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Lipid Nanoparticles (SLN®, NLC®) for Cutaneous Drug Delivery: Structure, Protection and Skin Effects

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Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) are new drug delivery systems composed of physiological lipid materials and surfactants accepted by regulatory authorities for application in cutaneous drug delivery, i.e., topical, dermal and transdermal. They have a whole set of unique advantages which make them very reliable in the development of topical and dermatological formulations. This review article focuses on the definitions and properties of these colloidal carriers including the production techniques and suitable formulations. Pharmaceutical considerations are also addressed on the basis of physicochemical stability of such carriers as well as of drug retention onto the lipid matrix. The drug diffusion enhancing effect of SLN and NLC into the skin is also discussed. Advantages of these carriers on minimizing/avoiding skin side effects are also emphasized.

Keywords: Solid Lipid Nanoparticles, SLN, Nanostructured Lipid Carriers, NLC, Cutaneous Drug Delivery, Topical Drugs, Polymorphism, Anisotropic Diffusion.

CONTENTS

1. INTRODUCTION

The development of topical and dermatological drug delivery systems for local or systemic effects has gained increased interest in the latest years. If the aim is the systemic drug therapy, transdermal administration of drugs shows some important advantages such as the avoidance of first-pass metabolism and minimization of side effects. Nonetheless, cutaneous administration is limited by the relative impermeability of the stratum corneum. The formulation should deliver the drug to the site of action in sufficient amounts over a suitable time scale. When using conventional topical semi-solids (e.g., creams, lotions, and ointments) several potentially valuable drugs cannot be delivered via topical route because they cannot cross the impermeable stratum corneum in sufficient quantities. To overcome such unfavourable physicochemical properties of these drugs, scientific research has developed several approaches. Examples of physical approaches used to enhance stratum corneum permeability are e.g., iontophoresis, sonophoresis, as well as microporation by means of microneedles.[1] Chemical methods include, e.g., penetration enhancers[2] and/or recurring to pro-drugs.[3] The former act by temporarily and reversibly diminution of the barrier functions of the stratum corneum so that the weakened horny layer allows the percutaneous absorption of drugs. When recurring to pro-drugs the aim is the chemical modification of the structure of the active molecules in order to increase the permeability within the stratum corneum. After crossing this barrier, the pharmaceutically active fragment of the compound is released near the site of action. However, very few chemical enhancers for transdermal administration have been approved for clinical use due to the risk of allergic reactions and toxicity.[4] Regarding the pro-drugs, the set of their physicochemical properties to obtain the optimum structure and self-penetration enhancing effects requires synthesis of the favourable shift of lipophilicity.[5]

The bioavailability of drugs penetrating into viable skin can be enhanced when using colloidal drug delivery

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systems because the small particle/vesicle size (i.e., below 1 μm) ensures close contact to the stratum corneum and thus, the amount of encapsulated drug reaching the site of action will be increased. Examples of such "classical" colloidal carriers are polymeric nanoparticles,[6,7] liposomes,[8,9] transfersomes,[10] oil-in-water (o/w)[11,12] and water-in-oil (w/o)[13] emulsions, multiple (w/o/w or o/w/o) emulsions,[14–16] and microemulsions.[17,18] In addition to these colloidal carriers, solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) have also been exploited intensively in cutaneous drug delivery.[19–21] The SLN and NLC are composed of a lipid matrix, which is solid at body and room temperature. Topical application of aqueous SLN or NLC dispersions is known to create a mono-layered lipid film onto the skin, which avoids water evaporation, and thus increases skin's moisture and hydration.[22,23] Due to their rigidity SLN and NLC are expected to be stable against coalescence. Such thermodynamic stability is enhanced by means of surfactant molecules used so stabilize SLN and NLC in aqueous dispersions. These molecules may also influence skin's permeability to a particular drug once they cause skin' structure disruption.[24] In addition, the matrix should reduce the mobility of incorporated drugs preventing drug leakage from the carrier and thus protecting sensitive molecules from the external environment. Since they are composed of biodegradable and physiological materials (fats and waxes) SLN and NLC can be prepared avoiding the use of organic solvents and other toxic additives, thus minimizing the toxicological risk.[25] Additionally, lipids are likely to minimize the danger of allergic contact dermatitis that might be induced by the drug.[10]

So far several physicochemically different drug molecules have been incorporated into lipid particles (Table I). The success of this incorporation depends mainly primarily on the chemical nature of the drug and on the lipid, which together will influence the selection of the production procedure for the lipid particles. To obtain a high payload the lipid selected should solubilize the drug, at least in its melted state. There are additionally a number of less obvious factors related to the production procedure itself, as well as to the final purpose of the developed formulation. The first product based on lipid nanoparticles (Nanobase®, Yamanouchi) was introduced to the market

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Table I. Examples of drugs incorporated into lipid particles intended for administration onto skin and/or mucosa.

Incorporated drugs	Refs.
Betamethasone valerate	[28–30]
Benzyl nicotinate	[31]
Bupivacaine	[32]
Chloramphenicol	[29]
Clobetasol propionate	[33–35]
Clotrimazole	[36–39]
Cortisone	[30]
Cyproterone acetate	[40]
Deoxycorticosterone acetate	[41]
Dexamethasone	[42, 43]
17β-o-estradiol derivatives	[44, 45]
Hydrocortisone	[46, 47]
Indomethacin	[46, 48]
Ketoconazole	[39, 49]
Metronidazole	[50]
Miconazole	[31, 51]
Pilocarpine	[52]
Podophyllotoxin	[53]
Prednicarbate	[30, 54, 55]
Prednisolone	[28, 56, 57]
Progesterone	[45, 47, 51, 58]
Timolol	[59]
Tobramycin	[60]
Triptolide	[61–64]
Ubidecarenone	[65–67]
Valdecoxib	[68]

in Poland and is patent protected.[26] Nanobase® exploits the special properties of placebo SLN, such as good application properties and adhesion leading to skin hydration. If active ingredients are included they will be dissolved in the aqueous phase of the cream. More recently, NLC have also reached the market.[27] A series of products from Cutanova (Dr. Rimpler GmbH) have recently been launched under the commercial designations of Nanorepair Q10 and Nanovital Q10. This Cutanova series provides a deep anti-aging treatment due to the higher Q10 concentrations achieved in the skin.

The aim of the present review is to present a detailed update of the use of SLN and NLC for cutaneous administration. Examples of several drugs entrapped into lipid particles for topical, dermal and transdermal application are given and the methods for their production are also described. Depending on the applied method, different morphological structures of lipid nanoparticles are obtained and their cutaneous features are discussed. The production of cutaneous formulations based on SLN and NLC is also addressed, as well as the cutaneous tolerability and toxicity of these carriers.

2. DEFINITIONS AND PROPERTIES OF LIPID NANOPARTICLES

The use of lipid particles intended for drug delivery is due to the research developed by Speiser et al. in Zurich,[69] who described their production applying high speed stirring of an o/w emulsion between a melted lipid phase and a hot aqueous surfactant solution. The obtained emulsion was cooled and the inner lipid phase formed solid particles. This product called "lipid nanopellets" was intended for oral drug delivery. A similar procedure was adapted by Domb, however applying ultrasounds for the emulsification phase producing the so-called "lipospheres."[70]

The use of stirring and sonication procedures in the emulsification of two immiscible phases is common. However, these procedures are associated with some disadvantages, such as the relatively broad size distribution and the relatively high concentrations of surfactants usually required to obtain a mean particle diameter in the nanometer range.

The first generation of submicron-sized lipid particles appeared in 1991 with the solid lipid nanoparticles (SLN), produced by high pressure homogenization (HPH),[71, 72] and by a microemulsion technique.[73]

Although the submicron size of the carriers and their low polydispersity index (PI) are the main factors affecting their long term stability, the yield of production is usually measured as a function of entrapment efficiency (EE) and loading capacity (LC), which are determined as follows:[34]

$$EE = \frac{W_a - W_s}{W_a} \times 100$$

$$LC = \frac{W_a - W_s}{W_a - W_s + W_L} \times 100$$

where W_a is the weight of drug added in the formulation, W_s is the weight of drug analysed in the supernatant (after separation of lipid and aqueous phases by centrifugation), and W_L is the weight of lipid added in the formulation. EE is thus defined as the ratio between the mass of entrapped drug and the total mass of drug, and LC is the ratio between the mass of entrapped drug and the total mass of lipid.

A problem related to SLN is that they are composed of solid lipids only and these can decrease both EE and LC due to the recrystallization process during storage time. A more intelligent carrier, the nanostructured lipid carriers (NLC), appeared to overcome such drawbacks.[74] NLC are composed of a blend of solid and liquid lipids, in a ratio that it is also solid at body and room temperatures. Such lipid mixture allows achieving release modulation of the encapsulated drug within NLC.[74]

2.1. Production Techniques

The scientific literature describes several production techniques which can be used to obtain both SLN and NLC.[75] HPH developed by Müller and Lucks,[72] leads to a product being relatively homogeneous in size, giving a higher physical stability of the aqueous dispersion. In general, dispersions with high PI show a greater tendency to aggregate or coalesce. The HPH is also a simple and very

cost-effective technique, and can be performed under hot or cold conditions. Briefly, for the hot HPH technique the lipid is melted at approx. 5–10 °C above its melting point, followed by drug dissolution or fine dispersion in the melted phase. Stirring in a hot surfactant solution then disperses this lipid phase. The obtained pre-emulsion is homogenized applying a pressure between 200–500 bar and 2–3 homogenization cycles. After homogenization a hot nanoemulsion is obtained. The nanoemulsion is cooled leading to recrystallization of the lipid and formation of lipid nanoparticles. For the cold HPH technique, an initial step of melting the solid lipid is required to dissolve the drug. Then, this phase is rapidly cooled using liquid nitrogen or dry ice, and after solidification it is ground by mortar milling to obtain lipid microparticles. These are then dispersed in a cold aqueous surfactant solution. The resulting pre-suspension is homogenized in the solid state at or below room temperature by cooling the high pressure homogenizer.

The microemulsion technique was described by Gasco.[73] Briefly, the lipid components are melted, the surfactant, co-surfactant and water are added in a ratio that a microemulsion results.[76] The size of the microemulsion region in the phase diagram is a function of temperature, i.e., the microemulsion can be converted to a different system when e.g., reducing the temperature. The microemulsion needs to be kept at high temperatures (e.g., 70 °C) during the process. The hot microemulsion is diluted into cold water (e.g., 4 °C), which breaks the microemulsion and forms an ultra-fine nanoemulsion. The dilution with water and the reduction of temperature narrowing the microemulsion region are the reasons for breaking of the microemulsion.[77]

The phase inversion-based technique described by Heurtault et al.[78, 79] also involves a melting process and it is based on a two-step method.[80] All components are placed on a magnetic stirrer using a temperature program from 25 °C to 85 °C. This is followed by progressive cooling to 60 °C. Three temperature cycles (85–60–85–60–85 °C) are applied to reach the inversion process defined by temperature range. In a second step, an irreversible shock is induced by dilution with cold water (e.g., 0 °C). This fast cooling-diluting process leads to the formation of stable nanoparticles.

A new process based on the use of a membrane contactor has been recently described by Charcosset et al.[81] In this case, the lipid phase is pressed at a temperature above the melting point of the solid lipid through the membrane pores allowing the formation of small droplets. Under cooling at room temperature these droplets recrystallize forming the lipid nanoparticles. This new technology has also been tested for the production of polymeric nanoparticles.[82]

Several other procedures have been adapted from the production of polymeric nanoparticles. Using the solvent emulsification-evaporation method described by Sjöström and Bergenståhl,[83] lipid material is dissolved in an organic solvent immiscible with water (e.g., cyclohexane,[83, 84] chloroform[84] or methylene chloride[85, 86]). The organic solution is dispersed in aqueous surfactant phase, and the solvent is removed by evaporation.[84] In the solvent displacement method described by Fessi et al.,[87] an organic solvent which is miscible with water is used. In this case, the lipid material is previously dissolved in a semi-polar water-miscible solvent, such as ethanol, acetone or methanol.[34, 35, 88–91] The emulsification-diffusion technique patented by Quintanar-Guerrero and Fessi,[92] the lipid material is dissolved in a water-saturated organic phase (benzyl alcohol[93] or tetrahydrofuran[94]), which is then used to prepare an o/w emulsion. Due to the saturation of the water phase with the organic solvent, no solvent diffuses from the droplets into the water phase. Removal of solvent from the droplets and particle formation is obtained by adding more water to the emulsion and extracting the solvent.[93]

2.2. Structure and Morphology of SLN and NLC

As previously mentioned, the structure and morphology of lipid nanoparticles depend on factors such as the selected lipid phase, the solubility properties of the drug in this organic phase, in addition to the production procedure and its variables (temperature, organic solvent, surfactants). In the literature, different theoretical models have been described for lipid nanoparticles.[19] Their physical shape is dependent on the selected lipids used for the production. If highly pure lipids are used, e.g., tristearin or cetyl palmitate, the nanoparticles have a more cubic shape because they will be reorganized in a crystal similar to a dense brick wall. When using crude mixtures, spherical shape is preferentially formed, because in this case larger and smaller and of different shape crystallized lipid molecules are present.

2.2.1. Structures and Models of SLN

Basically, the structure of lipid nanoparticles is composed of a solid core covered by a layer of surfactant molecules. For SLN three incorporation models have been proposed,[95] differing between them on the location and distribution of the drug molecules within the solid core (Fig. 1).

The SLN Type I is defined as the homogeneous matrix model, because the drug is molecularly dispersed in the lipid core or it is present in form of amorphous clusters. This model is obtained when applying the hot HPH in an optimized ratio of drug and lipid, or when using the cold HPH. As consequence of their structure, SLN Type I can show controlled release properties. The SLN Type II, or drug enriched shell model,[96] is obtained when applying the hot HPH technique and the drug concentration in the melted lipid is low. During the cooling of the

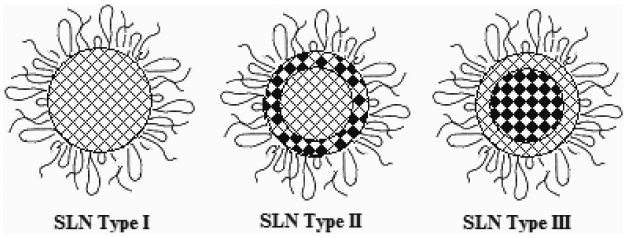

Fig. 1. Theoretical models for the structure of SLN. Black squares stand for drug molecules.

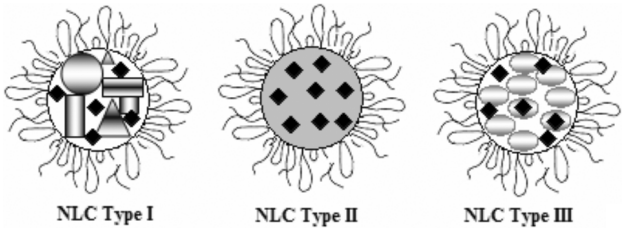

Fig. 2. Theoretical models for the structure of NLC. Black squares stand for drug molecules.

homogenized nanoemulsion, the lipid molecules precipitate first, leading to a steadily increasing concentration of drug in the remaining lipid melt with increased fraction of solidified lipid. A drug-free (or drug-reduced) lipid core is formed, when the drug reaches its saturation solubility in the remaining melt, an outer shell will solidify containing both drug and lipid. This model is not suitable for prolonged drug release, nonetheless it may be used to obtain a burst release of drug, in addition to the occlusive properties of the lipid core. The SLN Type III, or drug enriched core model,[28,37] is formed when the drug concentration is relatively close to or at its saturation solubility in the lipid melt. Under cooling of the nanoemulsion the solubility of the drug will decrease, when the saturation solubility is exceeded the drug precipitates which is then covered by a shell of lipid. This model is also useful for prolonged release purposes.

2.2.2. *Structures and Models of NLC*

NLC are also composed of a solid core covered by the emulsifying agent used during the production procedure (Fig. 2). For these carriers also three incorporation models are described in the literature, differing mainly on the type of lipid compounds used for their production.

The NLC Type I, or the imperfect crystal model, consists of a matrix with many voids and imperfections that are able to accommodate the drug molecules. This model is obtained when mixing solid lipids with sufficient amounts of liquid lipids (oils). Due to the different chain lengths of the fatty acids and the mixture of mono-, di- and triacylglycerols, the matrix of NLC is not able to form a highly ordered structure,[20] creating available spaces for the drug. The NLC Type II, or the amorphous model, is created when mixing special lipids (e.g., hydroxyoctacosanylhydroxystearate, isopropylmyristate, dibutyl adipate) that do not recrystallize after homogenization and cooling of the nanoemulsion. These lipids are able to create solid particles of amorphous structure, which can avoid the occurrence of recrystallization of lipid under cooling and during shelf life, minimizing drug expulsion during storage time. The NLC Type III is defined as the multiple model because it is composed of very small oil nanocompartments created inside the solid lipid matrix of the nanoparticles by a phase separation process.[20] It results when mixing solid lipids with oils (e.g., medium[34] and long chain triacylglycerols,[97] oleic acid[88]) in such a ratio that the solubility of the oil molecules in the solid lipid is exceeded. During the cooling of the nanoemulsion the lipid droplets reach the miscibility gap (40 °C), the oil precipitates forming tiny oil droplets in the melted solid lipid. Subsequent solidification of the solid lipid leads to fixation of the oily nanocompartments. The advantage of this model is the increase of LC for drugs that usually show higher solubility in liquid lipids than in solid lipids.[98]

These theoretical NLC models have been established based on analytical techniques, which can be used to discriminate between them afterwards. Differential scanning calorimetry (DSC) and X-ray diffraction analysis are useful to characterize the polymorphic forms of lipid molecules of the nanoparticle matrix, which are dependent on the lipid and surfactant composition. Magnetic resonance techniques such as NMR and ESR are useful to evaluate the dynamic phenomena and the presence of oily nanocompartments, which are characteristic of the NLC Type III.[95] Other analytical procedures include microscopy analysis, e.g., scanning (SEM) and transmission (TEM) electron microscopy, and atomic force microscopy (AFM).[99,100]

2.3. Pharmaceutical Considerations

Topical and dermatological formulations may be composed of free flowing powders (solids), semi-solids, or liquids, being the decision to employ a particular dosage form mainly dependent on considerations of its functional suitability. To develop an ideal formulation for cutaneous administration of drugs the major underlying principles are the flux of drug across the skin, the retention of the dosage form on the skin' surface, the reservoir capacity of the dosage form and the patients' acceptability of the formulation. Whenever this is accomplished, the formulation should be developed from the minimum number of ingredients giving the desired result. It is, therefore, wiser to employ well known materials in preference to novel, which are usually less well-known and poorly characterized. In this way, problems of incompatibility and instability on storage are easier resolved and the risk of adverse skin reactions is kept to a minimum.

2.3.1. Choice of Materials (Solubility Properties)

As mentioned above, the lipid materials used for SLN and NLC production resemble the physiologic lipids. Also, regulatory accepted lipids for topical administration are mostly used. Selection of lipid material is however dependent on the solubility properties of the drug. As expected, a higher EE and LC will be obtained if the drug is dissolved or solubilized in the melted lipid phase. Nevertheless, admixing very different lipids has the advantage of the creation of imperfections within the matrix that will also contribute to a higher loading. However, when mixing very chemically different lipid molecules supercooled melts can be obtained. The amount of supercooling (difference between melting and crystallization temperatures) is different for each particular lipid, and can be easily assessed by DSC. When working with triacylglycerols of high melting point and high crystallization temperature (pure lipid), the addition of lipid molecules with small-chain length will decrease the temperatures of both phenomena. This means that the amount of supercooling may increase, i.e., increases the difference between the melting and crystallization temperatures (Fig. 3). The same is valid for the opposite, i.e., if working with lipid materials of low crystallization temperature, the addition of long-chain length molecules will decrease the amount of supercooling.[101] During preparation, the emulsified dispersion must be cooled below the critical crystallization temperature of the lipid materials (i.e., much below their melting temperature) in order to crystallize and obtain solid lipid particles. If this critical temperature is not reached, the particles remain in the liquid state and an emulsion of supercooled, liquid particles is obtained rather than the desired SLN or NLC dispersion. Transition into a more stable polymorphic form is usually related to a rearrangement of the lipid molecules with increasing the lattice density. The type of crystal polymorph obtained and the kinetics of the transitions may thus have important consequences for the stability of the dispersion, as well as for the drug loading. DSC may be used to analyse supercooled melts because these do not display a melting event under heating above the melting peak of the lipid matrix.[102, 103]

There are some lipids that show the tendency to form supercooled melts, e.g., trilaurin (C_{12}), tricaprin (C_{14}) and some Witepsol bases.[101] These lipids should be avoided if SLN/NLC are intended for prolonged release purposes, or if their occlusive properties are to be exploited. In fact, supercooled melts are similar to o/w emulsions, showing high LC but low capacity to retain the drug molecules within the inner oil phase. Recrystallization can be however induced under storage at low temperatures, i.e., in the fridge (0–4 °C), which will maintain the physical stability of the systems. But under application the high temperature (skin' surface is at 32 °C) the inner lipid phase will liquefy.

2.3.2. Polymorphic Transformations

The incorporation of drug into the lipid matrix is dependent on the polymorphism of the crystallized matrix. In fact, drugs can be incorporated in between the lipid lamellae being only possible if the drug molecules are 20% smaller in size than lipid molecules.[104] Otherwise, drug molecules can be incorporated as amorphous clusters or in between imperfections of the lipid matrix created in the NLC structure, thus loading increases with increasing the number of voids within the matrix.[20]

Polymorphism is the ability of a compound to crystallize in more than one distinct crystalline species with

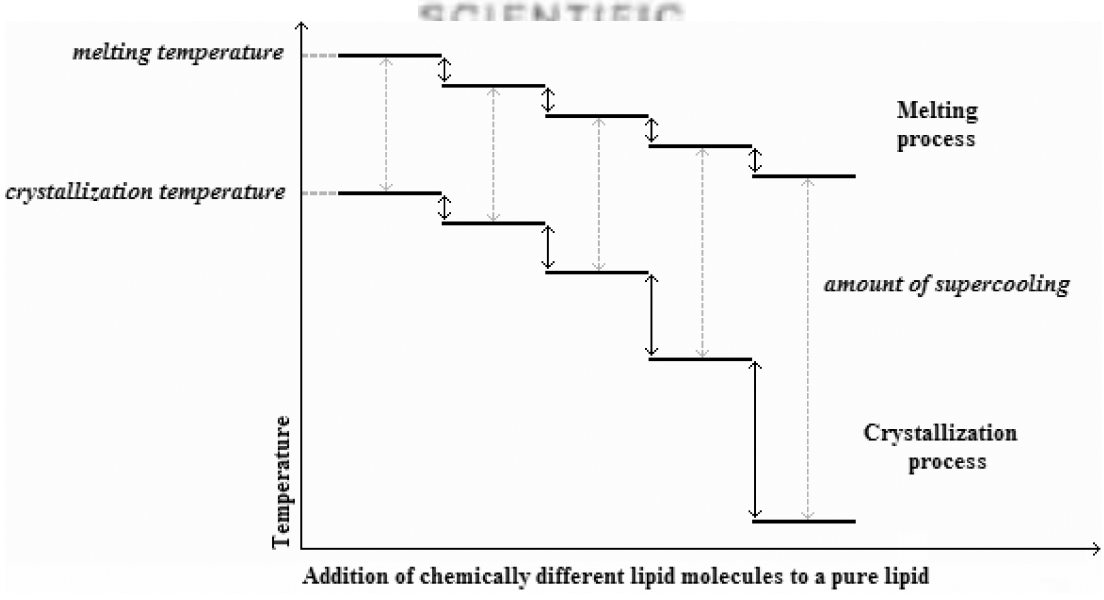

Fig. 3. Diagram describing the decrease of the melting temperature and crystallization temperature with the addition of very chemically different lipid molecules to a pure lipid.

different internal lattice structures. The internal structure of the lipid matrix can occur in a variety of three-dimensional structures, e.g., hexagonal (α), orthorhombic perpendicular (β') and triclinic parallel (β) are typical forms of triacylglycerols.[105]

Crystallization of bulk triacylglycerols from the melt after rapid cooling usually occurs in the less stable α form, which transforms via the β', into the more stable β form, upon heating or during storage time. In SLN/NLC dispersions, these transformations are faster than in the bulk material, which lead to a change in the relative fraction of the polymorphic forms.[103, 104] Depending on the chemical nature of the lipid and the production parameters, different fractions of α and β' forms can occur. This phenomenon can lead to a reduction of the melting temperature of the lipid. These polymorphic forms are not stable for longer periods of time, leading to a gradual transformation to more stable forms, which means increasing the content of β'/β_i and finally β. This phenomenon is not desired once it is responsible for drug expulsion during storage and changes in the release profile of incorporated drug, as well as in particle size parameters. Polymorphic transformations during storage time can be retarded when using co-surfactant molecules of high mobility to stabilise the system.

2.3.3. Gelation Phenomenon

The state of crystallinity of SLN/NLC will influence the physical stability of the dispersions,[95, 100] i.e., particle growth, particle aggregation and gel formation can happen due to polymorphic transformations which occur from highly energetic forms α to lower energetic forms β. The particle growth is accompanied by shape transformation, i.e., from spherical (α, hexagonal), to spheroid (β', orthorhombic), and finally to a platelet-like shape (β, triclinic). These changes in shape result in an increased surface area of each particle, and thus, particle aggregation. The next step after particle aggregation is the increase of viscosity and the formation of soft and finally relatively rigid gels. In order to avoid particle aggregation one interesting approach, especially concerning the development of topical formulations, is the entrapment of lipid nanoparticles in creams and hydrogels.[36]

The risk of gelation can also be due to the contact of the aqueous dispersion with odd-shaped surfaces, such as syringe needle used during particle size measurements, the increase of external shear forces, i.e., mechanical stress, during sample manipulation and transport, the exposure to high temperatures, light and oxidizing atmospheres, and due to formulations prepared with high lipid concentrations and high ionic strengths.[100]

To minimize the risk of gelation it is aimed to increase the physicochemical stability of the dispersions. Gel formation can be retarded or avoided by addition of co-emulsifying surfactants with high mobility.[49, 106] Storage at temperatures lower than at room temperature (25 °C), under dark conditions and nitrogen atmosphere can prevent particle growth.[107] When optimized formulations are developed and stored under protective conditions gelation phenomenon is unlikely to occur.

2.4. Production of Topical Formulations

Of all topical formulations, the most characteristic and common are the semi-solids because they are easy to apply. In addition, they are exploited as vehicles for a wide range of drugs in solution or suspension (with little tendency to separation), whether intended solely for superficial activity or for skin penetration. Spreading onto the skin may be controlled by adjusting the viscosity either for ease of application over a wide area or to confine the formulation to a circumscribed region. Semi-solids are devoid of mechanical irritant effect manifested by many particulate solids and, as they restrict the evaporation of moisture to a greater or less extent, they are smoothing to dry, fissured or inflamed skin (this property is retained longer with semi-solids than with liquids).

Aqueous SLN/NLC dispersions are usually produced with a lipid content of 20–30%, showing a low consistency, e.g., viscosity is usually 100 mPas.[108] To develop a suitable formulation for cutaneous application several approaches can be performed, e.g., incorporation of these carriers into hydrophilic bases, hydrophobic bases, or the production of lipid nanoparticle gels or creams.

2.4.1. Incorporation of Lipid Nanoparticles into Hydrophilic Bases

Aqueous SLN/NLC dispersions can be used as viscosity enhancers in topical hydrophilic formulations.[62, 68, 109] Hydrophilic bases include single phase systems (hydrogels) and biphasic systems (o/w creams and lotions). The hydrogels are aqueous solutions presented in semi-solid form as a gel, produced by the use of a protective colloid in high concentration. Examples are polyacrylates[62, 68] and hydroxyethylcellulose derivatives.[110] Whilst similar in consistency to the anhydrous o/w emulsifying bases, the hydrogels differ in having substantial water content and thereby exert a distinct cooling effect. The incorporation of lipid nanoparticles into hydrogels is obtained by mixing the gel components, and a concentrated nanoparticle dispersion is added before starting the gelation process. However, electrolytes can destabilize the lipid nanoparticles thus in case of preparation of polyacrylate gels it is recommended to use neutralising agents such as Tristan® (tromethamine) and Neutrol®TE (N,N,N,N-tetra(2-hydroxypropyl)ethylenediamine),[98] instead of sodium hydroxide, particularly previously to addition of lipid nanoparticles. Otherwise, the zeta potential (surface electric charge) of particles is reduced too strongly leading to aggregation.

To produce o/w formulations the lipid nanoparticles are added as a highly concentrated dispersion, i.e., with 50% solid content. This concentration is enough to create an occlusive effect[22] or to load a cream with chemically unstable compounds which are stabilized by incorporation in SLN/NLC.[98] Addition of lipid nanoparticles to creams can be performed during or after the production of the o/w cream. In the first case, a part of the water in the cream formulation is replaced by a highly concentrated dispersion, then the production process is run as usual. The lipid nanoparticles are sufficiently stabilized avoiding their coalescence with the oil droplets of the emulsion. If the production process of the emulsion is performed at a temperature higher than the melting point of the lipid nanoparticles, the latter will melt but will recrystallize during the cooling at the end of the process. In the second case, the cream is produced as usual, however with reduced water content in order to compensate the water added with the lipid nanoparticle dispersion. After production of the cream, the concentrated dispersion is admixed by stirring at $\geq$30 °C or at room temperature. This process avoids the melting of the nanoparticles, which may lead to an undesired change of the internal particle structure.

When lipid nanoparticles are dispersed in a gel network or in an o/w cream a three dimensional system similar to that of Figure 4 will be obtained. Figure 4 depicts a hydrogel network that encloses and it is interpenetrated by the lipid nanoparticles. Drug can be delivered entrapped into lipid nanoparticles, dissolved in the gel or in both. If the drug is present in both phases (i.e., lipid particles and gel network) a supersaturated system can be developed.[36] The principle of supersaturation has been exploited for topical microemulsions to increase drug penetration into the skin to the tissues underneath the stratum corneum. When applying microemulsions saturated with drug onto the skin, the water from the skin diffuses into the microemulsion increasing its water content. The increase in saturation solubility will lead to an increased diffusion pressure of the drug into the skin.[20] Similar systems can be developed when drug-loaded lipid nanoparticles are dispersed into semi-solid bases already saturated with drug. During storage, the drug remains in the lipid matrix because these particles preserve their modification. After application onto the skin, the increase in temperature and water loss observed leads to transformation to a more ordered lipid modification and are responsible for drug expulsion from the lipid matrix. The drug is expelled within the semi-solid vehicle (hydrogel or hydrophilic cream) already saturated with drug, leading to supersaturation and drug penetration onto the skin.[36]

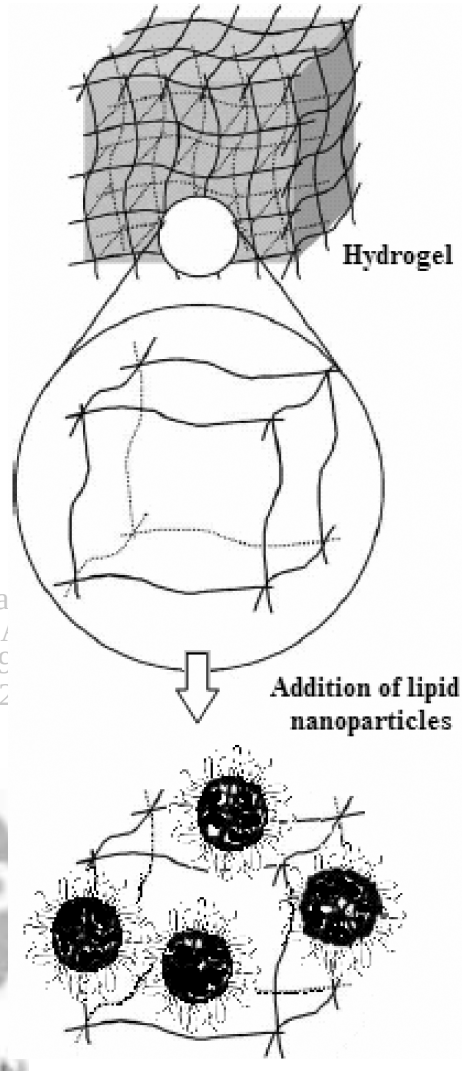

Fig. 4. Three-dimensional model network for the incorporation of lipid nanoparticles into hydrogels. Modified with permission from [36], E. B. Souto, SLN and NLC for Topical Delivery of Antifungals, Ph.D. Thesis (**2005**). © 2005, Institute of Pharmacy, Free University of Berlin, Germany.

2.4.2. Incorporation of Lipid Nanoparticles into Hydrophobic Bases

Hydrophobic bases such as paraffin, vegetable oils, and fatty alcohols can be used to disperse lipid nanoparticles to form w/o systems. These are suitable if the aim is to obtain only a topical effect avoiding systemic exposure of the drug. Being the lipid nanoparticles oily "soluble" constituents, when dispersed into hydrophobic bases skin penetration will be reduced in comparison to o/w systems. Moisture vapour transmission occurs more rapidly from w/o systems than when using only the hydrophobic bases. However, the former are sufficiently occlusive to have good emollient action. Hydrophobic bases may be characterized in terms of their composition, which usually includes a w/o emulsifying agent along with a lipophilic diluent. No aqueous phase is present except that added by admixture with lipid nanoparticle dispersions. Therefore, it would be feasible to use any w/o emulsifying agent, e.g., cholesterol and white wax. Although a w/o system will take up moderate quantities of water, it is not miscible

with a large excess; residues on the skin will not easily be removed with water alone and these bases are therefore not washable. The main advantage of lipid nanoparticles dispersed in hydrophobic bases is the reduction of their fatty character while providing sufficient occlusion without intensive penetration of drugs.

2.4.3. Production of Lipid Nanoparticle Gels

Topical formulations can be produced consisting only of lipid nanoparticle dispersions. This is a relatively fast procedure showing the advantage that the release of drug is only controlled by the lipid matrix, and not by the structure of the dispersed vehicle as occurs when using hydrophilic or hydrophobic vehicles.

For the production of lipid nanoparticle gels, first the aqueous dispersion is prepared, preferentially as highly concentrated as possible to be diluted to the desired final concentration. Then the gelling agent is added, preferentially non-electrolyte agents, such as cellulose derivatives, to avoid particle aggregation.[98] For many gel preparations a lipid nanoparticle content of approximately 4–10% is high enough.

2.4.4. Production of Lipid Nanoparticle Creams

Suspensions of lipid nanoparticles with a high concentration of lipid phase resemble the topical creams, due to the increase in viscosity. When using SLN, cream-like systems can be obtained above a maximum concentration of 30% lipid phase, depending on the chemical composition of SLN and NLC. However, when using NLC dispersions, creamy formulations are still obtained when using concentrations up to 50 or 60%, and not (as expected) an ointment-like system.

The viscosity of the preparations increases with the lipid concentration, i.e., at about 40–50% the preparations are cream-like, above 50% they become paste-like and when going up to a solid content of 80 or 90% the preparations become definitely solid. The rheological analysis of these systems shows the occurrence of a yield value with the increase of solid content. This parameter corresponds to the stress value below which the formulation behaves as elastic solid. In practice, the yield value needs to be sufficiently low to allow removal from the packaging and sufficiently high to retain the formulation onto the skin area. For this reason formulations containing 80 or 90% of lipid phase cannot be used for topical application but these can be exploited for oral administration.[111]

For the production of these creams, first a stock suspension is prepared by HPH containing rather 40% lipid nanoparticles, followed by stepwise addition of melted lipid achieving a concentration of up to 50% of solid content. In the melted state, the preparation is still liquid and thus it has a relatively low viscosity. Under cooling the viscosity will decrease and the system becomes creamy. Creams composed of highly concentrated lipid nanoparticle dispersions show distinct advantages of production facilities and possibility to load NLC with higher amount of liquid lipids (oils). Recently, cream-like NLC loaded with 70% of fish oil have been developed by our group.

3. TOPICAL AND DERMATOLOGICAL APPLICATIONS OF SLN AND NLC

Topical and dermatological formulations are applied onto the skin to deliver drugs which, depending on the therapeutic purposes, can have effect immediately on the local tissues beneath the application site, in deeper regions but near the application site, or far away from the application site if they are intended to reach the systemic circulation. When moving down this way (epidermis → dermis → blood), drug delivery becomes increasingly more difficult. For example, to reach the circulation through the skin, the drug has to be delivered over longer periods of time and in appropriate amounts to achieve the pharmacological effect. Thus, penetration and absorption differ in that the former means that the drug applied onto the skin has made its way into the epithelial layer, possibly only into the outer layer or possibly deeper. Absorption means that the drug has penetrated to the dermis and has been taken into the fluid in the blood or lymphatic capillaries and thus distributed throughout the body.

Cutaneous delivery systems are applied onto the skin in layers which are so thin that compositional changes of the formulation following their application are substantial. By changing the way of formulating conventional topicals and dermatologicals by using SLN and NLC, an increased penetration of drugs onto the skin has been observed. This issue has been intensively studied using several physicochemically different drugs.[54, 55, 98, 112–115]

Advantages of SLN and NLC over the other nanoparticle vehicles for cutaneous applications are manifold,[19] e.g.,
(i) their solid matrix is able to stabilize chemically labile drugs against hydrolysis and/or oxidation;
(ii) after topical application a monolayered nanoparticle film enhances skin hydration and elasticity due to their occlusive properties; and also
(iii) the possibility of modulation of release profile of entrapped drugs.

Spin-labelled compounds have been incorporated into lipid nanoparticles to study the possible penetration of particles onto the skin.[116] With this study, it was found that the lipid nanoparticles diffuse into the hair follicle, precisely into the gap surrounding the hair. This study shows the suitability of lipid nanoparticles for the treatment of acne and androgenetic alopecia.

The lipid organization of the stratum corneum is highly responsible for the transport properties of the skin (Fig. 5). The polar head groups of the lipids are organized in layers with the non-polar chains pointed in opposite directions,

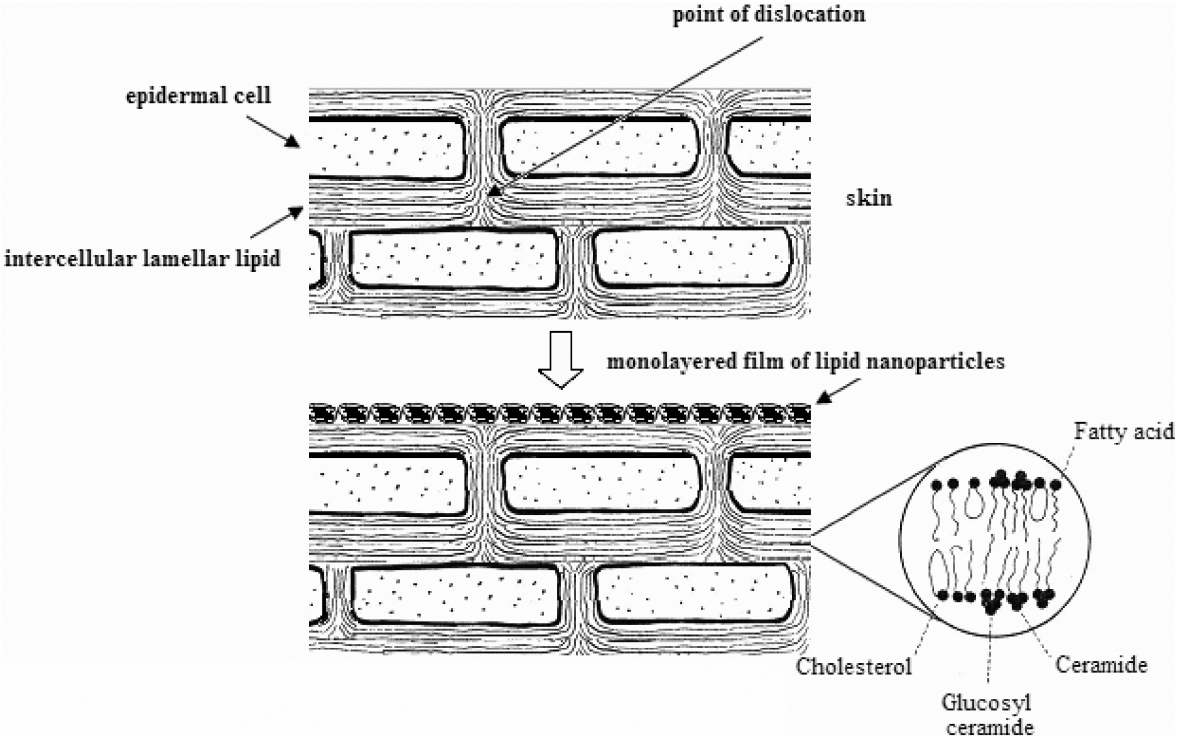

Fig. 5. Schematic representation of the layered structured of epidermal lipids and the monolayered film of lipid nanoparticles at the surface of the epidermal cells.

but not all the lipids are positioned with their polar groups localized in the polar layer. The diffusion in liquid crystals is highly anisotropic, i.e., it is not in the same direction. This layered structure is not a perfectly organized array of layers parallel to the skin' surface but, instead, a series of dislocations always occurs. If the particles are able to penetrate through the pores of the skin anisotropic diffusion of these particles can be assumed. The pores of the epidermis are of 10–70 μm size, thus lipid nanoparticles with a mean size of 200–400 nm might cross. It was found that liposomes can penetrate intact skin,[117] and that the decrease of particle size enhanced skin penetration.[118] This mechanism may be facilitated and enhanced due to the formation of a monolayered film of lipid nanoparticles at the surface of the skin, which exert an occlusive effect and thus increasing the skin hydration (Fig. 5).

The penetration profiles will depend on the chemical nature of the drug molecules, but they are intensively affected by the matrix structure of the particles. If assuming the existence of special interactions of the lipids of the particle matrix with the intercellular lamellar lipids of the epidermis, a pronounced effect on the penetration profile will be observed.

The matrix structure of lipid nanoparticles also affects the release rate of drug from SLN and NLC.[37] A burst release followed by a prolonged release profile rate has been described for NLC.[68] The SLN are composed solely of solid lipids thus a sustained released has been observed.[37] Burst release can be useful to improve the penetration of drugs, while a controlled release rate becomes important when it is necessary to supply the drug over a prolonged period of time, when it is necessary to reduce systemic absorption, and when the drug is irritating in high concentrations. The release of entrapped drugs from the lipid nanoparticles can thus be modulated according to the needs from very fast to very slow.

Figure 6 compares the release of clotrimazole obtained from SLN and NLC using Franz diffusion cells.[37] In NLC formulations lipid nanoparticles have a liquid core and clotrimazole is incorporated in the oil less tightly in comparison to the solid lipid matrix of SLN

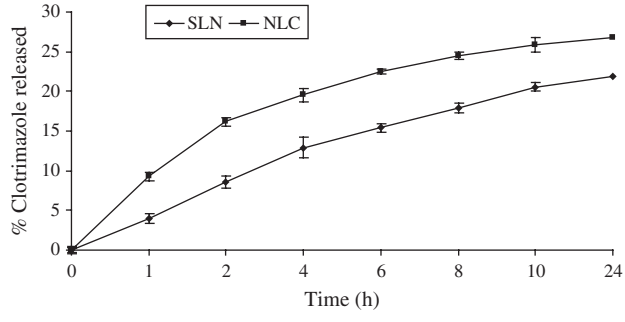

Fig. 6. Clotrimazole release profiles from tripalmitin based SLN and NLC. Modified with permission from [37], E. B. Souto et al., Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. *Int. J. Pharm.* 278, 71 (**2004**). © 2004, Elsevier.

formulations. In the latter, drug molecules are incorporated into the crystalline matrix and their diffusional mobility is decreased.

A comparison study has been performed to evaluate the delivery of a non-steroid anti-inflammatory drug (NSAID) from conventional hydrogels and from lipid nanoparticles. When applying drug-loaded lipid nanoparticles-based gels the anti-inflammatory effect was over two-fold higher than of conventional hydrogels.[62] An improved drug uptake has also been observed for the topical corticosteroid prednicarbate when delivered by means of SLN.[54] Due to their occlusive properties, SLN/NLC can increase skin penetration of corticosteroids. This is of major importance because these molecules are associated to severe side effects when applied topically. Skin atrophy is frequently reported and may be minimized if a lower dose of drug can be used maintaining the therapeutic efficiency.

4. TOPICAL TOLERABILITY/TOXICITY OF SLN AND NLC

Pharmaceutical particulate carriers intended for topical/dermatological applications should be biocompatible and physically stable. Local irritancy is one of the primary concerns associated with the drug and/or the delivery system. SLN and NLC are composed of biodegradable, well tolerated and physiological lipids, usually the same used in the pharmaceutical and cosmetic industry. The toxicity of excipients is also a major issue when developing a novel drug delivery system. Acylglycerols composed of fatty acids which are contained in oils of parenteral fat emulsions can be used for production of lipid nanoparticles. Thus, no toxic effects are expected from degradation products. Furthermore, due to the biodegradability and biocompatibility of the lipid materials only limited toxicity studies are expected to be required. Surfactants might also not be a problem once the majority of the used ones are also registered for cutaneous administration.

Therefore, cutaneous application of SLN/NLC reduces the risk of acute and chronic toxicity.[21] Valdecoxib-loaded SLN gel has shown no skin irritation on intact rabbit skin compared with the marketed valdecoxib-gel formulation which showed slight irritation at the end of 48 h.[68]

Due to the microbial flora of the skin' surface, enzymatic degradation is also likely to occur,[119] but the drug release can be modulated according to the therapeutic needs.

Recently citizen petition to FDA[120] raised some pertinent questions on the toxicity of nanoparticles. Claiming that nanomaterials possess fundamentally different properties and that making nanoparticles does not simply lead to an increase in compactness or refinement of the structure or properties, petitioners have called on FDA to develop an entirely new regulatory framework specifically designed for nanotechnology. In fact, nanoparticles exhibit numerous different fundamental properties, which create new risks that cannot be inferred from bulk material counterparts or the testing of them. Therefore, according to the petitioners, nanoparticles must be considered a new class of materials for regulation purposes. However, where topical formulations are concerned, only two sunscreen ingredients are singled out—titanium dioxide and zinc oxide—as evidence that nanoparticles are unsafe. Petitioners argue that these ingredients are dermally applied and absorbed, and therefore penetrate cells, tissue, and organs, presumably with toxic effects. Controversy remains since some studies provide evidence that titanium dioxide and zinc oxide do not penetrate the skin.[121, 122]

5. CONCLUSIONS

From outlined above lipid nanoparticles (SLN and NLC) appear to be suitable delivery systems intended for cutaneous administration of drugs. The incorporated drug can be released from the matrix into the skin using different diffusion mechanisms, either only from the lipid matrix, and either from both lipid and semi-solid vehicle. Physical and chemical properties of drug and lipid matrix components can be optimized to reach the desired release profile. Additionally, these systems are non-irritating and non-sensitizing, adhere onto the skin for a suitable time-length, and they are also manufacture reliable.

Promising examples of drugs to be formulated into semi-solid based SLN and NLC are the non-steroids anti-inflammatory drugs (NSAID) for local pain relief, topical anaesthetics and corticosteroids.

Several topical drugs require the use of organic solvents to improve drug solubility and skin penetration. However, a major disadvantage of this approach is the use of organic solvents to improve drug solubility and skin penetration with potential risk of skin irritation and/or allergic reactions. The formulation of drugs in SLN/NLC and the incorporation of the carriers in a semi-solid vehicle may improve the therapeutic efficiency of the drug, in addition to the reduction of the side-effect responses of irritating excipients.

LIST OF ABBREVIATIONS

AFM	atomic force microscopy
DSC	differential scanning calorimetry
EE	encapsulation efficiency
ESR	electron spin resonance
HPH	high pressure homogenization
LC	loading capacity
NLC	nanostructured lipid carriers
NMR	nuclear magnetic resonance
NSAID	non-steroids anti-inflammatory drugs
PI	polydispersity index
SEM	scanning electron microscopy
SLN	solid lipid nanoparticles
TEM	transmission electron microscopy

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