouton CW. Formulation of poorly water-soluble drugs for oral administration: physicochemical and physiological issues and the lipid formulation classification system. Eur J Pharm Sci. 2006;29 (3–4 SPEC. ISS):278–87.
5. Muller RH, Maassen S, Weyhers H, Mehnert W. Phagocytic uptake and cytotoxicity of solid lipid nanoparticles (SLN) sterically stabilized with poloxamine 908 and poloxamer 407. J Drug Target. 1996;4(3):161–70.
6. Ravi Kumar MN. Nano and microparticles as controlled drug delivery devices. J Pharm Pharm Sci. 2000;3(2):234–58.
7. Shidhaye SS, Vaidya R, Sutar S, Patwardhan A, Kadam VJ. Solid lipid nanoparticles and nanostructured lipid carriers—innovative generations of solid lipid carriers. Curr Drug Deliv. 2008;5 (4):324–31.
8. Chen DB, Yang TZ, Lu WL, Zhang Q. *In vitro* and *in vivo* study of two types of long-circulating solid lipid nanoparticles containing paclitaxel. Chem Pharm Bull. 2001;49(11):1444–7.
9. Fundaro A, Cavalli R, Bargoni A, Vighetto D, Zara GP, Gasco MR. Non-stealth and stealth solid lipid nanoparticles (SLN) carrying doxorubicin: pharmacokinetics and tissue distribution after i.v. administration to rats. Pharmacol Res. 2000;42(4): 337–43.
10. Freitas C, Muller RH. Effect of light and temperature on zeta potential and physical stability in solid lipid nanoparticle (SLN®) dispersions. Int J Pharm. 1998;168(2):221–9.
11. Freitas C, Muller RH. Correlation between long-term stability of solid lipid nanoparticles (SLN(TM)) and crystallinity of the lipid phase. Eur J Pharm Biopharm. 1999;47(2):125–32.
12. Freitas C, Muller RH. Stability determination of solid lipid nanoparticles (SLN®) in aqueous dispersion after addition of electrolyte. J Microencapsul. 1999;16(1):59–71.
13. Dingler A, Gohla S. Production of solid lipid nanoparticles (SLN): scaling up feasibilities. J Microencapsul. 2002;19(1): 11–6.
14. Gohla SH, Dingler A. Scaling up feasibility of the production of solid lipid nanoparticles (SLN). Pharmazie. 2001;56(1):61–3.
15. Manjunath K, Ready JS, Venkateswarlu V. Solid lipid nanoparticles as drug delivery systems. Meth Find Exp Clin Pharmacol. 2005;27(2):127–44.
16. Charman WN. Lipids, lipophilic drugs, and oral drug delivery—some emerging concepts. J Pharm Sci. 2000;89(8):967–78.
17. Charman WN, Porter CJH, Mithani S, Dressman JB. Physicochemical and physiological mechanisms for the effects of food on drug absorption: the role of lipids and pH. J Pharm Sci. 1997;86 (3):269–82.
18. Charman WN, Porter CJH. Lipophilic prodrugs designed for intestinal lymphatic transport. Adv Drug Deliv Rev. 1996;19 (2):149–69.
19. Holm R, Porter CJH, Mullertz A, Kristensen HG, Charman WN. Structured triglyceride vehicles for oral delivery of halofantrine: examination of intestinal lymphatic transport and bioavailability in conscious rats. Pharm Res. 2002;19(9):1354–61.
20. Porter CJH, Charman WN. Intestinal lymphatic drug transport: an update. Adv Drug Deliv Rev. 2001;50(1–2):61–80.
21. Kaur IP, Bhandari R, Bhandari S, Kakkar V. Potential of solid lipid nanoparticles in brain targeting. J Control Release. 2008;127(2):97–109.
22. Chen Y, Dalwadi G, Benson HA. Drug delivery across the blood–brain barrier. Curr Drug Deliv. 2004;1(4):361–76.
23. Souto EB, Doktorovova S, Gonzalez-Mira E, Egea MA, Garcia ML. Feasibility of lipid nanoparticles for ocular delivery of anti-inflammatory drugs. Curr Eye Res. 2010;35(7):537–52.
24. Pardeike J, Hommoss A, Müller RH. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. Int J Pharm. 2009;366(1–2):170–84.
25. Wissing SA, Kayser O, Muller RH. Solid lipid nanoparticles for parenteral drug delivery. Adv Drug Deliv Rev. 2004;56(9):1257–72.
26. Garcia-Fuentes M, Prego C, Torres D, Alonso MJ. A comparative study of the potential of solid triglyceride nanostructures coated with chitosan or poly(ethylene glycol) as carriers for oral calcitonin delivery. Eur J Pharm Sci. 2005;25(1):133–43.
27. Manjunath K, Venkateswarlu V. Pharmacokinetics, tissue distribution and bioavailability of clozapine solid lipid nanoparticles after intravenous and intraduodenal administration. J Control Release. 2005;107(2):215–28.
28. Tsai MJ, Huang YB, Wu PC, Fu YS, Kao YR, Fang JY, *et al.* Oral apomorphine delivery from solid lipid nanoparticleswith different monostearate emulsifiers: Pharmacokinetic and behavioral evaluations. J Pharm Sci. 2010 (in press).
29. Varshosaz J, Minayian M, Moazen E. Enhancement of oral bioavailability of pentoxifylline by solid lipid nanoparticles. J Liposome Res. 2010;20(2):115–23.
30. Varshosaz J, Tabbakhian M, Mohammadi MY. Formulation and optimization of solid lipid nanoparticles of buspirone HCl for enhancement of its oral bioavailability. J Liposome Res. 2010;20 (4):286–96.
31. Kumar VV, Chandrasekar D, Ramakrishna S, Kishan V, Rao YM, Diwan PV. Development and evaluation of nitrendipine loaded solid lipid nanoparticles: influence of wax and glyceride lipids on plasma pharmacokinetics. Int J Pharm. 2007;335(1–2):167–75.
32. Sarmento B, Martins S, Ferreira D, Souto EB. Oral insulin delivery by means of solid lipid nanoparticles. Int J Nanomedicine. 2007;2(4):743–9.

33. Kakkar V, Singh S, Singla D, Kaur IP. Exploring solid lipid nanoparticles to enhance the oral bioavailability of curcumin. Mol Nutr Food Res. 2010 (in press).
34. Yang R, Gao R, Li F, He H, Tang X. The influence of lipid characteristics on the formation, *in vitro* release, and *in vivo* absorption of protein-loaded SLN prepared by the double emulsion process. Drug Dev Ind Pharm. 2010 (in press).
35. Hu L, Xing Q, Meng J, Shang C. Preparation and enhanced oral bioavailability of cryptotanshinone-loaded solid lipid nanoparticles. AAPS PharmSciTech. 2010;11(2):582–7.
36. Hu L, Jia H, Luo Z, Liu C, Xing Q. Improvement of digoxin oral absorption in rabbits by incorporation into solid lipid nanoparticles. Pharmazie. 2010;65(2):110–3.
37. Hu L, Tang X, Cui F. Solid lipid nanoparticles (SLNs) to improve oral bioavailability of poorly soluble drugs. J Pharm Pharmacol. 2004;56(12):1527–35.
38. Paliwal R, Rai S, Vaidya B, Khatri K, Goyal AK, Mishra N, *et al*. Effect of lipid core material on characteristics of solid lipid nanoparticles designed for oral lymphatic delivery. Nanomedicine NBM. 2009;5(2):184–91.
39. Luo Y, Chen D, Ren L, Zhao X, Qin J. Solid lipid nanoparticles for enhancing vinpocetine's oral bioavailability. J Control Release. 2006;114(1):53–9.
40. Chen CC, Tsai TH, Huang ZR, Fang JY. Effects of lipophilic emulsifiers on the oral administration of lovastatin from nanostructured lipid carriers: physicochemical characterization and pharmacokinetics. Eur J Pharm Biopharm. 2010;74(3):474–82.
41. Martins S, Silva AC, Ferreira DC, Souto EB. Improving oral absorption of samon calcitonin by trimyristin lipid nanoparticles. J Biomed Nanotechnol. 2009;5(1):76–83.
42. Muller RH, Runge SA, Ravelli V, Thunemann AF, Mehnert W, Souto EB. Cyclosporine-loaded solid lipid nanoparticles (SLN®): drug-lipid physicochemical interactions and characterization of drug incorporation. Eur J Pharm Biopharm. 2008;68 (3):535–44.
43. Muller RH, Runge S, Ravelli V, Mehnert W, Thunemann AF, Souto EB. Oral bioavailability of cyclosporine: solid lipid nanoparticles (SLN®) *versus* drug nanocrystals. Int J Pharm. 2006;317 (1):82–9.
44. Saupe A, Gordon KC, Rades T. Structural investigations on nanoemulsions, solid lipid nanoparticles and nanostructured lipid carriers by cryo-field emission scanning electron microscopy and Raman spectroscopy. Int J Pharm. 2006;314(1):56–62.
45. Müller RH, Radtke M, Wissing SA. Nanostructured lipid matrices for improved microencapsulation of drugs. Int J Pharm. 2002;242(1–2):121–8.
46. Müller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Deliv Rev. 2002;54 (Supplement 1):S131–55.
47. Westesen K, Bunjes H, Koch MHJ. Physicochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential. J Control Release. 1997;48(2–3):223–36.
48. Jenning V, Lippacher A, Gohla SH. Medium scale production of solid lipid nanoparticles (SLN) by high pressure homogenization. J Microencapsul. 2002;19(1):1–10.
49. Cavalli R, Caputo O, Marengo E, Pattarino F, Gasco MR. The effect of the components of microemulsions on both size and crystalline structure of solid lipid nanoparticles (SLN) containing a series of model molecules. Pharmazie. 1998;53 (6):392–6.
50. Schwarz C. Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization. J Control Release. 1994;30(1):83–96.
51. Muchow M, Maincent P, Muller RH. Lipid nanoparticles with a solid matrix (SLN®, NLC®, LDC®) for oral drug delivery. Drug Dev Ind Pharm. 2008;34(12):1394–405.
52. Müller RH, Lucks JS, inventors; Arzneistoffträger aus festen Lipidteilchen, Feste Lipidnanosphären (SLN)/medication vehicles made of solid lipid particles (solid lipid nanospheres–SLN) patent EP 0605497 B1. 1996.
53. Gasco MR, inventor Gasco, M. R., assignee. Method for producing solid lipid microspheres having a narrow size distribution patent US5250236. 1993.
54. Cortesi R, Esposito E, Luca G, Nastruzzi C. Production of lipospheres as carriers for bioactive compounds. Biomaterials. 2002;23(11):2283–94.
55. Igartua M, Saulnier P, Heurtault B, Pech B, Proust JE, Pedraz JL, *et al*. Development and characterization of solid lipid nanoparticles loaded with magnetite. Int J Pharm. 2002;233(1–2):149–57.
56. Bondi ML, Azzolina A, Craparo EF, Lampiasi N, Capuano G, Giammona G, *et al*. Novel cationic solid-lipid nanoparticles as non-viral vectors for gene delivery. J Drug Target. 2007;15 (4):295–301.
57. Sjostrom B, Kaplun A, Talmon Y, Cabane B. Structures of nanoparticles prepared from oil-in-water emulsions. Pharm Res. 1995;12(1):39–48.
58. Shahgaldian P, Da Silva E, Coleman AW, Rather B, Zaworotko MJ. Para-acyl-calix-arene based solid lipid nanoparticles (SLNs): a detailed study of preparation and stability parameters. Int J Pharm. 2003;253(1–2):23–38.
59. Trotta M, Debernardi F, Caputo O. Preparation of solid lipid nanoparticles by a solvent emulsification–diffusion technique. Int J Pharm. 2003;257(1–2):153–60.
60. Hu FQ, Yuan H, Zhang HH, Fang M. Preparation of solid lipid nanoparticles with clobetasol propionate by a novel solvent diffusion method in aqueous system and physicochemical characterization. Int J Pharm. 2002;239(1–2):121–8.
61. Schubert MA, Muller-Goymann CC. Solvent injection as a new approach for manufacturing lipid nanoparticles—evaluation of the method and process parameters. Eur J Pharm Biopharm. 2003;55(1):125–31.
62. Charman WN, Stella VJ, editors. Lymphatic transport of drugs. Boca Raton: CRC Press; 1992.
63. Radomska-Soukharev A. Stability of lipid excipients in solid lipid nanoparticles. Adv Drug Deliv Rev. 2007;59(6):411–8.
64. Xie S, Pan B, Wang M, Zhu L, Wang F, Dong Z, *et al*. Formulation, characterization and pharmacokinetics of praziquantel-loaded hydrogenated castor oil solid lipid nanoparticles. Nanomedicine Lond. 2010;5(5):693–701.
65. Liu DH, Liu CX, Zou WW, Zhang N. Enhanced gastrointestinal absorption of *N*-3-O-toluyl-fluorouracil by cationic solid lipid nanoparticles. J Nanopart Res. 2010;12(3):975–84.
66. Zhang J, Fan Y, Smith E. Experimental design for the optimization of lipid nanoparticles. J Pharm Sci. 2009;98(5):1813–9.
67. Anton N, Benoit J-P, Saulnier P. Design and production of nanoparticles formulated from nano-emulsion templates—a review. J Control Release. 2008;128(3):185–99.
68. Sanjula B, Shah FM, Javed A, Alka A. Effect of poloxamer 188 on lymphatic uptake of carvedilol-loaded solid lipid nanoparticles for bioavailability enhancement. J Drug Target. 2009;17 (3):249–56.
69. Freitas C, Müller RH. Effect of light and temperature on zeta potential and physical stability in solid lipid nanoparticle (SLN (TM)) dispersions. Int J Pharm. 1998;168(2):221–9.
70. Mukherjee B, Santra K, Pattnaik G, Ghosh S. Preparation, characterization and *in vitro* evaluation of sustained release protein-loaded nanoparticles based on biodegradable polymers. Int J Nanomedicine. 2008;3(4):487–96.
71. Sahana B, Santra K, Basu S, Mukherjee B. Development of biodegradable polymer-based tamoxifen citrate-loaded nanoparticles and effect of some manufacturing process parameters on them: a physicochemical and *in vitro* evaluation. Int J Nanomedicine. 2010;5:621–30.
72. Zur Muhlen A, Zur Muhlen E, Niehus H, Mehnert W. Atomic force microscopy studies of solid lipid nanoparticles. Pharm Res. 1996;13(9):1411–6.
73. Souto EB, Mehnert W, Muller RH. Polymorphic behaviour of Compritol888 ATO as bulk lipid and as SLN and NLC. J Microencapsul. 2006;23(4):417–33.
74. Estella-Hermoso de Mendoza A, Campanero MA, Mollinedo F, Blanco-Prieto MJ. Lipid nanomedicines for anticancer drug therapy. J Biomed Nanotechnol. 2009;5(4):323–43.
75. Bunjes H, Steiniger F, Richter W. Visualizing the structure of triglyceride nanoparticles in different crystal modifications. Langmuir. 2007;23(7):4005–11.
76. Estella-Hermoso de Mendoza A, Rayo M, Mollinedo F, Blanco-Prieto MJ. Lipid nanoparticles for alkyl lysophospholipid edel-

fosine encapsulation: development and *in vitro* characterization. Eur J Pharm Biopharm. 2008;68(2):207–13.
77. Jenning V, Mader K, Gohla SH. Solid lipid nanoparticles (SLN) based on binary mixtures of liquid and solid lipids: a (1)H-NMR study. Int J Pharm. 2000;205(1–2):15–21.
78. Küchler S, Herrmann W, Panek-Minkin G, Blaschke T, Zoschke C, Kramer KD, *et al.* SLN for topical application in skin diseases—characterization of drug–carrier and carrier–target interactions. Int J Pharm. 2010;390(2):225–33.
79. Zimmermann E, Souto EB, Muller RH. Physicochemical investigations on the structure of drug-free and drug-loaded solid lipid nanoparticles (SLN) by means of DSC and 1H NMR. Pharmazie. 2005;60(7):508–13.
80. Braem C, Blaschke T, Panek-Minkin G, Herrmann W, Schlupp P, Paepenmüller T, *et al.* Interaction of drug molecules with carrier systems as studied by parelectric spectroscopy and electron spin resonance. J Control Release. 2007;119(1):128–35.
81. Lukowski G, Hoell A, Dingler A, Kranold R, Pflegel P. Fractal surface of solid lipid nanoparticles. Proc Control Release Society. 1997;24:631–2.
82. Muller RH, Mehnert W, Lucks JS, Schwarz C, Zur Muhlen A, Weyhers H, *et al.* Solid lipid nanoparticles (SLN)—an alternative colloidal carrier system for controlled drug delivery. Eur J Pharm Biopharm. 1995;41(1):62–9.
83. Zur Muhlen A, Mehnert W. Drug release and release mechanism of prednisolone loaded solid lipid nanoparticles. Pharmazie. 1998;53(8):552–5.
84. Chakraborty S, Shukla D, Mishra B, Singh S. Lipid—an emerging platform for oral delivery of drugs with poor bioavailability. Eur J Pharm Biopharm. 2009;73(1):1–15.
85. Crounse RG. Human pharmacology of griseofulvin: the effect of fat intake on gastrointestinal absorption. J Invest Dermatol. 1961;37:529–33.
86. Horter D, Dressman JB. Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. Adv Drug Deliv Rev. 2001;46(1–3):75–87.
87. Wagner D, Spahn-Langguth H, Hanafy A, Koggel A, Langguth P. Intestinal drug efflux: formulation and food effects. Adv Drug Deliv Rev. 2001;50 Suppl 1:S13–31.
88. Touitou E, Barry BW, editors. Enhancement in drug delivery. Florida: CRC Press; 2006.
89. Liversidge GG, Cundy KC. Particle size reduction for improvement of oral bioavailability of hydrophobic drugs: I. Absolute oral bioavailability of nanocrystalline danazol in beagle dogs. Int J Pharm. 1995;125(1):91–7.
90. Charman SA, Charman WN, Rogge MC, Wilson TD, Dutko FJ, Pouton CW. Self-emulsifying drug delivery systems: formulation and biopharmaceutic evaluation of an investigational lipophilic compound. Pharm Res. 1992;9(1):87–93.
91. Li C, Fleisher D, Li L, Schwier JR, Sweetana SA, Vasudevan V, *et al.* Regional-dependent intestinal absorption and meal composition effects on systemic availability of LY303366, a lipopeptide antifungal agent, in dogs. J Pharm Sci. 2001;90(1):47–57.
92. Martinez M, Amidon G, Clarke L, Jones WW, Mitra A, Riviere J. Applying the biopharmaceutics classification system to veterinary pharmaceutical products. Part II. Physiological considerations. Adv Drug Deliv Rev. 2002;54(6):825–50.
93. Trevaskis NL, Charman WN, Porter CJ. Lipid-based delivery systems and intestinal lymphatic drug transport: a mechanistic update. Adv Drug Deliv Rev. 2008;60(6):702–16.
94. Khoo SM, Shackleford DM, Porter CJH, Edwards GA, Charman WN. Intestinal lymphatic transport of halofantrine occurs after oral administration of a unit–dose lipid-based formulation to fasted dogs. Pharm Res. 2003;20(9):1460–5.
95. Wang D, Wang X, Li X, Ye L. Preparation and characterization of solid lipid nanoparticles loaded with α-asarone. PDA J Pharm Sci Technol. 2008;62(1):56–65.
96. Yang S, Zhu J, Lu Y, Liang B, Yang C. Body distribution of camptothecin solid lipid nanoparticles after oral administration. Pharm Res. 1999;16(5):751–7.
97. Bekerman T, Golenser J, Domb A. Cyclosporin nanoparticulate lipospheres for oral administration. J Pharm Sci. 2004;93(5):1264–70.
98. Hanafy A, Spahn-Langguth H, Vergnault G, Grenier P, Tubic Grozdanis M, Lenhardt T, *et al.* Pharmacokinetic evaluation of oral fenofibrate nanosuspensions and SLN in comparison to conventional suspensions of micronized drug. Adv Drug Deliv Rev. 2007;59(6):419–26.
99. Zhang N, Ping Q, Huang G, Xu W, Cheng Y, Han X. Lectin-modified solid lipid nanoparticles as carriers for oral administration of insulin. Int J Pharm. 2006;327(1–2):153–9.
100. Zhang Z, Lv H, Zhou J. Novel solid lipid nanoparticles as carriers for oral administration of insulin. Pharmazie. 2009;64 (9):574–8.
101. Zhang N, Ring Q, Huang G, Han X, Cheng Y, Xu W. Transport characteristics of wheat germ agglutinin-modified insulin-liposomes and solid lipid nanoparticles in a perfused rat intestinal model. J Nanosci Nanotechnol. 2006;6(9–10):2959–66.
102. Priano L, Esposti D, Esposti R, Castagna G, De Medici C, Fraschini F, *et al.* Solid lipid nanoparticles incorporating melatonin as new model for sustained oral and transdermal delivery systems. J Nanosci Nanotechnol. 2007;7(10):3596–601.
103. Liu C, Liu D, Bai F, Zhang J, Zhang N. *In vitro* and *in vivo* studies of lipid-based nanocarriers for oral N3-o-toluyl-fluorouracil delivery. Drug Deliv. 2010;17(5):352–63.
104. Yuan H, Chen J, Du YZ, Hu FQ, Zeng S, Zhao HL. Studies on oral absorption of stearic acid SLN by a novel fluorometric method. Colloids Surf B. 2007;58(2):157–64.
105. Yang L, Geng Y, Li H, Zhang Y, You J, Chang Y. Enhancement the oral bioavailability of praziquantel by incorporation into solid lipid nanoparticles. Pharmazie. 2009;64(2):86–9.
106. Li H, Zhao X, Ma Y, Zhai G, Li L, Lou H. Enhancement of gastrointestinal absorption of quercetin by solid lipid nanoparticles. J Control Release. 2009;133(3):238–44.
107. Pandey R, Sharma S, Khuller GK. Oral solid lipid nanoparticle-based antitubercular chemotherapy. Tuberculosis. 2005;85(5–6):415–20.
108. Langguth P, Hanafy A, Frenzel D, Grenier P, Nhamias A, Ohlig T, *et al.* Nanosuspension formulations for low-soluble drugs: pharmacokinetic evaluation using spironolactone as model compound. Drug Dev Ind Pharm. 2005;31(3):319–29.
109. Zhuang CY, Li N, Wang M, Zhang XN, Pan WS, Peng JJ, *et al.* Preparation and characterization of vinpocetine-loaded nanostructured lipid carriers (NLC) for improved oral bioavailability. Int J Pharm. 2010;394(1–2):179–85.
110. Westesen K. Novel lipid-based colloidal dispersions as potential drug administration systems—expectations and reality. Colloid Polym Sci. 2000;278(7):608–18.
111. Freitas C, Müller RH. Correlation between long-term stability of solid lipid nanoparticles (SLN(TM)) and crystallinity of the lipid phase. Eur J Pharm Biopharm. 1999;47(2):125–32.
112. Freitas C, Mullera RH. Spray-drying of solid lipid nanoparticles (SLN TM). Eur J Pharm Biopharm. 1998;46(2):145–51.
113. Kramer T, Kremer DM, Pikal MJ, Petre WJ, Shalaev EY, Gatlin LA. A procedure to optimize scale-up for the primary drying phase of lyophilization. J Pharm Sci. 2009;98(1):307–18.
114. Schwarz C, Mehnert W. Freeze-drying of drug-free and drug-loaded solid lipid nanoparticles (SLN). Int J Pharm. 1997;157 (2):171–9.
115. Heiati H, Tawashi R, Phillips NC. Drug retention and stability of solid lipid nanoparticles containing azidothymidine palmitate after autoclaving, storage and lyophilization. J Microencapsul. 1998;15(2):173–84.
116. Zimmermann E, Muller RH, Mader K. Influence of different parameters on reconstitution of lyophilized SLN. Int J Pharm. 2000;196(2):211–3.
117. Lim SJ, Kim CK. Formulation parameters determining the physicochemical characteristics of solid lipid nanoparticles loaded with all-*trans* retinoic acid. Int J Pharm. 2002;243(1–2):135–46.
118. del Pozo-Rodríguez A, Solinís MA, Gascón AR, Pedraz JL. Short- and long-term stability study of lyophilized solid lipid nanoparticles for gene therapy. Eur J Pharm Biopharm. 2009;71(2):181–9.
119. Pardeike J, Hommoss A, Muller RH. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. Int J Pharm. 2009;366(1–2):170–84.