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Lipid Nanoparticulate Drug Delivery Systems: A Revolution in Dosage Form Design and Development

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1. Introduction

Nanoparticulate drug delivery systems (DDS) have attracted a lot of attention because of their size-dependent properties. Among the array of nanoparticles being currently investigated by pharmaceutical scientists, lipid nanoparticles have taken the lead because of obvious advantages of higher degree of biocompatibility and versatility. These systems are commercially viable to formulate pharmaceuticals for topical, oral, pulmonary or parenteral delivery. Lipid nano formulations can be tailored to meet a wide range of product requirements dictated by disease condition, route of administration and considerations of cost, product stability, toxicity and efficacy. The proven safety and efficacy of lipid-based carriers make them attractive candidates for the formulation of pharmaceuticals, as well as vaccines, diagnostics and nutraceuticals [1].

The most frequent role of lipid-based formulations has traditionally been to improve the solubility of sparingly water soluble drugs especially Biopharmaceutics Classification System (BCS) Classes II & IV drugs. However, the spectrum of applications for lipid-based formulations has widened as the nature and type of active drugs under investigation vary. Lipid-based formulations may also protect active compounds from biological degradation or transformation, that in turn can lead to an enhancement of drug potency. In addition, lipid-based particulate DDS have been shown to reduce the toxicity of various drugs by changing the biodistribution of the drug away from sensitive organs. This reduction in toxicity may allow for more drug to be administered and forms the basis for the current success of several marketed lipid-based formulations of amphotericin B (Ambisome[®], Abelcet[®]) and doxorubicin (Doxil[®], Myocet[®]) [1].

Rapid advances in the ability to produce nanoparticles of uniform size, shape, and composition have started a revolution in science. The development of lipid-based drug carriers

has attracted increased attention over the last decade. Lipid nanoparticles (e.g. solid lipid nanoparticles, SLNs) are at the forefront of the rapidly developing field of nanotechnology with several potential applications in drug delivery, clinical medicine and research, as well as in other varied sciences. Due to their size-dependent properties, lipid nanoparticles offer the possibility to develop new therapeutics that could be used for secondary and tertiary level of drug targeting. Hence, lipid nanoparticles hold great promise for reaching the goal of controlled and site specific drug delivery and has attracted wide attention of researchers.

At the turn of the millennium, modifications of SLN, nanostructured lipid carriers (NLC) and lipid drug conjugate (LDC)-nanoparticles were introduced [2, 3] in addition to liquid crystal DDS. These carrier systems overcome observed limitations of conventional SLN and more fluid lipid DDS. Compared to liposomes and emulsions, solid particles possess some advantages, e.g. protection of incorporated active compounds against chemical degradation and more flexibility in modulating the release of the compound. This paper focuses on the different lipid based nano systems, their structure and associated features, stability, production methods, drug incorporation and other issues related to their formulation and use in drug delivery. The following advantages among others, could be ascribed to lipid based nanocarriers:

- ability to control and target drug release.
- ability to improve stability of pharmaceuticals.
- ability to encapsulate high drug content (compared to other carrier systems e.g. polymeric nanoparticles).
- the feasibility of carrying both lipophilic and hydrophilic drugs.
- most of the lipids used are biodegradable, and as such they have excellent biocompatibility, are non-toxic, non-allergenic and non-irritating.
- they can be formulated by water-based technologies and thus can avoid organic solvents.
- they are easy to scale-up and sterilize.
- they are less expensive than polymeric/surfactant based carriers.
- they are easy to validate.

2. Drug delivery systems

A drug delivery system is defined as a formulation or a device that enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time, and place of release of drugs in the body. The process of drug delivery includes the administration of the therapeutic product, the release of the active ingredients by the product, and the subsequent transport of the active ingredients across the biological membranes to the site of action. DDS interface between the patient and the drug. It may be a formulation of the drug or a device used to deliver the drug [4].

3. Lipids

The carboxylic acid group of a fatty acid molecule provides a convenient place for linking the fatty acid to an alcohol, via an ester linkage. If the fatty acid becomes attached to an

alcohol with a long carbon chain, the resultant substance is called a wax. When glycerol and a fatty acid molecule are combined, the fatty acid portion of the resultant compound is called an acyl group, and the glycerol portion is referred to as a glyceride. A triacylglyceride thus has three fatty acids attached to a single glycerol molecule. Sometimes, this name is shortened to triglyceride. Triglyceride substances are commonly referred to as fats or oils, depending on whether they are solid or liquid at room temperature [5]. A lipid is thus a fatty or waxy organic compound that is readily soluble in non-polar solvents (e.g. ether), but not in polar solvent (e.g. water). Examples of lipids are waxes, oils, sterols, cholesterol, fat-soluble vitamins, monoglycerides, diglycerides, triglycerides (fats), and phospholipids. Fatty acids (including fats) are a subgroup of lipids, hence, it will be inaccurate to consider the terms synonymous.

3.1. Classification of solid lipids for delivery of bioactives

Lipids can be grouped into the following categories based upon their chemical composition.

3.1.1. Homolipids

Homolipids are esters of fatty acids with alcohols. They are lipids containing only carbon (C), hydrogen (H), and oxygen (O), and as such are referred to as simple lipids. The principal materials of interest for oral delivery vehicle are long chain and medium chain fatty acids linked to a glycerol molecule, known as triacylglycerols. The long-chain fatty acids ranging from C14 to C24 appear widely in common fat while the medium chain fatty acids ranging from C6 to C12 are typical components of coconut oil or palm kernel oil [6]. Examples of homolipids include: cerides (waxes e.g. beeswax, carnauba wax etc.), glycerides (e.g. fats and oils) and sterides (e.g. the esters of cholesterol with fatty acids).

3.1.2. Heterolipids

Heterolipids are lipids containing nitrogen (N) and phosphorus (P) atoms in addition to the usual C, H and O e.g. phospholipids, glycolipids and sulfolipids. They are also known as compound lipids. The emphasis here will be on the phospholipids only. Two main classes of phospholipids occur naturally in quantities sufficient for pharmaceutical applications. These are the phosphoglycerides and phosphosphingolipids. Some phosphosphingolipids such as ceramide are used mainly in topical dosage forms. Phospholipids can be obtained from all types of biomass because they are essential structural components in all kinds of membranes of living organisms [6].

3.1.3. Complex lipids

The more complex lipids occur closely linked with proteins in cell membranes and subcellular particles. More active tissues generally have a higher complex lipid content. They may also contain phospholipids. Complex lipids in this context include lipoproteins, chylomicrons, etc. Lipoproteins are spherical lipid-protein complexes that are responsible

for the transport of cholesterol and other lipids within the body. Structurally, lipoprotein consists of an apolar core composed of cholesterol esters or triacylglycerols, surrounded by monolayer of phospholipid in which cholesterol and one or more specific apoproteins are embedded e.g. chylomicrons and lipoproteins

3.2. Lipid drug delivery systems

Lipid-based DDS are an accepted, proven, commercially viable strategy to formulate pharmaceuticals for topical, oral, pulmonary or parenteral delivery. Lipid formulations can be tailored to meet a wide range of product requirements. One of the earliest lipid DDS-liposomes have been used to improve drug solubility. Currently, some companies have established manufacturing processes for the preparation of large scale batches of sparingly soluble compounds, often at drug concentrations several orders of magnitude higher than the nominal aqueous solubility because of the introduction of novel lipid-based DDS [1].

3.3. Lipid nanoparticulate drug delivery systems

Lipid nanoparticles show interesting nanoscale properties necessary for therapeutic application. Lipid nanoparticles are attractive for medical purposes due to their important and unique features, such as their surface to mass ratio that is much larger than that of other colloidal particles and their ability to bind or adsorb and carry other compounds. Lipid nano formulations produce fine dispersions of poorly water soluble drugs and can reduce the inherent limitations of slow and incomplete dissolution of poorly water soluble drugs (e.g. BCS II & IV drugs), and facilitate formation of solubilised phases from which drug absorption occurs. In any vehicle mediated delivery system (whether the vehicle is an emulsion, liposome, noisome or other lipidic systems), the rate and mode of drug release from the system is important in relation to the movement of the delivery system *in vivo*.

Lipid particulate DDS abound depending on their architecture and particle size. Due to the large number of administration routes available, these delivery systems perform differently depending on the formulation type and route of administration. Figure 1 shows some of the different lipid particulate DDS available.

3.3.1. Solid lipid nanoparticles (SLN)

SLN are particulates structurally related to polymeric nanoparticles. However, in contrast to polymeric systems, SLN can be composed of biocompatible lipids that are physiologically well tolerated when administered *in vivo* and may also be prepared without organic solvents. The lipid matrices can be composed of fats or waxes (homolipids) that provide protection to the incorporated bioactive from chemical and physical degradation, in addition to modification of drug release profile. Typical formulations utilize lipids such as paraffin wax or biodegradable glycerides (e.g. Compritol 888 ATO) as the structural base of the particle [7].

Figure 1. Lipid particulate drug delivery systems

SLN were developed in the 1990s as an alternative carrier system to the existing traditional carriers, such as emulsions, liposomes and polymeric nanoparticles. SLN are prepared either with physiological lipids or lipids with generally regarded as safe (GRAS) status. Under optimized conditions they can incorporate lipophilic or hydrophilic drugs and seem to fulfil the requirements for an optimum particulate carrier system [8]. SLN have a potential wide application spectrum- parenteral administration and brain delivery, ocular delivery, rectal delivery, oral delivery, topical delivery and vaccine delivery systems etc., in addition to improved bioavailability, protection of sensitive drug molecules from the outer environment and even controlled release characteristics. Common disadvantages of SLN are particle growth, unpredictable gelation tendency, unexpected dynamics of polymorphic transitions and inherent low incorporation rate due to the crystalline structure of the solid lipid [9].

3.3.2. Nanostructured lipid carriers (NLC)

NLC are colloidal carriers characterized by a solid lipid core consisting of a mixture of solid and liquid lipids, and having a mean particle size in the nanometer range. They consist of a lipid matrix with a special nanostructure [10]. This nanostructure improves drug loading and firmly retains the drug during storage. NLC system minimizes some problems associated with SLN such as low payload for some drugs; drug expulsion on storage and high water content of SLN dispersions.

The conventional method for the production of NLC involves mixing of spatially very different lipid molecules, i.e. blending solid lipids with liquid lipids (oils). The resulting matrix of the lipid particles shows a melting point depression compared with the original solid lipid but the matrix is still solid at body temperature. Depending on the method of production and the composition of the lipid blend, different types of NLC are obtained. The

basic idea is that by giving the lipid matrix a certain nanostructure, the payload for active compounds is increased and expulsion of the compound during storage is avoided. Ability to trigger and even control drug release should be considered while mixing lipids to produce NLC. Newer methods of generating NLC are being developed.

3.3.3. Lipid drug conjugates (LDC)-nanoparticles

A major problem of SLN is the low capacity to load hydrophilic drugs due to partitioning effects during the production process. Only highly potent low dose hydrophilic drugs may be suitably incorporated in the solid lipid matrix [11]. In order to overcome this limitation, LDC nanoparticles with drug loading capacities of up to 33% were developed [8]. An insoluble drug-lipid conjugate bulk is first prepared either by salt formation (e.g. with a fatty acid) or by covalent linking (e.g. to ester or ethers). The obtained LDC is then processed with an aqueous surfactant solution to nanoparticle formulation by high pressure homogenization (HPH). Such nanoparticles may have potential application in brain targeting of hydrophilic drugs in serious protozoal infections [12].

3.3.4. Liposomes

Liposomes are closed vesicular structures formed by bilayers of hydrated phospholipids. The bilayers are separated from one another by aqueous domains and enclose an aqueous core. As a consequence of this alternating hydrophilic and hydrophobic structure, liposomes have the capacity to entrap compounds of different solubilities. Additionally, the basic liposome structure of hydrated phospholipid bilayers is amenable to extensive modification or 'tailoring' with respect to the physical and chemical composition of the vesicle. This versatility has resulted in extensive investigation into the use of liposomes for various applications such as in radiology, cosmetology and vaccinology.

Liposomes used in drug delivery typically range from 25 nm to several micrometers and are usually dispersed in an aqueous medium. There are various nomenclatures for defining liposome subtypes based either on structural parameters or the method of vesicle preparation. These classification systems are not particularly rigid and a variation exists in use of these terms, particularly with respect to size ranges. Liposomes are often distinguished according to their number of lamellae and size. Small unilamellar vesicles (SUV), large unilamellar vesicles and large multilamellar vesicles or multivesicular vesicles are differentiated. SUVs with low particle sizes in the nanometer range are of interest as liposomal nanocarriers for drug and antigen delivery [13, 14].

3.3.5. Transfersomes

Transfersome technology was developed with the intention of providing a vehicle to allow delivery of bioactive molecules through the dermal barrier. Transfersomes are essentially ultra-deformable liposomes, composed of phospholipids and additional 'edge active' amphiphiles such as bile salts that enable extreme distortion of the vesicle shape. The vesicle

diameter is in the order of 100 nm when dispersed in buffer [15]. These flexible vesicles are thought to permeate intact through the intact dermis under the forces of the hydrostatic gradient that exists in the skin [16]. Drug or antigen may be incorporated into these vesicles in a manner similar to liposomes.

3.3.6. Niosomes

Niosomes are vesicles composed mainly of non-ionic bilayer forming surfactants [17]. They are structurally analogous to liposomes, but the synthetic surfactants used have advantages over phospholipids in that they are significantly less costly and have higher chemical stability than their naturally occurring phospholipid counterparts [18]. Niosomes are obtained on hydration of synthetic non-ionic surfactants, with or without incorporation of cholesterol or other lipids. Niosomes are similar to liposomes in functionality and also increase the bioavailability of the drug and reduce the clearance like liposomes. Niosomes can also be used for targeted drug delivery, similar to liposomes. As with liposomes, the properties of the niosomes depend both on the composition of the bilayer and the method of production. Antigen and small molecules have also been delivered using niosomes [19, 20].

3.3.7. Liquid crystal drug delivery systems

The spontaneous self assembly of some lipids to form liquid crystalline structures offers a potential new class of sustained release matrix. The nanostructured liquid crystalline materials are highly stable to dilution. This means that they can persist as a reservoir for slow drug release in excess fluids such as the gastrointestinal tract (GIT) or subcutaneous space, or be dispersed into nanoparticle form, while retaining the 'parent' liquid crystalline structure. The rate of drug release is directly related to the nanostructure of the matrix. Lyotropic liquid crystal systems that commonly consist of amphiphilic molecules and solvents can be classified into lamellar (L_α), cubic, hexagonal mesophases, etc. In recent years, lyotropic liquid crystal systems have received considerable attention because of their excellent potential as drug delivery vehicles [21]. Among these systems, reversed cubic (Q_{II}) and hexagonal mesophases (H_{II}) are the most important and have been extensively investigated for their ability to sustain the release of a wide range of bioactives from low molecular weight drugs to proteins, peptides and nucleic acids.

3.3.8. Nanoemulsions

Lipid-based formulations present a large range of optional delivery systems such as solutions, suspensions, self-emulsifying systems and nanoemulsions. Among these approaches, oral nanoemulsions offer a very good alternative because nanoemulsions can improve the bioavailability by increasing the solubility of hydrophobic drugs and are now widely used for the administration of BCS class II and class IV drugs. Oral nanoemulsions use safe edible materials (e.g., food-grade oils and GRAS-grade excipients) for formulation of the delivery system. Nanoemulsions possess outstanding ability to encapsulate active

compounds due to their small droplet size and high kinetic stability [22, 23]. Nanoemulsions have sizes below 1 μm and have been extensively investigated as novel lipid based DDS [22] together with microemulsions.

3.4. Functional properties of lipids used in formulating lipid drug delivery systems

3.4.1. Crystallinity and polymorphism of lipids

Many pharmaceutical solids exist in different physical forms. It is well recognised that drug substances and excipients can be amorphous, crystalline or anhydrous, at various degrees of hydration or solvated with other entrapped solvent molecules, as well as varying in crystal hardness, shape and size. Amorphous solids consist of disordered arrangements of molecules and do not possess a distinguishable crystal lattice. In the crystalline state (polymorphs, solvates/hydrates, co-crystals), the constituent molecules are arranged into a fixed repeating array built of unit cells, which is known as lattice. Possession of adequate crystallinity is a prerequisite for a good lipid particulate DDS.

Triglycerides, which are mainly used as lipid matrices crystallize in different polymorphic forms. The most important forms are the α and β forms. Since the formulation of lipid particulate DDS may involve melting at some point, recrystallization from the melt results in the metastable α -polymorph, which subsequently undergoes a polymorphic transition into the stable β -form via a metastable intermediate form (β') [24]. The β -polymorph especially consists of a highly ordered, rigid structure with low loading capacity for drugs. The formation of all these polymorphic forms has been proved amongst solid triglyceride nanoparticles [25].

3.4.2. Melting characteristics of lipid matrices

A pure triacylglycerol has a single melting point that occurs at a specific temperature. Nevertheless, certain lipids contain a wide variety of different triacylglycerols, with different melting points and as a result, they melt over a wide range of temperatures, producing a wide endothermic transition in differential scanning calorimeter. High purity lipids with sharp melting transitions exclude drugs on recrystallization. In addition to the solidity or melting point of each individual triglyceride, in drug delivery, we are interested and concerned with the combination of triglycerides throughout the fat mixture. This impacts the plasticity and the melting point range. In the development of lipid nanoparticles, lipids with melting points well above the body temperature are preferred. This will enable among others, sustained release of the encapsulated drug.

3.5. Crystallinity and polymorphism vs drug loading capacity and drug release

Crystallinity and polymorphism have a lot of influence on some properties of lipid matrices used in lipid DDS. Parameters like drug loading capacity and drug release depend highly on the crystallinity and the polymorphic form of the lipids. The crystalline order and density

increase from α to β forms and are highest for the β -forms of polymorphic lipids [24]. An increasing crystalline order has a great impact on the drug loading capacity, since an increase in order reduces the ability to incorporate different molecules including drugs [24]. Hence, the drug loading capacity of the poorly organized polymorphic forms is high [26]. However, this advantage goes along with the particles being in a metastable form which are able to transform into the stable β -polymorph upon storage. As a consequence of this transformation, often drug expulsion occurs. The increasing order of the matrix also reduces the diffusion rate of a drug molecule within the particle and hence reduces the rate of drug release.

3.6. Strategies to improve drug loading in lipid particulate drug delivery systems

The high crystallinity of SLN leads to a rather low drug loading capacity for many drugs, a problem still being addressed. However, for lipophilic drugs the incorporation into the particles is much easier and often results in rather high drug loading. In order to overcome the disadvantage of low loading capacity, many investigations have been done. Müller *et al.* [10] introduced NLC. In these formulations, lipids of highly ordered crystalline structure are combined with chemically different lipids of amorphous structure, giving rise to structured matrices that accommodate more drug.

Friedrich *et al* [27] reported a different method to increase the drug payload by incorporating amphiphilic phospholipids into the lipid matrix. This resulted in a much higher solubility of the drug in the matrix, which was attributed to the formation of a solidified reverse micellar solution within the matrix. In this case, the nanoparticles were prepared by cold homogenization which may have prevented a redistribution of the lecithin to the surface of the particles or into the aqueous phase. Such a behaviour could be observed for a similar system after high pressure homogenization of the molten lipids [28].

Another mechanism of increasing the drug loading capacity of SLN has been recently developed [29-32]. In these works, the researchers used mixtures of solid lipids of natural origin possessing fatty acids of different chain lengths. In the analytical characterization of the lipid mixtures using differential scanning calorimetry (DSC), X-ray diffraction and isothermal microcalorimetry, it was observed that the mixtures were able to form matrices of imperfect structure composed of mixed crystals and mixtures of crystals, which enhanced drug incorporation compared with the single lipids.

3.7. Ingredients used in the formulation of lipid based particulate drug delivery systems

3.7.1. Emulsifiers

Emulsifiers are essential to stabilize lipid nanoparticle dispersions and prevent particle agglomeration. The choice of the ideal surfactant for a particular lipid matrix is based on the surfactant properties such as charge, molecular weight, chemical structure, and respective hydrophile-lipophile balance (HLB). The HLB of an emulsifier is given by the balance

between the size and strength of the hydrophilic and the lipophilic groups. Table 1 shows some of the emulsifiers employed in the production of lipid nanoparticles. The choice of the emulsifiers depends on the route of administration of the formulation, for e.g. for parenteral formulations, there are limits of the emulsifiers to be used [33]. For topical and ocular route the issue of skin sensitization has to be considered, while for oral route, the emulsifier should not produce any physiological effect at the use concentration. Emulsifiers could be used in combination to produce synergistic effect and better stabilize the formulation [22].

Emulsifiers/coemulsifiers	HLB	References
Lecithin	4-9	[9]
Poloxamer 188	29	[34]
Poloxamer 407	21.5	[35]
Polysorbate 20	16.7	[36]
Polysorbate 65	10.5	[7]
Polysorbate 80	15	[9]
Cremophor EL	12-14	[37]
Solutol HS 15	15	[28]

Table 1. Some emulsifiers used for the production of lipid nanoparticles

3.7.2. Lipids

The matrices for lipid nanoparticle preparation are natural, semi-synthetic or synthetic lipids which can be biodegradable, including triglyceride (tri-stearic acid, tri-palmitic acid, tri-lauric acid and long-chain fatty acid), steroid and waxes (e.g. beeswax, carnauba wax, etc) and phospholipids. They could be used singly or in combination. Lipids for the production of nanoparticles may be grouped into two: bilayer and non-bilayer lipids.

3.7.3. Bilayer lipids used in drug delivery

Some lipids are capable of adopting a certain orientation depending on the processing condition. Compounds that have approximately equal-sized heads and tails e.g. phospholipids (Figures 2 and 3) tend to form bilayers instead of micelles in aqueous system. In these structures, two monolayers of lipid molecules associate tail to tail, thus minimizing the contact of the hydrophobic portions with water and maximizing hydrophilic interactions [38] (Figure 3). The phospholipid molecules can move about in their half the bilayer, but there is a significant energy barrier preventing migration to the other side of the bilayer. Cholesterol can insert into the bilayer, and this helps to regulate the fluidity of the membrane. The self-assembled nature of lipid bilayers implies that they are normally in a tension-less state.

Figure 2. Structure of phospholipid (phosphatidylcholine) [Ref. 39].

Figure 3. Interaction of phospholipid with water [38].

Phospholipids with certain head groups e.g. phosphatidylcholine (Figure 2) [39], can alter the surface chemistry of a lipid particle. The packing of phospholipid chains within the surface of the particle also affects its mechanical properties, including swelling, stretching,

bending and deformability. Many of these properties have been taken advantage of in the design of novel lipid particulate DDS such as surface modification for improved drug loading capacity [31, 32, 40]. Deformability has been utilized in the development of ultra-deformable liposomes termed transfersomal DDS (transfersomes). Cholesterol strengthens the bilayer but decrease its permeability. Bilayer lipids when present in lipid particulate DDS may define the boundaries of the particle and its environment (aqueous), and are often involved in many complex processes occurring at the interface.

3.7.4. Non-bilayer lipids used in drug delivery

In many biological systems, the major lipids are non-bilayer lipids, which in purified form cannot be arranged in a lamellar structure in the presence of aqueous systems. The structural and functional roles of these lipids in drug delivery are mainly in their utilization as matrix-forming lipids. They include such lipids as homolipids e.g. triglycerides and waxes. Their functional properties in lipid nanotechnology differs depending partly on their melting points, crystallinity and polymorphic characteristics. However, they may have absorption promoting properties especially for lipophilic drugs. Table 2 shows some of the non-bilayer lipids used in the formulation of lipid micro- and nanoparticles.

Hard fats e.g.	Natural hard fats e.g.
Stearic acid [Ref. 41] Palmitic acid [Ref. 42] Behenic acid [Ref. 43]	Goat fat [Ref. 45] Theobroma oil [Ref. 53]
Triglycerides e.g.	Waxes e.g.
Trimyristin (Dynasan 114) [Ref. 44] Tripalmitin (Dynasan 116) [Ref. 45] Tristearin (Dynasan 118) [Ref. 44] Trilaurin [Ref. 46]	Beeswax [Ref. 9] Cetyl palmitate [Ref. 47] Carnauba wax [Ref. 89]
Mono, di and triglycerides mixtures e.g.	
Witepsol bases [Ref. 48] Glyceryl monostearate (Imwitor 900) [Ref. 49] Glyceryl behenate (Compritol 888 ATO) [Ref. 50] Glyceryl palmitostearate (Precirol ATO 5) [Ref. 51] Softisan 142 and Softisan 154 [Refs. 52, 27]	

Table 2. Some non-bilayer lipids used in the formulation of lipid nanoparticles

3.8. Characterization and selection of lipids for particulate drug delivery systems

There is an increasing interest in lipid-based DDS due to factors such as better characterization of lipidic excipients and formulation versatility and the choice of different DDS. Apart from the fatty acid profile of previously undefined lipids, many different

analytical procedures are used to characterize lipids. These technologies provide different scales of analysis and may be used in combination to select the appropriate lipid matrix for use in formulation. DSC is the most widely used thermo-analytic technique for studying fats, oils and their mixtures. It gives information about the temperatures and energy associated with their fusion and crystallization, phase behaviour, polymorphic transformations, and data to estimate solid fat contents. DSC reports the destruction of structures in recordings obtained in a permanent out-of-equilibrium state. X-ray diffraction (small angle X-ray diffraction, SAXD and wide angle X-ray diffraction, WAXD) is also an essential tool for elucidating properties of fats and their mixtures. However, it complements DSC. XRD recordings provide both short and long spacings at a given temperature at which the sample is supposed to be in equilibrium. Since lipid systems are quite sensitive to their preparation history, only simultaneous recordings of SAXD, WAXD, and DSC circumvent the problem of reproducibility and guarantee identical conditions for all three measurements whatever may be the thermal treatment of the sample. Polarized light microscopy (PLM) is an analytical technique used in characterization of fats to observe the microstructures of the various polymorphic forms of fats. With a hot stage coupled, it is used to observe the microstructural changes in fats during melting, as the lipid passes from crystalline phase to isotropic phase, to visualize crystallization from isotropic melts and to visually detect undissolved drug crystals in the lipid matrix.

Isothermal microcalorimetry (IMC) is a recent and an important tool in studying the time dependent crystallization of lipids and lipid matrices. It has been applied in both pure and mixed systems. Isothermal crystallization kinetics studies of mixtures of lipids using IMC also address the question of how the crystallinity of one component affects the crystallization behaviour of the other [29, 52, 53]. Atomic force microscopy (AFM) can provide invaluable information about the physicochemical characteristics of the carriers that play an important role in determining the performance of the DDS. A lot of this information cannot be obtained from other characterization techniques due to the unique ability of the atomic force microscope to probe nanometer scale features at the molecular level.

3.9. Preparation of lipid nanoparticulate drug delivery systems

There many methods for the preparation of lipid nanoparticulate DDS. The method used is dictated by the type of drug especially its solubility and stability, the lipid matrix, route of administration, etc. Liposomal preparation follows a different method as described by Mozafari [54]. In this section, emphasis was laid on the production of SLN, NLC and LDC-nanoparticles, with methods that can also be applied to the formulation of liquid crystal DDS.

3.9.1. High pressure homogenization

High pressure homogenisation (HPH) is a suitable method for the preparation of SLN, NLC and LDC-nanoparticles and can be performed at elevated temperature (hot HPH technique)

or at and below room temperature (cold HPH technique) [11, 55]. The particle size is decreased by impact, shear, cavitation and turbulence. Briefly, for the hot HPH, the lipid and drug are melted (approximately 10 °C above the melting point of the lipid) and combined with an aqueous surfactant solution at the same temperature. A hot pre-emulsion is formed by homogenisation (e.g. using Ultra-Turrax). The hot pre-emulsion is then processed in a temperature-controlled high pressure homogenizer at 500 bar (or more) and predetermined number of cycles. The obtained nanoemulsion recrystallizes upon cooling down to room temperature forming SLN, NLC or LDC-nanoparticles. The cold HPH is a suitable technique for processing heat-labile drugs or hydrophilic drugs. Here, lipid and drug are melted together and then rapidly ground under liquid nitrogen forming solid lipid microparticles. A pre-suspension is formed by homogenisation of the particles in a cold surfactant solution. This pre-suspension is then further homogenised in a HPH at or below room temperature at predetermined homogenisation conditions to produce SLN, NLC or LDC-nanoparticles. The possibility of a significant increase in temperature during cold homogenisation should be borne in mind. Both HPH techniques are suitable for processing lipid concentrations of up to 40% and generally yield very narrow particle size distributions [56]. A schematic representation of HPH method of lipid particle preparation is shown in Figure 4.

3.9.2. *Production of SLN via microemulsions*

Gasco [57] developed and optimised a suitable method for the preparation of SLN via microemulsions. In a typical process, a warm microemulsion is prepared and thereafter, dispersed under stirring in excess cold water (typical ratio about 1:50) using a specially developed thermostated syringe. The excess water is removed either by ultra-filtration or by lyophilisation in order to increase the particle concentration. Experimental process parameters such as microemulsion composition, dispersing device, effect of temperature and lyophilisation on size and structure of the obtained SLN should be optimized. The removal of excess water from the prepared SLN dispersion is a difficult task with regard to the particle size. Also, high concentrations of surfactants and cosurfactants (e.g. butanol) are necessary for the formulation, but less desirable with respect to regulatory purposes and application.

3.9.3. *SLN prepared by solvent emulsification/evaporation*

For the production of nanoparticle dispersions by solvent emulsification/evaporation, the lipophilic material is dissolved in water immiscible organic solvent (e.g. cyclohexane) that is emulsified in an aqueous phase [58]. Upon evaporation of the solvent, nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium. Siekmann and Westesen [59] produced cholesterol acetate nanoparticles with a mean size of 29 nm using solvent emulsification/evaporation technique.

Other methods of lipid nanoparticle preparation include phase inversion and supercritical fluid (SCF) technology.

Figure 4. Production of lipid nanoparticles (SLN) by high pressure homogenisation (HPH)

3.10. Characterization of lipid nanoparticle quality

Quantitative analysis of particle characteristics such as morphological features can be very informative as biophysical properties are known to influence biological activity, biodistribution and toxicity. Several techniques are often used to assess nanoparticle characteristics such as lamellarity, size, shape and polydispersity [60]. Adequate and proper characterization of the lipid nanoparticles is necessary for quality control. However, characterization of lipid nanoparticles is a serious challenge due to the colloidal size of the particles and the complexity and dynamic nature of the delivery system.

The important parameters which need to be evaluated for the lipid nanoparticles are particle size, size distribution kinetics (zeta potential), degree of crystallinity and lipid modification (polymorphism), coexistence of additional colloidal structures (micelles, liposome, super cooled melts, drug nanoparticles), time scale of distribution processes, drug content

(encapsulation efficiency and loading capacity), *in vitro* drug release and surface morphology.

Particle size and size-distribution may be studied using photon correlation spectroscopy (PCS) otherwise known as dynamic light scattering (DLS), static light scattering (SLS), transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM), scanning tunneling microscopy (STM), freeze fracture electron microscopy (FFEM) or cryoelectron microscopy (Cryo-EM). These microscopy techniques are also used to study the morphology of nanoparticles.

Among the imaging techniques, AFM has been widely applied to obtain the size, shape and surface morphological information on nanoparticles. It is capable of resolving surface details down to 0.01 nm and producing a contrasted and three-dimensional image of the sample. X-ray diffraction and differential scanning calorimetric analysis give information on the crystalline state and polymorphic changes in the nanoparticles. Confocal laser scanning microscopy (CLSM) gives information on interaction of nanoparticles with cells. Nuclear magnetic resonance (NMR) can be used to determine both the size and the qualitative nature of nanoparticles. The selectivity afforded by chemical shift complements the sensitivity to molecular mobility to provide information on the physicochemical status of components within the nanoparticle.

An important characterization technique for lipid nanoparticles is determination of solid state properties. This is very important in order to detect the possible modifications in the physicochemical properties of the drug incorporated into the lipid nanoparticles or the lipid matrix. It has been proven that although particles were produced from crystalline raw materials, the presence of emulsifiers, preparation method and high shear encountered (e.g. HPH) may result in changes in the crystallinity of the matrix constituents compared with bulk materials. This may lead to liquid, amorphous or only partially crystallized metastable systems [61]. Polymorphic transformations may cause chemical and physical changes (e.g. shape, solubility, melting point) in the active and auxiliary substances. The solid state analysis of lipid nanoparticles is usually carried out using the following procedures: DSC, X-ray diffraction, hot stage microscopy, Raman spectroscopy and Fourier-transform infrared spectroscopy.

3.11. Drug incorporation and loading capacity

Many different drugs have been incorporated in lipid nanoparticles. A very important point to judge the suitability of a drug carrier system is its loading capacity. The loading capacity is generally expressed in percent related to the lipid phase (matrix lipid and drug). Westesen *et al.* [61] studied the incorporation of drugs using loading capacities of typically 1 - 5%, but for ubidecarenone loading capacities of up to 50% were reported. Different loading capacities were obtained for other drugs [62]. Factors determining the loading capacity of a drug in the lipid include among others, solubility of drug in melted lipid; miscibility of drug melt and lipid melt; chemical and physical structure of solid lipid matrix; and polymorphic state of lipid material.

The prerequisite to obtain a sufficient loading capacity is a sufficiently high solubility of the drug in the lipid melt. This is why it is necessary to determine the solubility of a drug in the lipid matrix as a preformulation strategy. Typically, the solubility should be higher than required because it decreases when cooling the melt and might even be lower in the solid lipid. Solubility of a drug in the lipid melt could be enhanced by the addition of solubilizers.

3.12. Drug release from lipid nanoparticles

There have been many studies dealing with drug incorporation, however, there are distinctly less data available about drug release, especially information about the release mechanisms. Drug release from lipid nanoparticles could be conducted using different models and biorelevant media. Generally, artificial membranes, tissue constructs or excised skin are used as barriers. A major problem encountered with lipid nanoparticles is the burst release observed with these systems, however, a prolonged drug release could also be obtained with these systems. It is possible to modify the release profiles as a function of lipid matrix, surfactant concentration and production parameters (e.g. temperature) and by surface modification [40].

The release profiles of drugs from lipid nanoparticles could be modulated to obtain prolonged release without burst effect, or to generate systems with tailored burst, followed by prolonged release. The burst can be exploited to deliver an initial dose when desired. It is important to note that the release profiles are not or only slightly affected by the particle size. Predominant factors for the shape of the release profiles are the production parameters (surfactant concentration, temperature) and the nature of the lipid matrix. During particle production of lipid nanoparticles by the hot homogenization technique, drug partitions from the liquid oil phase to the aqueous water phase. The amount of drug partitioning to the water phase will increase with the solubility of the drug in the water phase, that means with increasing temperature of the aqueous phase and increasing surfactant concentration as these two parameters directly affect the solubility of drugs in aqueous system.

It was reported that there was a decrease in release with decrease in temperature for a drug encapsulated in lipid nanocapsule [63]. This decrease in drug release with decreasing temperature was attributed to an increased microviscosity of the oil delaying the drug diffusion out of lipid nanocapsule core into the aqueous release medium. The effect of ambient temperature on viscosity, however, was not limited to the internal oily core material but applies also to the external aqueous phase.

Investigation of drug release from different wax matrix pellets using theophylline as a lipophilic drug showed that as the hydrophobicity of the wax increased, the drug release rate decreased. The more hydrophilic is the wax, the more it is susceptible to hydration by the release medium and therefore the faster the drug release. The drug release pattern was also highly dependent on the drug aqueous solubility. The release process was mainly affected by the relative affinity of the drug to the wax and the aqueous release medium [63]. Therefore, wax could be modified with compatible hydrophilic material to moderate drug release from lipid-based wax matrices. It was also found that minor temperature changes around 37 °C will not significantly influence the drug release from these lipid nanocapsules,

which excluded the option of a temperature-dependent release under *in vivo* conditions. zur Mühlen *et al.* [64] reported that interactions between drug and lipid molecules plays important role in controlling the drug release. These interactions could affect the viscosity of the solid lipid matrix leading to different release rates for different drugs although having similar lipophilic properties. They demonstrated that the development of sustained release SLN is possible by the proper choice of the drug and the lipid and their degree of interaction.

3.13. Challenges of lipid nanoparticle drug delivery system

One major problem with the intravenous administration of colloidal particles is their interaction with the reticulo-endothelial system (RES). Nanoparticles for medical applications are frequently given via parenteral administration. As with any foreign material, the body mounts a biological response to an administered nanoparticle. This response is the result of a complex interplay of factors, not just the intrinsic characteristics of the nanoparticle. In particular, most materials, upon contact with biological matrices, are immediately coated by proteins, leading to a protein “corona” [65]. Protein coronas are complex and variable. Certain components of the nanoparticle corona, called opsonins, may enhance uptake of the coated material by cells of the RES. The presence of opsonins on the particle surface creates a “molecular signature” which is recognized by immune cells and determines the route of particle internalization. The route of internalization affects the eventual fate of the nanoparticle in the body (i.e. its rate of clearance from the bloodstream, volume of distribution, organ disposition, and rate and route of clearance from the body). The cells of RES are capable of mopping up particles that they do not recognise as self i.e. particles they recognise as foreign. The negative effect can however, be remedied by linking of polyethylene glycol molecules to the lipid nanoparticles, thus increasing their hydrophilicity and the residence time of these particles in circulation. Alternatively, the lipid nanoparticles could be engineered to evade these RES cells by limiting the particle sizes to about 200 nm or less. It is believed that these cells do not recognise low nanometer-sized particles as foreign. Several other varied and unrelated challenges are encountered in lipid nanoparticle technology. These challenges constitute a serious research question which current strategies are targeted to address.

3.13.1. Formation of perfect crystalline structure during storage

Triglycerides crystallize in different polymorphic forms such as α , γ , β' , and β - forms. Recrystallization from the melt results in the metastable α -polymorph which subsequently undergoes a polymorphic transition into the stable β -form via a metastable intermediate. The β -polymorph especially consists of a highly ordered, rigid structure with low loading capacity of drugs. Transition to the β -form via a metastable intermediate form leads to drug expulsion and inability to protect or prolong the release of the encapsulated drug.

3.13.2. Physical stability

The stability of lipid particulate systems is influenced by the size of the nanoparticles. Lipid nanoparticles are prevented from sedimentation by Brownian motion, but other instabilities

like Ostwald ripening and aggregation may occur. Since nanoparticles have a large specific surface area, stabilization of the surface with sufficient amounts of emulsifier(s) is necessary. The formulation of the colloidal carriers themselves is a difficult task due to many problems that arise from their colloidal state and specific pharmaceutical demands on such formulations. Stability in a pharmaceutical sense refers to a shelf life of usually 3-5 years. Shorter shelf lives will only be accepted in very special cases. However, for most systems of pharmaceutical interest the colloidal state is at its best metastable. The colloidal state may cause several additional instabilities, for example, due to the presence of large interfaces (adsorption-desorption processes, interactions in the stabilizer layer, higher risk of chemical instabilities, etc.). Since many colloidal administration systems are intended for intravenous use, stability is very crucial. Ability to be sterilized is also an added advantage.

3.13.3. *Gel formation*

The change in morphology of lipid nanoparticles from spheres to platelets is responsible for the gelation of solid lipid nanoparticle dispersions [66]. Depending on the composition, especially of the emulsifier(s) and the amount of lipid matrix, a gelation of the normally liquid dispersions can be observed on storage [66, 67]. By means of TEM and synchrotron measurements, the reason for the gelation was found. The gelation may derive from a reversible self-association of the particles due to a stacking of the platelets [68, 69]. This gel-like feature of highly concentrated dispersions favours application as dermal drug delivery system since the viscoelastic features resemble those of semisolid creams [56]. In contrast, the lipid nanoparticle formulations for intravenous and ocular administration have to remain fluid. Phase transitions that would lead to an unusual lamellar gel phase (L_β) should be investigated for in parenteral formulations.

3.13.4. *High water content of dispersions*

The high water content of lipid nanoparticles (70-95%) could lead to drug degradation and high cost of energy input during lyophilisation. During lyophilisation, the integrity of the nanoparticles could be affected if adequate croprotectant or lyoprotectant was not included in the formulation. Water free nanoparticles could be used in tablet production or the nanoparticle dispersion used as granulating fluid during production. SLN can be transformed into a powder by spray-drying. In any case, it is beneficial to have a higher solid content to avoid the need of having to remove too much water. For cost reasons, spray drying might be the preferred method for transforming SLN dispersions into powders, with the previous addition of a protectant [26].

3.13.5. *Dosing problems*

Selection of appropriate dosage form for lipid nanoparticle may be a problem. Outside injectables, there is need to package lipid nanoparticles in appropriate dosage units e.g. dispersible powders or hard/soft gelatine capsules especially for oral administration. Since the stomach acidic environment and its high ionic strength favour particle aggregation, aqueous dispersions of lipid nanoparticles might not be suitable to be administered orally as

a dosage form. In addition, the presence of food will also have a high impact on their performance [70]. Packaging of SLN in a sachet for redispersion in water or juice prior to administration will allow an individual dosing by volume of the reconstituted SLN. This means additional step in packaging, which might result in introduction of additional technology and increase in the cost of the product.

3.13.6. Coexistence of other colloidal structures in the system (e.g. liposome and vesicles in SLN and NLC containing phospholipids)

Considerable amounts of emulsifiers are needed for the stabilization of lipid nanoparticles. If the emulsifiers redistribute from the particles into the aqueous phase, they can form colloidal structures like micelles or liposomes or other vesicular structures by self-assembly. Drugs can be solubilized within these structures, affecting drug release as well as drug loading capacity [70]. Hence the formation of additional colloidal structures has to be investigated for each formulation to enable further precautions to be taken to avoid it. The coexistence of liposomes and oil droplets was detected in an intravenous o/w nanoemulsion [71]. In solid lipid nanoparticle dispersion, additional liposomes were observed by means of cryo-TEM, although the amount was lower than in a corresponding emulsion [72]. In contrast to this, Schubert *et al.* [73] performed NMR, TEM and small angle X-ray scattering (SAXS) measurements and showed that no additional colloidal structures were formed. Drug nanocrystals could also be formed when the amount of the drug present far exceeds its solubility limit in the lipid matrix.

3.13.7. Supercooling of nanoparticles

Lipid nanoparticles prepared from triglycerides which are solid at room temperature may not necessarily crystallize on cooling to common storage temperatures. The particles can remain liquid for several months without crystallization (supercooled melt) [74]. Dispersions with lower melting points, in particular, monoacid triglycerides such as trilaurin or trimyristin, do not display melting transitions upon heating in differential scanning calorimeter or reflections due to crystalline nature in X-ray diffractometer after storage at room temperature. As confirmed by studies with quantitative ^1H NMR spectroscopy, the matrix of the dispersed particles consists of liquid triglycerides in such particles. The particles have a high tendency toward supercooling. The critical crystallization temperature is mainly dependent on the composition of the triglyceride matrix and can also be modified by incorporated drugs. The degree of supercooling is much higher in the nanoparticles than for the bulk triglyceride. This supercooling could be taken advantage of and utilized as a delivery system of its own but this has to be planned for *ab initio*.

3.14. Applications of lipid particulate drug delivery systems

During the last decade, different substances have been entrapped into lipid nanoparticles ranging from lipophilic to hydrophilic molecules and including difficult compounds such as proteins and peptides.

3.14.1. *Lipid nanoparticles as carriers for oral drug delivery*

Lipid nanoparticles such as SLN can be administered orally as dispersion, SLN-based tablet, pellets or capsules [70] or even as lyophilized unit dose powders for reconstitution for oral delivery. The stability of the particles in the GIT has to be thoroughly tested, since low pH and high ionic strength in the GIT may result in aggregation of the particles. In order to prove this, an investigation of the effect of artificial gastric fluids on different lipidic nanoparticle formulations was performed. The authors showed that a zeta potential of at least 8-9 mV in combination with a steric stabilization hinders aggregation under these conditions [75]. Additionally, for oral drug delivery, a release upon enzymatic degradation has to be taken into account [3, 76].

The routes for particle uptake after oral application are transcellular (via the M cells in the Peyer's patches or enterocytes) or paracellular (diffusion between the cells). However, the uptake via M cells is the major pathway, resulting in the transport of the particles to the lymph [77]. Uptake into the lymph and the blood was demonstrated by means of TEM and gamma counting of labelled SLN. It was found that uptake to the lymph was considerably higher than to the plasma [78], and as such, a reduced first pass effect concludes, as the transport via the portal vein to the liver is bypassed [79].

SLN containing the antituberculosis drugs rifampicin, isoniazid and pyrazinamide have been studied in animals model and it was found that administration every 10 days could be successful for the management of tuberculosis [80].

3.14.2. *Lipid nanoparticles for parenteral drug delivery*

Lipid nanoparticles can be formulated for subcutaneous, intramuscular or intravenous administration. For intravenous administration, the small particle size is a prerequisite as passage through the needle and possibility of embolism should be considered. SLN offer the opportunity of a controlled drug release and the possibility to incorporate poorly soluble drugs. Additionally, especially for intravenous application, drug targeting via modification of the particle surface is possible, and for SLN formulation with a controlled release, higher plasma concentrations over a prolonged period of time can be obtained. Such systems form an intravenous depot. Further studies with different drugs such as idarubicin, doxorubicin, tobramycin, clozapine or temozolomide also showed a sustained release as described in a review paper by Harms and Müller-Goymann [81].

3.14.3. *Lipid nanoparticles as carriers for peptides and proteins drugs*

Lipid nanoparticles have been extensively studied for the delivery of proteins and peptides [82]. Therapeutic application of peptides and proteins is restricted by their high molecular weight, hydrophilic character and limited chemical stability, which cause low bioavailability, poor transfer across biological membranes and low stability in the bloodstream. Most of the available peptides and proteins are delivered by injection, but their short half life demands repeated doses that are costly, painful and not well tolerated by patients. Lipid nanoparticles could be useful for peptide and protein delivery due to the

stabilizing effect of lipids and to the absorption promoting effect of the lipidic material that constitute this kind of nanoparticles [83]. The use of niosomes and liposomes as adjuvants for the delivery of Newcastle disease virus to chicks has been reported [14].

3.14.4. Lipid nanocarriers for nasal vaccination

The use of lipid nanocarriers provides a suitable way for the nasal delivery of antigenic molecules. Besides improved protection and facilitated transport of the antigen, nanoparticulate delivery systems could also provide more effective antigen recognition by immune cells. These represent key factors in the optimal processing and presentation of the antigen, and therefore in the subsequent development of a suitable immune response. In this sense, the design of optimized vaccine nanocarriers offers a promising way for nasal mucosal vaccination [82].

3.14.5. Lipid nanoparticles as carriers in cosmetic and dermal preparation

Lipid nanoparticles can be incorporated into a cream, hydrogel or ointment to obtain semisolid systems for dermal applications. Another possibility is to increase the amount of lipid matrix in the formulation above a critical concentration, resulting in semisolid formulations [84]. The substances used for the preparation of dermal SLN are rather innocuous since they are mostly rated as GRAS and many of them are used in conventional dermal formulations. This resulted in the first dermal formulations containing SLN for cosmetic purposes entering the market [85].

Due to the adhesiveness of small particles, SLN adhere to the stratum corneum forming a film as this films have been shown to possess occlusive properties [86]. It was shown that the degree of crystallinity has a great impact on the extent of occlusion by the formulation. With increasing crystallinity the occlusion factor increases as well [87]. This explains why liquid nanoemulsions in contrast to SLN do not show an occlusive effect and why the extent of occlusion by NLC compared to SLN is reduced. Other parameters influencing the occlusion factor are the particle size and the number of particles. Whilst with increasing size the factor decreases, an increase in number results in an increase in the extent occlusion [88]. The occlusive effect leads to reduced water loss and increased skin hydration. Highly crystalline SLN can be used for physical sun protection due to scattering and reflection of the UV radiation by the particles. A high crystallinity was found to enhance the effectiveness and was also synergistic with UV absorbing substances used in conventional sunscreens [89]. Similarly synergism was observed on the sun protection factor and UV-A protection factor exhibited by the incorporation of the inorganic sunscreen, titanium-dioxide in NLC of carnauba wax and decyl oleate [90].

3.14.6. Lipid nanoparticles for ocular application

The eye possesses unique challenges with respect to drug delivery especially with respect to the posterior segment and treating vision threatening diseases. Poor bioavailability of drugs from ocular dosage form is mainly due to the pre-corneal loss factors which include tear

dynamics, non-productive absorption, transient residence time in the cul-de-sac, and relative impermeability of the corneal epithelial membrane. Due to the adhesive nature of the small nanoparticles, these negative effects can be reduced. For ocularly administered SLN an increase in bioavailability was observed in rabbits by Cavalli *et al.* [91] using tobramycin ion pair as the model drug. Various *in vitro* studies show a prolonged and enhanced permeation when the drug is incorporated in lipid nanoparticles [39]. For systems containing phospholipids, a further improved permeation of diclofenac sodium was observed [31].

3.14.7. Pulmonary application of lipid nanoparticles

Pulmonary drug application offers the advantage of minimizing toxic side effects if a local impact is intended. Systemic delivery can be achieved through pulmonary delivery, offering the advantage of bypassing the first pass effect, as well as offering a large absorptive area, extensive vasculature, easily permeable membrane and low extracellular and intracellular enzyme activities [92]. A problem of this method of administration is the low bioavailability. SLN can easily be nebulized to form an aerosol of liquid droplets containing nanoparticles for inhalation [82]. *In vivo* studies showed that the administered drugs (rifampicin, isoniazid and pyrazinamide for the treatment of tuberculosis) resulted in a prolonged mean residence time and a higher bioavailability than the free drug [93, 94].

3.14.8. Application of liquid crystal drug delivery systems

The spontaneous self assembly of some lipids to form liquid crystalline structures offers a potential new class of sustained release matrix. The nanostructured liquid crystalline materials are highly stable to dilution. This means that they can persist as a reservoir for slow drug release in excess fluids such as the GIT or subcutaneous space, or be dispersed into nanoparticle form, while retaining the parent liquid crystalline structure. The rate of drug release is directly related to the nanostructure of the matrix. The particular geometry into which the lipids assemble can be manipulated through either the use of additives to modify the assembly process, or through modifying conditions such as temperature, thereby providing a means to control drug release.

Liquid crystal depot could be injected as a low-viscosity solution. Once in the body, it self-assembles and encapsulates the drug in a nanostructured, viscous liquid crystal gel. The drug substance is then released from the liquid crystal matrix over a time period, which can be tuned from days to months. The liquid crystal depot system is capable of providing *in vivo* sustained release of a wide range of therapeutic agents over controlled periods of time. Liquid crystal nanoparticles can be combined with controlled-release and targeting functionalities. The particles are designed to form *in situ* at a controlled rate, which enables an effective *in vivo* distribution of the drug. The system has been shown to give more stable plasma levels of peptides in comparison to competing microsphere and conventional oil-depot technologies [21, 95].

Oral liquid crystal DDS are designed to address the varied challenges in oral delivery of numerous promising compounds including poor aqueous solubility, poor absorption, and

large molecular size. Compared with conventional lipid or non-lipid carriers, these show high drug carrier capacity for a range of sparingly water-soluble drugs. For drugs susceptible to *in vivo* degradation, such as peptides and proteins, liquid crystal nanoparticles protect the sensitive drug from enzymatic degradation. The system also addresses permeability limitations by exploiting the lipid-mediated absorption mechanism. For water-soluble peptides, typical bioavailability enhancements range from twenty to more than one hundred times. In an alternative application large proteins have been encapsulated for local activity in the GIT. Liquid crystal nanoparticle systems can be designed to be released at different absorption sites (e.g., in the upper or lower intestine) which is important for drugs that have narrow regional absorption windows.

With regards to topical application, liquid crystal systems form a thin surface film at mucosal surfaces consisting of a liquid crystal matrix, whose nanostructure can be controlled for achieving an optimal delivery profile. The system also provides good temporary protection for sore and sensitive skin. Their unique solubilizing, encapsulating, transporting, and protecting capacity is advantageously exploited in liquid and gel products used to increase transdermal and nasal bioavailability of small molecules and peptides.

3.15. Lipid nanoparticles in drug targeting

Nanoscale lipid materials containing drugs and diagnostics are being developed to image the distribution of tumour cells in the body, target and attach to cancerous cells, destroy unwanted cells via ablation or interference with cellular functions. Drug targeting might overcome the problem of repeated administration by facilitating the efficacy of drug administered systemically and attenuating side effects on healthy tissues. Ultimately, lipid nanoparticles represent versatile DDS, with the ability to overcome physiological barriers and guide the drug to specific cells or intracellular compartments by means of passive or ligand-mediated targeting mechanisms.

There are many research reports dealing with targeting of drugs to certain cellular targets in the organs using lipid nanoparticles. The knowledge of the pathophysiological/passive targeting approaches used in cancer chemotherapy is used to develop nanocarriers for targeting drug to appropriate cancer cells in the body. Small molecules administered systemically are easily eliminated through the kidney into the urine and distributed equally not only in the target tissue but also in other healthy tissues, resulting in less efficacy and more possible side effects [96]. On the other hand, large molecules have longer half-life in the blood and tend to be passively delivered to the lesions with highly permeable vessels in neovascularization and inflammation. This tendency is remarkable especially in solid tumors because of high density of neovascular vessels and immature lymph systems, and is termed enhanced permeability and retention (EPR) effect. Because of the successes being recorded from *in vitro* experiments, lipid nanoparticles have been subject of further investigation for drug targeting to the brain, several types of cancer, posterior segments of the eye, otic (inner ear) diseases, and delivery of nucleic acids and genes, utilizing the both active and passive targeting opportunities [97].

3.16. Scale up issues and ultimate dosage form development

Large scale production of lipid nanoparticles is possible using lines available in pharmaceutical plants. In spite of intensive research on colloidal drug carrier systems, in some cases for several decades (e.g., with lipid emulsions or liposomes), remarkably few have been introduced into the market [98, 99] as presented in Table 3 indicating that there seem to be problems either with the underlying concepts or with the formulation of adequate colloidal carriers, or both. Most of the products are cosmetic products. In this context, it has, of course, to be taken into account that the development and registration of a new type of administration system may take as long for a new drug or novel formulation thereof; that means up to 10 years or even more. Therefore, products will appear on the market only after considerable period of time when the concept has been successfully realized.

Product name	Producer	Market entry date	Main active ingredients
Cutanova Cream Nano Repair Q10 Intensive Serum NanoRepair Q10 Cutanova Cream NanoVital Q10	Dr. Rimpler	10/2005	Q 10, polypeptide, Hibiscus extract, ginger extract, ketosugar
		10/2005	Q 10, polypeptide, mafane extract
		06/2006	Q 10, TiO ₂ , polypeptide, ursolic acid, oleanolic acid, sunflower seed extract
SURMER Crème Légère Nano-Protection SURMER Crème Riche Nano-Restructurante SURMER Elixir du Beauté Nano- Vitalisant SURMER Masque Crème Nano-Hydratant	Isabelle Lancray	11/2006	Kukuinut oil, Monoi Tiare Tahiti®, pseudopeptide, milk extract from coconut, wild indigo, noni extract
NanoLipid Restore CLR Nanolipid Q10 CLR Nanolipid Basic CLR NanoLipid Repair CLR	Chemisches Laboratorium Dr. Kurt Richter, (CLR)	04/2006 07/2006 07/2006 02/2007	Black currant seed oil containing ω -3 and ω -6 unsaturated fatty acids coenzyme Q10 and black currant seed oil caprylic/capric triglycerides black currant seed oil and manuka oil
IOPE SuperVital - Cream - Serum - Eye cream	Amore Pacific	09/2006	coenzyme Q10, ω -3 und ω -6 unsaturated fatty acids

Product name	Producer	Market entry date	Main active ingredients
- Extra moist softener - Extra moist emulsion			
NLC Deep Effect Eye Serum NLC Deep Effect Repair Cream NLC Deep Effect Reconstruction Cream NLC Deep Effect Reconstruction Serum	Beate Johnen	12/2006	coenzyme Q10, highly active oligo saccharides Q10, TiO ₂ , highly active oligo saccharides Q10, Acetyl Hexapeptide-3, micronized plant collagen, high active oligosaccharides in polysaccharide matrix
Regenerationscreme Intensiv	Scholl	6/2007	Macadamia Ternifolia seed oil, Avocado oil, Urea, Black currant seed oil
Swiss Cellular White Illuminating Eye Essence Swiss Cellular White Intensive Ampoules	la prairie	1/2007	Glycoprotiens, Panax ginseng root extract, Equisetum Arvense extract, Camellia Sinensis leaf extract, Viola Tricolor Extract
SURMER Creme Contour Des Yeux Nano-Remodelante	Isabelle Lancray	03/2008	Kukuinut oil, Monoi Tiare Tahiti®, pseudopeptide, hydrolized wheet protien
Olivenöl Anti Falten Pflegekonzentrat Olivenöl Augenpflegebalsam	Dr. Theiss	02/2008	Olea Europaea Oil, Panthenol, Acacia Senegal, Tocopheryl Acetate Olea Europaea Oil, Prunus Amygdalus Dulcis Oil, Hydrolized Milk Protein, Tocopheryl Acetate, Rhodiola Rosea Root Extract, Caffeine

Table 3. Examples of cosmetic products currently on the market containing lipid nanoparticles (Adapted from [98, 99]).

4. Future prospects of novel lipid particulate drug delivery systems

Novel lipid based nanoparticles offer a highly versatile platform that should be considered when working with drugs that present solubility and/or bioavailability challenges. An increasing number of drugs under development are poorly water soluble and therefore have poor bioavailability. These are designated BCS class II and class IV drugs. Creative formulation techniques are required to produce finished drug products of these drugs that have acceptable pharmacokinetics. A common formulation approach with such compounds

is to focus on creating and stabilizing very small particles of the drug in an attempt to increase the surface area available for dissolution *in vivo*, and hence the rate of dissolution, and consequently plasma or tissue levels of drug. Shelf-life stability and enzymatic degradation are two main areas of concern, and formulation design focuses on stabilizing the drug in storage and protecting it from endogenous enzyme degradation until it reaches its therapeutic target. These novel DDS are well suited for the formulation of these bioactives.

Lipid nanoparticle DDS can be employed for the delivery of phytomedicines intended for oral and topical administration, which are the main routes of administration of phytomedicinals. This application holds great promise in the development and use of phytomedicines considering the difficulties of their delivery owing to their physicochemical properties. Since many phytomedicines usually possess different pharmacological activities, the delivery can be targeted to specific part(s) of the body where action is desired by means of lipid nanoparticle technology [60]. Thus, undesired effects and wastage of materials would be avoided. The implication is that with efficiency of preparation, only required quantity is utilized for formulation and adequate dose is absorbed and successfully delivered to the target for the desired activity. Lipid nanoparticle formulation of phytomedicinals would find useful applications in nanomedicine especially where targeted delivery is important such as in the treatment of cancer.

5. Conclusions

The use of lipid particles as drug carrier systems has been favoured recently as result of the GRAS status of the excipients and their traditional use in other food and pharmaceutical products. Lipids and lipid nanoparticles are promising delivery systems for oral administration of small molecule drugs, proteins and peptides. Lipid formulations of drugs are able to control the release of drugs and reduce absorption variability. The oral administration of lipid nanoparticles is possible as aqueous dispersion or alternatively transformed into a traditional dosage forms such as tablets, pellets, capsules, or powders in sachets. The ability to incorporate drugs into lipid nanocarriers offers a new prototype in drug delivery that could be used for passive and active drug targeting. Lipid nanoparticles for topical application could be formulated with high content of lipid matrix or dispersed in creams or gels to give it 'body'. With the development and interest in lipid particulate drug delivery systems shown by pharmaceutical formulation scientists, a future full of lipid nanoparticle products in the market is envisaged.

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6. References

- [1] Northern Lipids Inc. (2008) Lipid Based Drug Formulation. <http://www.northernlipids.com/ourfacilities.htm>. (accessed 17 April 2012).
- [2] Muller 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(Suppl. 1): S131–S155.
- [3] Muller RH. Medicament Vehicle for the Controlled Administration of an Active Agent, Produced from Lipid Matrix-Medicament Conjugates. 2000; WO0067800.
- [4] Jain KK. Drug Delivery Systems - An Overview. In: Jain KK. (ed.) *Drug Delivery Systems*. Totowa: Humana Press; 2008. p1-50.
- [5] Lipids-Chemistry Encyclopaedia- structure, water, proteins, number, name, molecule. <http://www.chemistryexplained.com/Kr-Ma/Lipids.html#ixzz1sJCO5V00>. (accessed 17 April 2012).
- [6] Stuchlík M, Žák S. Lipid-based Vehicle for Oral Drug Delivery. *Biomed. Papers* 2001; 145(2): 17–26.
- [7] Attama AA, Nkemnele MO. *In Vitro* Evaluation of Drug Release from Self Micro-Emulsifying Drug Delivery Systems Using a Novel Biodegradable Homolipid from *Capra hircus*. *Int. J. Pharm.* 2005; 304 4-10.
- [8] Müller RH, Mehnert W, Lucks JS, Schwarz C, Mühlen A, Weyhers H, Freitas C, Rühl D. Solid Lipid Nanoparticles (SLN)- An Alternative Colloidal Carrier System for Controlled Drug Delivery. *Eur. J. Pharm. Biopharm.* 1995;41: 62-69.
- [9] Attama AA, Müller-Goymann CC. Effect of Beeswax Modification on the Lipid Matrix and Solid Lipid Nanoparticle Crystallinity. *Colloids Surf. A: Physicochem. Eng. Aspects.* 2008;315: 189-195.
- [10] Müller RH, Souto EB, Radtke M. PCT application PCT/EP00/04111. 2000.
- [11] Schwarz C, Mehnert W, Lucks JS, Muller RH. Solid Lipid Nanoparticles (SLN) for Controlled Drug Delivery: I. Production, Characterization and Sterilization. *J. Control. Rel.* 1994;30: 83–96.
- [12] Olbrich C, Geßner A, Kayser O, Muller RH. Lipid-Drug Conjugate (LDC) Nanoparticles as Novel Carrier System for the Hydrophilic Antitrypanosomal Drug Diminazene diacetate. *J. Drug Target.* 2002;10(5): 387–396.
- [13] Onuigbo EB, Okore VC, Ngene AA, Esimone CO, Attama AA. Preliminary Studies of a Stearylamine-based Cationic Liposome. *J. Pharm. Res.* 2011;10(1): 25-29.
- [14] Onuigbo EB, Okore VC, Esimone CO, Ngene A, Attama AA. Preliminary Evaluation of the Immunoenhancement Potential of Newcastle Disease Vaccine Formulated as a Cationic Liposome. *Avian Pathology* 2012. DOI: 10.1080/03079457.2012.691154.
- [15] Cevc G, Gebauer D, Stieber J, Schatzlein A, Blume G. Ultraflexible Vesicles Transfersomes, have an Extremely Low Pore Penetration Resistance and Transport

- Therapeutic Amounts of Insulin Across the Intact Mammalian Skin. *Biochim. Biophys. Acta* 1998;1368: 201–215.
- [16] Cevc G, Schaltzlein A, Richardsen H. Ultradeformable Lipid Vesicles can Penetrate the Skin and Other Semi-Permeable Barriers Unfragmented. Evidence from Double Label CLSM Experiments and Direct Size Measurements. *Biochim. Biophys. Acta* 2002;1564: 21–30.
- [17] Uchegbu IF, Vyas SP. Non-ionic Surfactant Based Vesicles (Niosomes) in Drug Delivery. *Int. J. Pharm.* 1998;172: 33–70.
- [18] Conacher M, Alexander J, Brewer JM. Niosomes as Immunological Adjuvants. In: Uchegbu I. (ed.) *Synthetic Surfactant Vesicles*. Singapore: International Publishers Distributors; 2000. p185–205.
- [19] Onuigbo EB. Evaluation of Cationic Liposome- or Niosome-Based Antigen Delivery Systems for Newcastle Disease Vaccine. Ph.D. thesis. University of Nigeria, Nsukka; 2011.
- [20] Lakshmi PK, Devi GS, Bhaskaran S, Sacchidanand S. Niosomal Methotrexate Gel in the Treatment of Localized Psoriasis: Phase I and phase II Studies. *Indian J. Dermatol. Venereal. Leprol.* 2007;73(3): 157–161.
- [21] Guo C, Wang J, Cao F, Lee RJ, Zhai G. Lyotropic Liquid Crystal Systems in Drug Delivery. *Drug Discov. Today* 2010;15: 1032–1040.
- [22] Charles L, Attama AA. Current State of Nanoemulsions in Drug Delivery. *J. Biomat. Nanobiotechnol.* 2011;2: 626–639.
- [23] Kotta S, Khan AW, Pramod K, Ansari SH, Sharma RK, Ali J. Exploring Oral Nanoemulsions for Bioavailability Enhancement of Poorly Water-soluble Drugs. *Expert Opin. Drug Deliv.* 2012;9(5): 585–598.
- [24] Hagemann JW. Thermal Behaviour and Polymorphism of Acylglycerides. In: Garti N, Sato K. (eds.) *Crystallization and Polymorphism of Fats and Fatty Acids*. Surfactant Science Series, Vol. 31. New York: Marcel Dekker; 1988. p9–95.
- [25] Westesen K, Siekmann B, Koch MHJ. Characterization of Submicron-sized Drug Carrier Systems Based on Solid Lipids by Synchrotron Radiation X-ray Diffraction. *Prog. Colloid Polym. Sci.* 1993;93: 356.
- [26] Jennings V, Thünemann AF, Gohla SH. Characterisation of a Novel Solid Lipid Nanoparticle Carrier System Based on Binary Mixtures of Liquid and Solid Lipids. *Int. J. Pharm.* 2000;199(2): 167–177.
- [27] Friedrich I, Reichl S, Müller-Goymann CC. Drug Release and Permeation Studies of Nanosuspensions Based on Solidified Reverse Micellar Solutions (SRMS). *Int. J. Pharm.* 2005;305(1–2): 167–175.
- [28] Schubert MA, Harms M, Müller-Goymann CC. Structural Investigations on Lipid Nanoparticles Containing High Amounts of Lecithin. *Eur. J. Pharm. Sci.* 2006;27(2–3): 226–236.
- [29] Attama AA, Schicke BC, Müller-Goymann CC. Further Characterization of Theobroma Oil-Beeswax Admixtures as Lipid Matrices for Improved Drug Delivery Systems. *Eur. J. Pharm. Biopharm.* 2006;64: 294–306.

- [30] Attama AA, Müller-Goymann CC. A Critical Study of Novel Physically Structured Lipid Matrices Composed of a Homolipid from *Capra hircus* and Theobroma Oil. *Int. J. Pharm.* 2006;322: 67-78.
- [31] Attama AA, Reichl S, Müller-Goymann CC. Diclofenac Sodium Delivery to the Eye: *In Vitro* Evaluation of Novel Solid Lipid Nanoparticle Formulation Using Human Cornea Construct. *Int. J. Pharm.* 2008;355: 307-313.
- [32] Attama AA, Weber C, Müller-Goymann CC. Assessment of drug permeation from SLN formulated with a novel structured lipid matrix through artificial skin construct bio-engineered from HDF and HaCaT cell lines. *J. Drug Deliv. Sci. Technol.* 2008;18(3) 181-188.
- [33] Severino P, Andreani T, Sofia-Macedo A, Fangueiro JF, Santana M-H A, Silva AM, Souto EB. Current State-of-Art and New Trends on Lipid Nanoparticles (SLN and NLC) for Oral Drug Delivery. *J. Drug Deliv.* 2012; doi:10.1155/2012/750891.
- [34] Kalam MA, Sultana Y, Ali A, Aqil M, Mishra AK, Chuttani K. Preparation, Characterization and Evaluation of Gatifloxacin Loaded Solid Lipid Nanoparticles as Colloidal Ocular Drug Delivery System. *J. Drug Target.* 2010; 18(3): 191-204.
- [35] Göppert TM, Müller RH. Adsorption Kinetics of Plasma Proteins on Solid Lipid Nanoparticles for Drug Targeting. *Int. J. Pharm.* 2005; 302(1-2): 172-186.
- [36] 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): 286-296.
- [37] Pandita D, Ahuja A, Velpandian T, Lather V, Dutta T, Khar RK. Characterization and *In Vitro* Assessment of Paclitaxel Loaded Lipid Nanoparticles Formulated using Modified Solvent Injection Technique. *Pharmazie* 2009; 64(5): 301-310.
- [38] Lipids - Chemistry Encyclopedia - structure, water, proteins, number, name, molecule <http://www.chemistryexplained.com/Kr-Ma/Lipids.html#ixzz1sJC5I4CP> (accessed 17 April 2012).
- [39] <http://www.web-books.com/MoBio/Free/Ch1B2.htm> (accessed 3 June 2012).
- [40] Attama AA, Reichl S, Müller-Goymann CC. Sustained Release and Permeation of Timolol from Surface Modified Solid Lipid Nanoparticles through Bio-engineered Human Cornea. *Current Eye Res.* 2009;34(8): 698-705.
- [41] Xie S, Zhu L, Dong Z, Wang Y, Wang X, Zhou W. Preparation and Evaluation of Ofloxacin-loaded Palmitic Acid Solid Lipid Nanoparticles. *Int. J. Nanomed.* 2011; 6: 547-555.
- [42] Battaglia L, Gallarate M, Cavalli R, Trotta M. Solid Lipid Nanoparticles Produced Through a Coacervation Method. *J. Microencapsul.* 2010; 27(1): 78-85.
- [43] Severino P, Pinho SC, Souto EB, Santana MHA. Crystallinity of Dynasan114 and Dynasan118 Matrices for the Production of Stable Miglyol-loaded Nanoparticles. *J. Thermal Analysis Calorimetry* 2012; 108:101-108.
- [44] Başaran E, Demirel M, Sirmagül B, Yazan Y. Cyclosporine A Incorporated Cationic Solid Lipid Nanoparticles for Ocular Delivery. *J. Microencapsul.* 2010; 27(1): 37-47.

- [45] Attama AA, Müller-Goymann CC. Investigation of Surface-modified Solid Lipid Nanocontainers Formulated with a Heterolipid-templated Homolipid. *Int. J. Pharm.* 2007; 334: 179-189.
- [46] Montenegro L, Campisi A, Sarpietro M G et al. *In Vitro* Evaluation of Idebenone-loaded Solid Lipid Nanoparticles for Drug Delivery to the Brain. *Drug Dev. Ind. Pharm.* 2011; 37(6): 737-746.
- [47] Vivek K, Reddy H, Murthy RSR. Investigations of the Effect of the Lipid Matrix on Drug Entrapment, *In Vitro* Release, and Physical Stability of Olanzapine-loaded Solid Lipid Nanoparticles. *AAPS PharmSciTech.* 2007; 8(4) Article no. 83.
- [48] Rawat MK, Jain A, Singh S. *In Vivo* and Cytotoxicity Evaluation of Repaglinide-loaded Binary Solid Lipid Nanoparticles after Oral Administration to Rats. *J. Pharm. Sci.* 2011; 100(6): 2406-2417.
- [49] Shah M, Chuttani K, Mishra AK, Pathak K. Oral Solid Compritol 888 ATO Nanosuspension of Simvastatin: Optimization and Biodistribution Studies. *Drug Dev. Ind. Pharm.* 2011; 37(5): 526-537.
- [50] del Pozo-Rodríguez A, Delgado D, Solinís M A et al. Solid Lipid Nanoparticles as Potential Tools for Gene Therapy: *In Vivo* Protein Expression after Intravenous Administration. *Int. J. Pharm.* 2010; 385(1-2): 157-162.
- [51] Nnamani PO, Adikwu MU, Attama AA, Ibezim EC (2010). SRMS142 Based Solid Lipid Microparticles: Application in Oral Delivery of Glibenclamide to Diabetic Rats. *Eur. J. Pharm. Biopharm.* 76(1) 68 - 74.
- [52] Attama AA, Schicke BC, Müller-Goymann CC. Novel Physically Structured Lipid Matrices of Beeswax and a Homolipid from *Capra hircus* (Goat Fat): A Physicochemical Characterization. *J. Drug Deliv. Sci. Technol.* 2007;17(2): 103-112.
- [53] Attama AA, Schicke BC, Paepenmüller T, Müller-Goymann CC. Solid Lipid Nanodispersions Containing Mixed Lipid Core and a Polar Heterolipid: Characterization. *Eur. J. Pharm. Biopharm.* 2007; 67: 48-57.
- [54] Mozafari MR. Liposomes: an Overview of Manufacturing Techniques. *Cellular Mol. Biol. Letters* 2005;10: 711-719.
- [55] Cortesi R, Esposito E, Luca G, Nastruzzi C. Production of Lipospheres as Carriers for Bioactive Compounds. *Biomaterials* 2002;23: 2283- 2294.
- [56] Lippacher A, Müller RH, Mäder K. Semisolid SLN Dispersions for Topical Application: Influence of Formulation and Production Parameters on Viscoelastic Properties. *Eur. J. Pharm. Biopharm.* 2002;53(2): 155-160.
- [57] Gasco MR. Method for Producing Solid Lipid Microspheres having a Narrow Size Distribution, US Patent No. 5250236; 1993.
- [58] Sjostrom B, Bergensthal B. Preparation of Submicron Drug Particles in Lecithin-stabilized o/w Emulsion I: Model Studies of the Precipitation of Cholesteryl acetate. *Int. J. Pharm.* 1992;88: 53-62.
- [59] Siekmann B, Westesen K. Investigation on Solid Lipid Nanoparticle Prepared by Precipitation in o/w Emulsion. *Eur. J. Pharm. Biopharm.* 1996;43: 104-109.
- [60] Attama AA. SLN, NLC, LDC: State of the Art in Drug and Active Delivery. *Recent Patents Drug Deliv. Form.* 2011;5(3): 178-187.

- [61] Westesen K, Bunjes H, Koch MH. Physicochemical Characterization of Lipid Nanoparticles and Evaluation of their Drug Loading Capacity and Sustained Release. *J. Control. Rel.* 1997;48: 223-236.
- [62] Müller RH, Mäder K, Gohla SH. Solid Lipid Nanoparticles (SLN) for Controlled Drug Delivery- A Review of the State of the Art. *Eur. J. Pharm. Biopharm.* 2000;50: 161-177.
- [63] Abdel-Mottaleb MMA, Neumann D, Lamprecht A. *In Vitro* Drug Release Mechanism from Lipid Nanocapsules (LNC). *Int. J. Pharm.* 2010;390: 208-213.
- [64] zur Mühlen A, Schwarz C, Mehnert W. Solid Lipid Nanoparticles (SLN) for Controlled Drug Delivery- Drug Release and Release Mechanism. *Eur. J. Pharm. Biopharm.* 1998;45: 149-155.
- [65] Aggarwal P, Hall JB, McLeland CB, Dobrovolskaia MA, McNeil SE. Nanoparticle Interaction with Plasma Proteins as it Relates to Particle Biodistribution, Biocompatibility and Therapeutic Efficacy. *Adv. Drug Deliv. Rev.* 2009;61: 428-437.
- [66] Westesen K, Siekmann B. Investigation of the Gel Formation of Phospholipid-stabilized Solid Lipid Nanoparticles. *Int. J. Pharm.* 1997;151: 35-45.
- [67] Lippacher A, Müller RH, Mäder K. Investigation on the Viscoelastic Properties of Lipid Based Colloidal Drug Carriers. *Int. J. Pharm.* 2000;196(2): 227-230.
- [68] Unruh T, Westesen K, Bösecke P, Lindner P, Koch MHJ. Self-assembly of Triglyceride Nanocrystals in Suspension. *Langmuir* 2002;18(5): 1796-1800.
- [69] Illing A, Unruh T, Koch MHJ. Investigation on Particle Self Assembly in Solid Lipid-based Colloidal Drug Carrier Systems. *Pharm. Res.* 2004;21(4): 592-597.
- [70] Mehnert W, Mäder K. Solid Lipid Nanoparticles: Production, Characterization and Applications. *Adv. Drug Deliv. Rev.* 2001;47(2-3): 165-196.
- [71] Westesen K, Wehler T. Physicochemical Characterization of a Model Intravenous Oil-in-water Emulsion. *J. Pharm. Sci.* 1992;81: 777-786.
- [72] Heiati H, Phillips NC, Tawashi R. Evidence for Phospholipid Bilayer Formation in Solid Lipid Nanoparticles Formulated with Phospholipid and Triglyceride. *Pharm. Res.* 1996;13: 1406-1410.
- [73] Schubert MA, Harms M, Müller-Goymann CC. Structural Investigations on Lipid Nanoparticles Containing High Amounts of Lecithin. *Eur. J. Pharm. Sci.* 2006;27(2-3): 226-236.
- [74] Westesen K, Bunjes H. Do Nanoparticles Prepared from Lipids Solid at Room Temperature Always Possess a Solid Lipid Matrix. *Int. J. Pharm.* 1995;115: 129-131.
- [75] Zimmermann E, Müller RH. Electrolyte-and pH-stabilities of Aqueous Solid Lipid Nanoparticle (SLN) Dispersions in Artificial Gastrointestinal Media. *Eur. J. Pharm. Biopharm.* 2001;52(2): 203-210.
- [76] Olbrich C, Müller RH. Enzymatic Degradation of SLN- Effect of Surfactant and Surfactant Mixtures. *Int. J. Pharm.* 1991;180(1): 31-39.
- [77] des Rieux A, Fievez V, Garinot M, Schneider YJ, Preat V. Nanoparticles as Potential Oral Delivery Systems of Proteins and Vaccines: A Mechanistic Approach. *J. Control. Rel.* 2006;116(1): 1-27.

- [78] Bargoni A, Cavalli R, Caputo O, Fundaro A, Gasco MR, Zara GP. Solid Lipid Nanoparticles in Lymph and Plasma after Duodenal Administration to Rats. *Pharm. Res.* 1998;15(5): 745-750.
- [79] Souto EB, Doktorovova S. Solid Lipid Nanoparticle Formulations, Pharmacokinetic and Biopharmaceutical Aspects in Drug Delivery. *Methods Enzymol.* 2009;464(C): 105-129.
- [80] Pandey R, Sharma S, Khuller GK. Oral Solid Lipid Nanoparticle-based Antitubercular Chemotherapy. *Tuberculosis* 2005;85(5-6): 415-420.
- [81] Harms M, Müller-Goymann CC. Solid Lipid Nanoparticles for Drug Delivery. *J. Drug Del. Sci. Tech.* 2011;21(1): 89-99.
- [82] Almeida AJ, Souto EB. Solid Lipid Nanoparticles as a Drug Delivery System for Peptides and Proteins. *Adv. Drug Deliv. Rev.* 2007;59: 478-490.
- [83] del Pozo-Rodriguez A, Delgado D, Solinís MA, Gascón AR. Lipid Nanoparticles as Vehicles for Macromolecules: Nucleic Acids and Peptides. *Recent Pat. Drug Deliv. Formul.* 2011;5: 214-226.
- [84] Hussein R. Charakterisierung von Hartfettmatrices und Lipidnanosuspensionen mit Phospholipon 90H. Ph.D. thesis. TU Braunschweig, Braunschweig. 2009.
- [85] Müller RH, Petersen RD, Hommoss A, Pardeike J. Nanostructured Lipid Carriers (NLC) in Cosmetic Dermal Products. *Adv. Drug Del. Rev.* 2007;59(6): 522-530.
- [86] Pardeike J, Hommoss A, Müller RH. Lipid Nanoparticles (SLN, NLC) in Cosmetic and Pharmaceutical Dermal Products. *Int. J. Pharm.* 2009;366(1-2): 170-184.
- [87] Wissing SA, Müller RH. The Influence of the Crystallinity of Lipid Nanoparticles on their Occlusive Properties. *Int. J. Pharm.* 2002;242(1-2): 377-379.
- [88] Wissing SA, Müller RH. Cosmetic Applications for Solid Lipid Nanoparticles (SLN). *Int. J. Pharm.* 2003;254(1): 65-68.
- [89] Villalobos-Hernandez JR, Müller-Goymann CC. Sun Protection Enhancement of Titanium Dioxide Crystals by the Use of Carnauba Wax Nanoparticles: The Synergistic Interaction Between Organic and Inorganic Sunscreens at Nanoscale. *Int. J. Pharm.* 2006;322(1-2): 161-170.
- [90] Villalobos-Hernandez JR, Müller-Goymann CC. *In vitro* Erythematous UV-A Protection Factors of Inorganic Sunscreens Distributed in Aqueous Media Using Carnauba Wax-Decyl Oleate Nanoparticles. *Eur. J. Pharm. Biopharm.* 2007;65(1): 122-125.
- [91] Cavalli R, Gasco MR, Chetoni P, Burgalassi S, Saettone MF. Solid Lipid Nanoparticles (SLN) as Ocular Delivery System for Tobramycin. *Int. J. Pharm.* 2002;238(1-2): 241-245.
- [92] Bur M, Henning A, Hein S, Schneider M, Lehr CM. Inhalative Nanomedicine- Opportunities and Challenges. *Inhalation Toxicol.* 2009;21(Suppl. 1): 137-143.
- [93] Nassimi M, Schleh C, Lauenstein HD, Hussein R, Hoymann HG, Koch W, Pohlmann G, Krug N, Sewald K, Rittinghausen S, Braun A, Müller-Goymann CC. A Toxicological Evaluation of Inhaled Solid Lipid Nanoparticles Used as a Potential Drug Delivery System for the Lung. *Eur. J. Pharm. Biopharm.* 2010;75(2): 107-116.
- [94] Pandey R, Khuller GK. Solid Lipid Particle-based Inhalable Sustained Drug Delivery System Against Experimental Tuberculosis. *Tuberculosis* 2005;85(4): 227-234.
- [95] Innovative Nanoscale Therapeutics. Camurus. <http://www.camurus.com/> (accessed 17 April 2012).

- [96] Yasukawa T, Tabata Y, Kimura H, Ogura Y. Recent Advances in Intraocular Drug Delivery Systems. *Recent Patents Drug Deliv. Formul.* 2011;5: 1-10.
- [97] Battaglia L, Gallarate M. Lipid Nanoparticles: State of the Art, New Preparation Methods and Challenges in Drug Delivery. *Expert Opin. Drug Deliv.* 2012;9(5): 497-508.
- [98] Hommoss A. Nanostructured Lipid Carriers (NLC) in Dermal and Personal Care Formulations. Ph.D. thesis. Freie Universität Berlin, Berlin. 2008.
- [99] Puri D, Bhandari A, Sharma P, Choudhary D. Lipid Nanoparticles (SLN, NLC): A Novel Approach for Cosmetic and Dermal Pharmaceutical. *J. Global Pharma. Technol.* 2010; 2(5):1-15.