7 Delivery of Lipophilic Compounds with Lipid Nanoparticles — Applications in Dermatics and for Transdermal Therapy

Monika Schäfer-Korting and Wolfgang Mehnert

CONTENTS

7.1 Introduction 128
7.2 General Ingredients of SLN 129
7.3 Preparation of SLN 129
7.3.1 High-Shear Homogenization and Ultrasound 129
7.3.2 Microemulsion-Based SLN Preparations 129
7.3.3 SLN Prepared by Solvent Emulsification/Evaporation 129
7.3.4 High-Pressure Homogenization 130
7.3.5 Hot Homogenization 130
7.3.6 Cold Homogenization 130
7.4 Alternative Systems: Nanostructured Lipid Carriers 130
7.5 Physicochemical Characterization of Lipid Nanoparticles 131
7.5.1 Measurement of Particle Size, Particle Shape, and Zeta Potential 131
7.5.2 Determination of Crystallinity and Lipid Modifications 132
7.6 Drug Release 133
7.7 Storage Stability 134
7.8 Incorporation of Lipid Nanodispersions into Dermatics for Topical Use 135
7.9 Methods for Evaluating Cutaneous Uptake 135
7.10 Features of SLN Dispersions for Topical Use 136
7.10.1 Improved Chemical Stability 136
7.10.2 Occlusive Properties 136

0-8493-1692-8/05/$0.00+$1.50

7.10.3 Influence of Carrier Systems on Epidermal Input and Skin Permeation 136
7.10.4 Colloidal Carriers to Improve Eczema Therapy 137
7.10.5 Antiacne Drugs and Cosmetics 137
7.10.6 Transdermal Application of High–Molecular Weight Agents 138
7.10.7 Colloidal Carriers in Cosmetics 138
7.11 Outlook 139
References 139

7.1 INTRODUCTION

Human skin (cutis) consists of three different layers: the superficial stratum corneum (horny layer; about 10 µm), viable epidermis (50 to 100 µm), and dermis (1 to 2 mm). The outermost stratum corneum is a buildup of dead cells (corneocytes) surrounded by extremely hydrophobic epidermal lipids, mainly consisting of ceramides, cholesterol, and long-chain (predominantly C22 and C24) free fatty acids, but also cholesteryl sulfate. The water content is low (20%). In healthy skin, epidermal lipids form lamellae of orthorhombic packing but also a liquid phase and build up an efficient barrier against excessive water loss and harmful environmental effects (for review, see [1]). Hair follicles reaching from the dermis or even the subcutis to the skin surface are the most important appendages of human skin; the orifices of the hair follicles cover 0.1% of the surface. Hair follicles occur much more frequently in the skin of furry animals.

The horny layer also forms an efficient barrier against the uptake of drugs topically applied for skin diseases or intended for transdermal therapy. Drugs penetrate and permeate the skin via the intercellular pathway between the corneocytes, in addition to using the orifices of hair follicles. Hair follicles passing through the epidermis allow direct access of the xenobiotics to the blood vessels. Because the orifices cover only about 0.1% of the surface of human skin, the impact of the follicles on cutaneous uptake in general is limited [2], yet follicular uptake may be more relevant with rigid particulate carriers [3] with a size of 3 to 10 µm [2,4]. However, follicular uptake is neglected with respect to regulatory human studies [4].

Despite the less efficient barrier function resulting from many skin diseases (e.g., atopic eczema and psoriasis), piercing through the horny layer is still an important challenge in pharmaceutical technology. This is because conventional drug carrier systems such as creams and ointments result in drug uptake of only a small percentage. A rather high interindividual variation of uptake rates may be the consequence.

At present, research in the field of pharmaceutical technology focuses on micro- and nanoparticulate systems to improve or control uptake rates and induce drug targeting to the skin or even to its substructures, and thus allow for an improved benefit/risk ratio of drug therapy. In addition to innovative carrier systems, the formation of prodrugs facilitating the use of carrier systems may add to the improvement of topical therapy of skin diseases.

In the following text, we describe the production and characterization of solid lipid nanoparticles (SLN, Nanopearls) to be used in dermatotherapy and cosmetics. This is an innovative drug carrier system first developed for application via the

intravenous route. In recent years its suitability for other administration routes (e.g., peroral and transdermal application) has been intensively investigated. SLN are an alternative to polymer nanoparticles, liposomes, and nanoemulsions [5–10]. The solid state of the particle matrix should influence the fate of incorporated compounds. Chemically labile agents should be protected from degradation, and the release profile of drugs can be modulated.

7.2 GENERAL INGREDIENTS OF SLN

SLN for the topical application to the skin are made up from lipids such as glyceryl behenate (Compritol 888 ATO), glyceryl monostearate (Imwitor 900), glyceryl palmitostearate (Precirol ATO 5), triglycerides (trimyristin, tripalmitin, tristearin), or the wax cetyl palmitate. Nanodispersions contain 5 to 40% lipid; the higher-concentrated preparations have a semisolid appearance. These nanodispersions are cosmetically acceptable as they are, whereas the fluid nanodispersions with lower lipid content should be incorporated into, for example, a cream that facilitates the application.

Mean particle size ranges 50 to 1000 nm. Depending on the type and concentration of the lipid, 0.5 to 5% emulsifier (surfactant) has to be added for physical stabilization. For dermal use, surfactants are very often poloxamer 188, polysorbate 80, lecithin, tyloxapol, polyglycerol methylglucose distearate (Tegocare 450), sodium cocoamphoacetate (Miranol Ultra C32), or saccharose fatty acid ester.

7.3 PREPARATION OF SLN

7.3.1 High-Shear Homogenization and Ultrasound

Lipid microparticles and "nanopellets for oral use" were first described by Speiser [11]. Nanopellets are prepared by dispersing melted lipids with high-speed mixers or via ultrasound techniques. Lipospheres developed by Domb are also prepared from dispersed lipids by stirring and sonication [12]. These preparations may contain a high degree of microparticles, which thus excludes an intravenous injection. For other routes of application (e.g., peroral administration), these microparticles might not be a serious problem. Furthermore, the dispersions may be contaminated by metal shed. With optimized conditions, however, mean particles sizes of 100 to 200 nm are possible [13].

7.3.2 Microemulsion-Based SLN Preparations

Lipid particles can also be prepared by dispersing a hot microemulsion in cold water (2 to 3°C) under stirring. Drawbacks of this process are the frequent need for organic solvents and the relative low particle concentration as a result of the dilution with water [14].

7.3.3 SLN Prepared by Solvent Emulsification/Evaporation

Moreover, nanoparticles can be prepared by precipitation in oil/water (o/w) emulsions [15]. Lipids dissolved in a water-immiscible organic solvent (e.g., cyclohexane) are

emulsified in water. Through evaporation of the solvent, lipid nanoparticles precipitate in the remaining aqueous phase. Depending on the surfactant, mean particle sizes vary from 25 to 100 nm. Although a heating step is avoided, which protects thermolabile drugs, the need for organic solvents is disadvantageous.

7.3.4 High-Pressure Homogenization

Organic solvents are avoidable for the production of SLN if high-pressure homogenization is used. Because of the production conditions, these dispersions are characterized by an average particle size below 500 nm and a low microparticle content. The drug has to be dissolved or dispersed in the melted lipid, and lipids sufficiently dissolving the drug are suitable for nanoparticles prepared according to this technique.

7.3.5 Hot Homogenization

The drug dissolved or dispersed in the melted lipid is poured into an aqueous emulsifier phase of the same temperature. By means of a rotor-stator homogenizer (e.g., an Ultra-Turrax), an o/w preemulsion is prepared and is then homogenized at high pressure and at a temperature at least 10°C above the melting point of the lipid. In most cases, nanoemulsion arises after only three to five homogenization cycles at 500 bar. Nanoparticles are formed by cooling the nanoemulsion to room temperature.

7.3.6 Cold Homogenization

The drawback of hot homogenization is that hydrophilic drugs may diffuse into the aqueous phase during the dispersion step. Moreover, labile drugs may degrade as a result of the thermal load. With these drugs, however, nanoparticles may be prepared by cold homogenization, which includes a very rapid cooling step of the drug–lipid melt that is then milled at reduced temperature. The resulting microparticles of about 50 to 100 μm are suspended in a cold emulsifier solution. The microparticle suspension is subjected to high-pressure homogenization at or below room temperature. In general, larger particle sizes and a broader size distribution are observed in cold homogenized samples than in hot homogenization. A common feature of these dispersing techniques is that mean particle size and particle size distribution depend not only on production procedures but also on the type and amount of lipid as well as on the emulsifier.

7.4 ALTERNATIVE SYSTEMS: NANOSTRUCTURED LIPID CARRIERS

A major drawback of SLN is the frequently low drug incorporation. In addition to the physicochemical features of the drug, the composition of the lipid influences the capacity for drug incorporation. Because of the limited space in the forming crystal lattice, drug expulsion from the matrix during lipid crystallization may occur. This is most relevant with homogenous lipids, which form lattices almost free of any imperfections (the lack of which impedes drug incorporation). In contrast, mixed

lipids — such as mixtures of mono-, di-, and triglycerides or polyacid triglycerides — form less densely packed lattices, which should favor drug incorporation. Numbers of imperfections should increase also in mixtures of lipids that are solid and liquid at room temperature. Moreover, drug solubility is often higher in an oil phase. On the basis of these considerations, nanostructured lipid carriers (NLC) recently have been developed [10]. Three different types of NLC are discussed here: imperfect crystal type NLC (type I), solid amorphous NLC (type II), and multiple NLC (type III).

Drug incorporation increases with type I and type II NLC because of a reduced or lacking crystallinity, whereas drugs dissolve in oily nanocompartments located in the solid matrix of type III particles. The distinct advantage of NLC compared with SLN should be the higher drug loading capacity.

7.5 PHYSICOCHEMICAL CHARACTERIZATION OF LIPID NANOPARTICLES

Lipid nanodispersions (SLN and NLC) are complex, thermodynamically unstable systems. The colloidal size of the particles alters physical features (e.g., increasing solubility and the tendency to form supercooled melts). The complex structured lipid matrix may include liquid phases and various lipid modifications that differ in the capacity to incorporate drugs. Lipid molecules of variant modifications may differ in their mobility. Moreover, the high amount of emulsifier used may result in liposome or micelle formation in addition to the nanoparticles.

Therefore, extensive characterization is required, as the physicochemical properties of lipid nanodispersions influence not only drug incorporation and release but also the physical stability of the preparation: for example, drug localization in the matrix. Several methods have to be combined for characterization to allow detection of dynamic processes such as changes in lipid modifications, particle aggregation, and the formation of nanostructures of other kinds.

7.5.1 Measurement of Particle Size, Particle Shape, and Zeta Potential

Both photon correlation spectroscopy and laser diffraction are used for the routine measurement of particle size. Because photon correlation spectroscopy measures particle sizes of about 3 nm to 3 μm, it is most suitable to characterizing nanoparticles. Larger particles, however, are to be detected only by laser diffraction, which covers a broader range of particle size (40 nm to 2000 μm). Difficulties arise with dispersions containing populations of different size. Furthermore, uncertainties may result from nonspherical particle shapes. Platelet structures commonly occur during lipid crystallization and have also been suggested for lipid nanoparticles [6]. Therefore, additional procedures have to be used. Light microscopy allows a rapid detection of microparticles. In addition, it is possible to differentiate between microparticles of unit form and microparticles emerging from an aggregation of nanoparticles. Particle shapes are shown by electron microscopy [16], yet the investigator has to

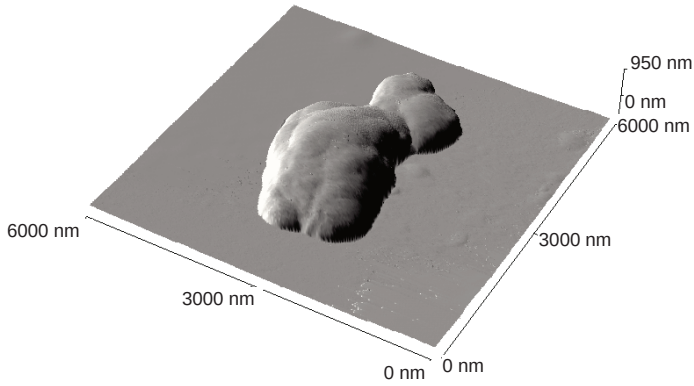

FIGURE 7.1 Atomic force microscopy image of prednisolone-loaded Compritol nanoparticles produced by cold homogenization. Imaging was performed by using the noncontact mode. The formulation is composed of 5% Compritol, 1% prednisolone, 2.5% poloxamer 188, and 92.5% water. (From zur Mühlen, A. and Mehnert, W., *Pharmazie,* 53, 552–55, 1998. With permission.)

be aware of possible artifacts caused by the sample preparation. Atomic force microscopy (Figure 7.1) is another increasingly important technique to depict particle morphology and surface features [17–19]. Quantification of the zeta potential allows us to predict physical stability of a colloidal dispersion. Because of electrical repulsion, aggregation is less likely to occur when the zeta potential exceeds –30 mV.

7.5.2 Determination of Crystallinity and Lipid Modifications

In addition to particle size, the degree of crystallinity and the modification of the lipid are of relevance for drug incorporation and release. Lipid crystallization and a change of the modification can be delayed with very small particles and in the presence of emulsifiers [20,21].

Differential scanning calorimetry and x-ray scattering are well established as means to characterize the crystallinity of lipids [22,23]. Differential scanning calorimetry is based on the fact that different lipid modifications possess different melting points and melting enthalpies. X-ray scattering allows us to determine the length of the long and short spacing of the lipid lattices as well as the influence of storage conditions (temperature, time) on this parameter. The colloidal size of the particles and the addition of emulsifiers may change the features of the nanodispersion considerably as compared to the bulk lipid. Characteristics of lipid particles are influenced by surface-related phenomena and lipid–surfactant interactions [24]. As compared to thermodynamically stable modifications, lipid molecules of unstable modifications are of higher mobility. Because of the lower packing density of the lipid molecules, guest molecules (e.g., drugs) can be incorporated more easily. During storage, unstable lipid modifications may change to their thermodynamically

more stable form, possibly leading to an expulsion of the incorporated drug. These processes can follow water evaporation and may result in an increased cutaneous penetration if a lipid nanodispersion is applied to the surface of skin [25].

To detect dynamic features of colloidal preparations, additional methods are required. Nuclear magnetic resonance spectroscopy allows a rapid, repeatable, and noninvasive measurement of the physical parameters of lipid matrices without sample preparation (e.g., dilution of the probe) [26,27]. Decreased lipid mobility results in a remarkable broadening of the signals of lipid protons, which allows the differentiation of SLN and supercooled melts. Because of the different chemical shifts, it is possible to attribute the nuclear magnetic resonance signal to particular molecules or their segments.

Less frequently used at present is electron spin resonance spectroscopy, which is based on the use of spin probes as model compounds or covalent spin labeling of drugs. Microviscosity and micropolarity of the molecular environment of the probe can be derived from electron spin resonance spectra. Moreover, the spectra allow us to differentiate isotropic and anisotropic movements, which result from the incorporation of the probe into liposomal structures. Quantitative distribution of the spin probes between the internal lipid layer, the surfactant, and the external water phase is to be determined noninvasively. On the basis of the chemical degradation of drugs released from the lipid compartment, agents with reductive features (e.g., ascorbic acid) allow us to measure the exchange rate of the drugs between lipophilic compartments and the water phase [27,28].

7.6 DRUG RELEASE

Drug release from SLN has been evaluated using local anesthetics and steroids as model drugs. Tetracaine and etomidate have been released spontaneously from the solid lipid matrix [29], and a release over 6 weeks has been observed with prednisolone [30]. The preparation (cold and hot homogenization) and the ingredients of the nanodispersion (lipid, emulsifier) influence the release profile. As shown with prednisolone, emulsifier and higher temperature–enhancing drug solubility in the aqueous phase favor the enrichment of the steroid in superficial layers of the nanoparticles during cooling of the preparation and crystallization of the lipid. Superficially entrapped prednisolone is available for the initial burst release (Figure 7.2). The solubility of the drug determines whether the crystallization of the melt results in a monolithic solid solution or a solid dispersion containing the drug in a homogenous distribution or in clusters. Depending on the melting point of the lipid and the drug, as well as its solubility and concentration, the compound may be enriched in the core or in the more outer layers of the particle. A drug-enriched core will be formed if the drug precipitates before the lipid recrystallizes. In summary, the particle structure obtained (Figure 7.3) is a function of the ingredients and the production conditions.

The interpretation of *in vitro* drug release profiles also has to take the specific *in vivo* environment into account. Then a possible enzymatic degradation of lipid particles may be influenced to a relevant extent by the composition of the particles [31].

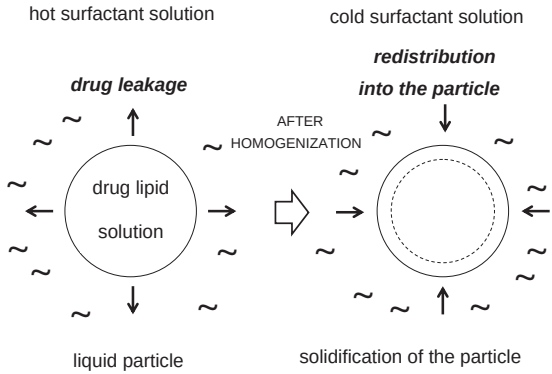

FIGURE 7.2 Distribution processes of drug, occurring during and after the production of prednisolone-loaded SLN, applying the hot homogenization. During the production process, the drug dissolved in the hot aqueous phase. After the production, the solubility in the aqueous phase decreases and the drug is enriched in the outer particle shell. (From zur Mühlen, A. and Mehnert, W., *Pharmazie,* 53, 552–55, 1998. With permission.)

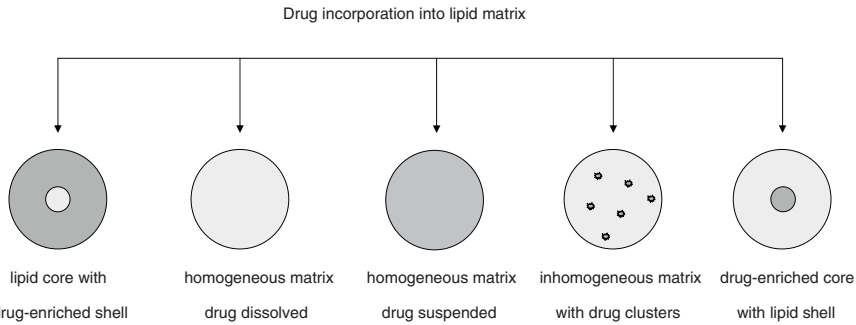

FIGURE 7.3 Models of drug incorporation into solid lipid nanoparticles.

7.7 STORAGE STABILITY

Although nanoparticles and nanoemulsions are closely related with respect to ingredients and production method, SLN should not be regarded just as colloidal lipid dispersions of solidified droplets. Both drug carrier systems may contain additional colloidal structures, such as micelles, mixed micelles, liposomes, or drug nanoparticles, that can influence the physical stability of the lipid dispersion. Other colloidal structures may make up additional distribution compartments for the drug that compete with the intended carrier system. With respect to SLN, the situation is even more complex, as supercooled melts, different lipid modifications, and nonspherical particle shapes may also occur and influence the stability of the colloidal dispersion. Moreover, particle aggregation and gelation of the dispersion are frequently found

with drug-loaded and drug-free SLN preparations. Gelation appears to depend on exposure to light, temperature, and air as well as on density of particle and electrolyte concentration. The reason for this unwanted gelation process, however, is not fully understood yet [32]. Finally, the amount of the lipid in crystalline form and changes in lipid modifications influence physical stability [33–35].

7.8 INCORPORATION OF LIPID NANODISPERSIONS INTO DERMATICS FOR TOPICAL USE

The generally low lipid content and the poor viscosity of lipid nanodispersions make these preparations, as they are, less suitable for dermal drug application. The handling of the preparation by the patient is improved by SLN incorporation into ointments, creams, and gels. Alternatively, ready-to-use preparations may be obtained by one-step production, increasing the lipid phase to at least 30%. However, increasing the lipid frequently results in an unwanted increase in particle size. Surprisingly, it has been found that very concentrated (30 to 40%) semisolid cetyl palmitate formulations preserve the colloidal particle size [10].

If SLN are incorporated into vehicles, interactions with the vehicle constituents may induce physical instabilities such as dissolution or aggregation of lipid particles. Therefore, during storage, particle sizes and the solid character of the particles have to be followed.

7.9 METHODS FOR EVALUATING CUTANEOUS UPTAKE

Because the measurement of clinical efficiency is very expensive and time consuming, less laborious procedures have been tested for comparing multiple formulations of a drug. With respect to topical dermatics, plasma levels are not to be followed because they are generally very low and the drug passes through the target organ skin before entering the blood stream. Alternatives to be used in humans include the tape-stripping procedure to determine drug levels in the horny layer, which has to be passed through by any topical dermatic for the drug to become active. Drug levels in the horny layer are reported to parallel drug levels in the viable epidermis and dermis. This way, bioequivalence can be determined with many like topical formulations (e.g., cream vs. cream). Glucocorticoid formulations are compared by the blanching assay, based on the surrogate parameter vasoconstriction, which is closely correlated with efficiency (for review, see [4]). The test protocol is given by a Food and Drug Administration guidance document released in 1995 [36].

For *in vitro* testing the Organisation for Economic Cooperation and Development (OECD) approved in 2004 test guideline 428 [37], which currently advocates the use of human, rat, and pig skin to measure cutaneous absorption by a vertical diffusion system (Franz cell). Drug concentrations are followed in an acceptor fluid separated by the skin from the donor vehicle, which is applied to the external surface of the skin. Instead of human or animal skin, human skin models could be used as soon as the equivalence of their results are proven. Comparative studies indicate a correlation of penetration data *in vitro* and in humans [38].

7.10 FEATURES OF SLN DISPERSIONS FOR TOPICAL USE

7.10.1 Improved Chemical Stability

The solid matrix of SLN protects the drug from hydrolysis and oxidation. Chemical stability of tocopherol and retinol improves considerably [17,39], with tocopherol improving by 57% compared with an aqueous dispersion. The degree of retinol stabilization depends on the nature of lipid and surfactant [39]. For each drug, the optimal preparation has to be defined individually.

7.10.2 Occlusive Properties

Following the evaporation of water from the lipid nanodispersion applied to the skin surface, lipid particles form an adhesive layer, applying occlusion to the surface [17,40]. Therefore, the hydration of the stratum corneum may increase, which can facilitate drug penetration into deeper skin strata and even systemic availability of the drug. Occlusive effects are strongly related to particle size. Nanoparticles have turned out 15-fold more occlusive than microparticles [17], and particles smaller than 400 nm in a dispersion containing at least 35% high-crystallinity lipid proved to be most potent [41].

7.10.3 Influence of Carrier Systems on Epidermal Input and Skin Permeation

At present, *in vitro* and *in vivo* studies with SLN-based dermatics are clearly lower in number than studies with liposome applications to the skin. Therefore, to describe particle effects, liposome preparations also will be referred to in the following text. Investigations into the mode of cutaneous uptake indicate that liposomal drug application, in general, results in the use of the transepidermal pathway [1,2]. Other studies, however, indicate the follicular pathway [42,43] to be more relevant than the minor coverage of skin surface by the hair follicle orifices indicates. High lipophilicity apparently promotes follicular deposition [44]. Liquid-state vesicles pertubating the lipid organization in the deeper horny layer are more effective than gel-state liposomes in increasing skin permeation. The most elastic Transfersomes appear especially active with respect to skin permeation — even with peptides and proteins. Obviously interacting with the more superficial horny layer, gel-state particles appear to inhibit permeation, whereas liposome size and lamellarity appear less relevant (for review, see [1]).

Because of the rigid nature of SLN, cutaneous drug uptake should be closer to the cutaneous penetration of drugs from gel-state liposomes. Indeed, an increase in glucocorticoid uptake over a conventional cream and a relative increase in drug concentrations in human stratum corneum plus epidermis as compared to the dermis has been reported [45]. This fits nicely with the interaction of skin and rigid liposomes. The improved uptake may result from occlusive effects, as described. Moreover, the small particle size and the intense dispersion of the drug with the particle or drug adsorption to the particle surface favor the contact of the active agent with the skin surface. This may be of relevance, too.

7.10.4 Colloidal Carriers to Improve Eczema Therapy

Lipid-based carrier systems have been investigated to improve treatment of inflammatory skin diseases such as atopic eczema and psoriasis by glucocorticoids and T-cell inhibitors such as ciclosporin and tacrolimus.

Indeed, glucocorticoids were the first drugs tested as liposomal preparations for cutaneous application [46,47]. The improved uptake of liquid-state liposomes as compared to gel-state particles described above was observed with glucocorticoids, as well (for review, see [1]). Liposomes made up from epidermal lipids more efficiently delivered glucocorticoid to the deeper skin than did phospholipid-based vesicles [48]. We could demonstrate an improved prednicarbate uptake by human skin *in vitro* if the steroid is applied as a SLN dispersion or a cream containing prednicarbate-loaded SLN. Most importantly, a prednicarbate targeting to the epidermis occurred [45]. This is most relevant with respect to eczema treatment, as it is the glucocorticoid in the dermis that is most important for induction of skin atrophy. Complete regeneration of skin thickness after the application of potent conventional glucocorticoids takes time [49] and may not be complete before the next exacerbation of disease requires another glucocorticoid treatment [50]. Because targeting is not seen with drug-free SLN added to prednicarbate cream, particle association of the drug is an essential feature [45]. Further clinical studies in patients with repeated eczema eruptions have to show whether the SLN-improved kinetics of prednicarbate and possibly other topical glucocorticoids transfers into benefits for the chronically ill patient. Despite the clearly lower atrophogenicity of prednicarbate, there is still place for a further increase in the benefit/risk ratio.

Although ciclosporin and tacrolimus applied systemically improve psoriatic lesions, they are clearly less active when applied topically. Therefore, liposomal preparations have been developed. Indeed, ciclosporin penetrates deeper strata of rodent and human cadaver skin more efficiently when incorporated into liposomes [51]. Moreover, tacrolimus concentrations in murine skin have increased ninefold, and skin graft survival prolonged, if the drug is liposome encapsulated [52]. This indicates that topical psoriasis therapy with tacrolimus may become possible. At present, topical tacrolimus is confined to the less recalcitrant forms of mild eczema.

7.10.5 Antiacne Drugs and Cosmetics

Liposome-encapsulated tretinoin has been tested in hairless mice as well as in man. The animal experiment has demonstrated the favorable uptake of the retinoid, whereas the liposomal lipids appear to be more retained in the horny layer [53]. Moreover, with phospholipid-based liposomes belonging to the gel-state type, tretinoin penetration in murine skin appears to be confined to the epidermis [54] and, thus, is close to prednicarbate penetration described above. In patients with acne vulgaris, we could demonstrate a better tolerability of liposomal tretinoin as compared to a commercial gel while efficiency remains the same [55].

Not infrequently, retinol is used for mild acne, and the more active retinoids such as retinoic acid, isotretinoin, and adapalene serve for the treatment of the more severe forms. Retinol incorporated into Compritol-based SLN has been released more rapidly and to a higher extent compared with conventional vehicles and with a

nanoemulsion [25,56]. This effect appears to result from a burst release from the solid particles following water evaporation on the skin surface, as well as from the change of lipid modification.

7.10.6 Transdermal Application of High–Molecular Weight Agents

Investigations by Yarosh over almost two decades have proven that liposomal carriers allow uptake of a DNA repair enzyme into the skin [57]. This uptake significantly reduces the number of new actinic keratoses and new lesions of basal cell carcinoma in patients with xeroderma pigmentosum who were treated for 12 months [58]. Moreover, in a mice model, transdermal vaccination by antigen incorporation into liposomes has also been demonstrated [59,60].

On the basis of the phagocytotic activity of human keratinocytes [61], liposomes also appear to be of interest with respect to gene therapy. Cationic lipids are especially interesting because they spontaneously form complexes with DNA. In hairless mice, skin liposomal lipids have been tested as a nonviral transfer system for gene therapy using the Franz cell system for application. Reporter genes could be expressed in viable epidermis [62]. Serious side effects (T-cell leukemia) of retroviral vectors possibly as a result of proto-oncogen activation [63,64] are of high relevance.

Therefore, liposomes, and also nanoparticles, may allow for the development of needle-less vaccination systems. Studies on mice inoculated with influenza DNA vaccine complexes with liposomes and SLN already demonstrated a clear T-cell (predominantly Th1-type) response. Therefore, the immune response appears to be mediated by Langerhans cells, which is the immune competent cell in the skin (for review, see [65]).

Particles from cationic lipids may also be useful for antisense therapy of skin disease — a nontoxic increase in the oligonucleotide uptake by cultivated keratinocytes and a sebocyte cell line has been reported [66]. Moreover, cationic dendrimers also efficiently transfer reporter gene DNA to human keratinocytes cultivated *in vitro*. In the skin of hairless mice, *in vivo* transfection was possible with complexes, yet reporter gene expression was localized to perifollicular areas. Transfection, however, failed with the naked plasmid. For prolonged contact, biodegradable membranes coated with dendrimer/DNA complexes were used [67]. This hints at a follicular uptake of these complexes and indicates that gene transfection also may be possible with human skin, which has a thicker stratum corneum compared with mouse skin (eight to ten vs. two to three layers [58]).

7.10.7 Colloidal Carriers in Cosmetics

Nonloaded and loaded SLN were already investigated with respect to use in cosmetics. Although adequate controls are difficult to prepare, first experiments indicate an increase in skin hydration and a reduction in wrinkle depth following SLN application [68]. Moreover, cetyl palmitate-nanodispersions act both as particulate ultraviolet (UV) blockers themselves and as carriers for UV absorbing agents (e.g., 2-hydroxy-4-methoxy benzophenone; Eusolex 4360). This results in a threefold

increase in UV protection, which allows a reduction in the concentration of the UV absorber. This is particularly important as UV absorbers are currently in discussion because of their possible estrogenic activity and long-term effects in the environment [69,70]. SLN may also be suitable for long-lasting perfume and insect repellent formulations (for review, see [10]). As with drugs, an improved uptake of cosmetic agents (Q_{10}, tocopherol) into the horny layer has been described. The relation of cutaneous penetration to particle size indicates that the increase is caused by an occlusive effect.

7.11 OUTLOOK

The efficiency of liposomes as drug carriers to the skin has been investigated for two decades. Until today, their limited physical stability is the major drawback of this system. SLN and NLC developed to overcome the stability problems of liposomes should result in a higher number of approved drugs with improved stability, high and constant cutaneous absorption, or even drug targeting to specific skin layers or appendages in the near future. Improved skin levels may be due to enhanced contact of drug and skin resulting from the large particle surface, film formation, and skin hydration. Improved uptake may also be due to the interaction of the particle lipids and surfactants with epidermal lipids or sebum. This interaction has to be evaluated in detail because these carrier systems should present remarkable therapeutic progress. Moreover, SLN and NLC may also favorably influence the effects of active compounds in cosmetics.

REFERENCES

[1] Bouwstra, J. A., et al., Structure of the skin barrier and its modulation by vesicular formulations. *Prog. Lipid Res.,* 42, 1–36, 2003.
[2] Barry, B. W., Drug delivery routes in skin: a novel approach. *Adv. Drug Deliv. Rev.,* 54 (Suppl. 1), S31–40, 2002.
[3] Hoffman, R. M., Topical liposome targeting of dyes, melanins, genes, and proteins selectively to hair follicles. *J. Drug Target.,* 5, 67–74, 1998.
[4] Shah, V. P., Progress in methodologies for evaluating bioequivalence of topical formulations. *Am. J. Clin. Dermatol.,* 2, 275–80, 2001.
[5] Müller, R. H. and Lucks, J. S., Arzneistoffträger aus festen Lipidteilchen, Feste Lipidnanosphären (SLN). European Patent, EP 0605497, 1996.
[6] Siekmann, B. and Westesen, K., Sub-micron sized parenteral carrier systems based on solid lipids. *Pharm. Pharmacol. Lett.,* 1, 123–26, 1992.
[7] Müller, R. H., et al., Solid lipid nanoparticles (SLN): an alternative colloidal drug carrier system for controlled drug delivery. *Eur. J. Pharm. Biopharm.,* 41, 1995.
[8] Müller, R. H., Mäder, K. and Gohla, S., Solid lipid nanoparticles (SLN) for controlled drug delivery: a review of the state of the art. *Eur. J. Pharm. Biopharm.,* 50, 161–77, 2000.
[9] Mehnert, W. and Mäder, K., Solid lipid nanoparticles: production, characterization and applications. *Adv. Drug Deliv. Rev.,* 47, 165–96, 2001.

[10] Müller, R. H., Radtke, M. and Wissing, S. A., Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Adv. Drug Deliv. Rev.,* 54 (Suppl. 1), S131–55, 2002.

[11] Speiser, P., Lipidnanopellets als Trägersystem für Arzneimittel zur peroralen Anwendung. European Patent, EP 0167825, 1990.

[12] Domb, A. J., Lipospheres for controlled delivery of substances. United States Patent, US 5,188,337, 1993.

[13] Ahlin, P., Kristl, J. and Smid-Kobar, J., Optimization of procedure parameters and physical stability of solid lipid nanoparticles in dispersions. *Acta Pharm.,* 48, 257–67, 1998.

[14] Gasco, M. R., Solid lipid nanospheres from warm microemulsions. *Pharm. Technol. Eur.,* 9, 52–58, 1997.

[15] Sjöström, B. and Bergenståhl, B., Preparation of submicron drug partides in lecithin-stabilized o/w emulsions. I. Model studies of the precipitation of cholesteryl acetate. *Int. J. Pharm.,* 88, 53–62, 1992.

[16] Siekmann, B. and Westesen, K., Electron-microscopic characterization of melt-homogenized solid lipid nanoparticles. *Eur. J. Pharm. Biopharm.,* 2, 190, 1994.

[17] Dingler, A., et al., Solid lipid nanoparticles (SLN/Lipopearls): a pharmaceutical and cosmetic carrier for the application of vitamin E in dermal products. *J. Microencapsul.,* 16, 751–67, 1999.

[18] Dubes, A., et al., Scanning electron microscopy and atomic force microscopy imaging of solid lipid nanoparticles derived from amphiphilic cyclodextrins. *Eur. J. Pharm. Biopharm.,* 55, 279–82, 2003.

[19] zur Mühlen, A., et al., Atomic force microscopy studies of solid lipid nanoparticles. *Pharm. Res.,* 13, 1411–16, 1996.

[20] Westesen, K. and Bunjes, H., Do nanoparticles prepared from lipids solid at room temperature always possess a solid lipid matrix? *Int. J. Pharm.,* 115, 129–31, 1995.

[21] Bunjes, H., Koch, M. H. J. and Westesen, K., Influence of emulsifiers on the crystallization of solid lipid nanoparticles. *J. Pharm. Sci.,* 92, 1509–20, 2003.

[22] Siekmann, B. and Westesen, K., Thermoanalysis of the recrystallization of melt-homogenized glyceride nanoparticles. *Colloids Surf. B.,* 3, 159–75, 1994.

[23] Laggner, P., X-ray diffraction of lipids, in: *Spectral properties of lipids*, Hamilton, R. J. and Cast, J., Eds., Sheffield Academic Press, Sheffield, 1999, 327–67.

[24] Unruh, T., et al., Observation of size-dependent melting in lipid nanoparticles. *J. Phys. Chem. B,* 103, 10373–77, 1999.

[25] Jenning, V., et al., Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin. *Eur. J. Pharm. Biopharm.,* 49, 211–18, 2000.

[26] Jenning, V., Mäder, K. and Gohla, S. H., Solid lipid nanoparticles (SLN) based on binary mixtures of liquid and solid lipids: a ^{1}H-NMR study. *Int. J. Pharm.,* 205, 15–21, 2000.

[27] Jores, K., Mehnert, W. and Mäder, K., Physicochemical investigations on solid lipid nanoparticles and on oil-loaded solid lipid nanoparticles: a nuclear magnetic resonance and electron spin resonance study. *Pharm. Res.,* 20, 1274–83, 2003.

[28] Ahlin, P., Kristl, J. and Sentjurc, M., EPR study of loading capacity of spin-labelled lipophilic substance in different SLN. *Proc. Int. Symp. Control. Release Bioact. Mater.,* 25, 334–35, 1998.

[29] zur Mühlen, A., Schwarz, C. and Mehnert, W., Solid lipid nanoparticles (SLN) for controlled drug delivery: drug release and release mechanism. *Eur. J. Pharm. Biopharm.,* 45, 149–55, 1998.

[30] zur Mühlen, A. and Mehnert, W., Drug release and release mechanism of prednisolone loaded solid lipid nanoparticles. *Pharmazie,* 53, 552–55, 1998.
[31] Olbrich, C. and Müller, R. H., Enzymatic degradation of SLN-effect of surfactant and surfactant mixtures. *Int. J. Pharm.,* 180, 31–39, 1999.
[32] Freitas, C. and Müller, R. H., Stability determination of solid lipid nanoparticles (SLN) in aqueous dispersion after addition of electrolyte. *J. Microencapsul.,* 16, 59–71, 1999.
[33] Bunjes, H., Westesen, K. and Koch, M. H. J., Crystallization tendency and polymorphic transitions in triglyceride nanoparticles. *Int. J. Pharm.,* 129, 159–73, 1996.
[34] Freitas, C. and Müller, R. H., Correlation between long-term stability of solid lipid nanoparticles (SLN) and crystallinity of the lipid phase. *Eur. J. Pharm. Biopharm.,* 47, 125–32, 1999.
[35] Westesen, K. and Siekmann, B., Investigation of the gel formation of phospholipid-stabilized solid lipid nanoparticles. *Int. J. Pharm.,* 151, 35–45, 1997.
[36] FDA Guidance: Topical dermatologic corticosteroids: *in vivo* bioequivalence, available online at http://www.fda.gov/cder/guidance/old098fn.pdf, June 2, 1995.
[37] OECD, Skin absorption: *in vitro* method. OECD Test Guideline 428, Paris, 2004.
[38] Wagner, H., et al., Drug distribution in human skin using two different *in vitro* test systems: comparison with *in vivo* data. *Pharm. Res.,* 17, 1475–81, 2000.
[39] Jenning, V. and Gohla, S. H., Encapsulation of retinoids in solid lipid nanoparticles (SLN). *J. Microencapsul.,* 18, 149–58, 2001.
[40] de Vringer, T. and de Ronde, H. A. G., Preparation and structure of a water-in-oil cream containing lipid nanoparticles. *J. Pharm. Sci.,* 84, 466–72, 1995.
[41] Wissing, S. A., Lippacher, A. and Müller, R. H., Investigations on the occlusive properties of solid lipid nanoparticles (SLN). *J. Cosmet. Sci.,* 52, 313–24, 2001.
[42] Weiner, N., Targeted follicular delivery of macromolecules via liposomes. *Int. J. Pharm.,* 162, 29–38, 1998.
[43] Otberg, N., et al., Visualization of topically applied fluorescent dyes in hair follicles by laser scanning microscopy. *Laser Phys.,* 13, 761–64, 2003.
[44] Grams, Y. Y. and Bouwstra, J. A., Penetration and distribution of three lipophilic probes *in vitro* in human skin focusing on the hair follicle. *J. Control. Release,* 83, 253–62, 2002.
[45] Santos Maia, C., et al., Drug targeting by solid lipid nanoparticles for dermal use. *J. Drug Targeting,* 10, 489–95, 2002.
[46] Mezei, M. and Gulasekharam, V., Liposomes: a selective drug delivery system for the topical route of administration: gel dosage form. *J. Pharm. Pharmacol.,* 34, 473–74, 1982.
[47] Mezei, M. and Gulasekharam, V., Liposomes — a selective drug delivery system for the topical route of administration: lotion dosage form. *Life Sci.,* 26, 1473–77, 1980.
[48] Fresta, M. and Puglisi, G., Survival rate improvement in a rat ischemia model by long circulating liposomes containing cytidine-5I-diphosphate choline. *Life Sci.,* 61, 1227–35, 1997.
[49] Korting, H. C., Vieluf, D. and Kerscher, M., 0.25% prednicarbate cream and the corresponding vehicle induce less skin atrophy than 0.1% betamethasone-17-valerate cream and 0.05% clobetasol-17-propionate cream. *Eur. J. Clin. Pharmacol.,* 42, 159–61, 1992.
[50] Schäfer-Korting, M., Schmid, M. H. and Korting, H. C., Topical glucocorticoids with improved risk-benefit ratio: rationale of a new concept. *Drug Saf.,* 14, 375–85, 1996.
[51] Egbaria, K., Ramachandran, C. and Weiner, N., Topical application of liposomally entrapped cyclosporin evaluated by *in vitro* diffusion studies with human skin. *Skin Pharmacol.,* 4, 21–28, 1991.

[52] Erdogan, M., Wright, J. R., Jr. and McAlister, V. C., Liposomal tacrolimus lotion as a novel topical agent for treatment of immune-mediated skin disorders: experimental studies in a murine model. *Br. J. Dermatol.,* 146, 964–67, 2002.

[53] Masini, V., et al., Cutaneous bioavailability in hairless rats of tretinoin in liposomes or gel. *J. Pharm. Sci.,* 82, 17–21, 1993.

[54] Michel, C., et al., Penetration of spin-labeled retinoic acid from liposomal preparations into the skin of SKH1 hairless mice: measurement by EPR tomography. *Int. J. Pharm.,* 98, 131–39, 1993.

[55] Schäfer-Korting, M., Korting, H. C. and Ponce-Pöschl, E., Liposomal tretinoin for uncomplicated acne vulgaris. *Clin. Investig.,* 72, 1086–91, 1994.

[56] Jenning, V., Schäfer-Korting, M. and Gohla, S., Vitamin A-loaded solid lipid nanoparticles for topical use: drug release properties. *J. Control. Release,* 66, 115–26, 2000.

[57] Yarosh, D., et al., Effect of topically applied T4 endonuclease V in liposomes on skin cancer in xeroderma pigmentosum: a randomised study. *Lancet,* 357, 926–29, 2001.

[58] Yarosh, D. B., Liposomes in investigative dermatology. *Photodermatol. Photoimmunol. Photomed.,* 17, 203–12, 2001.

[59] Paul, A., Cevc, G. and Bachhawat, B. K., Transdermal immunisation with an integral membrane component, gap junction protein, by means of ultradeformable drug carriers, transfersomes. *Vaccine,* 16, 188–95, 1998.

[60] Baca-Estrada, M. E., et al., Effects of IL-12 on immune responses induced by transcutaneous immunization with antigens formulated in a novel lipid-based biphasic delivery system. *Vaccine,* 18, 1847–54, 2000.

[61] Schaller, M., Korting, H. C. and Schmid, M. H., Interaction of cultured human keratinocytes with liposomes encapsulating silver sulphadiazine: proof of the uptake of intact vesicles. *Br. J. Dermatol.,* 134, 445–50, 1996.

[62] Birchall, J. C., et al., Gene expression in an intact ex-vivo skin tissue model following percutaneous delivery of cationic liposome-plasmid DNA complexes. *Int. J. Pharm.,* 197, 233–38, 2000.

[63] Williams, D. A. and Baum, C., Medicine: gene therapy — new challenges ahead. *Science,* 302, 400–01, 2003.

[64] Hacein-Bey-Abina, S., et al., LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science,* 302, 415–19, 2003.

[65] Choi, M. J. and Maibach, H. I., Topical vaccination of DNA antigens: topical delivery of DNA antigens. *Skin Pharmacol. Appl. Skin Physiol.,* 16, 271–82, 2003.

[66] Fimmel, S., et al., Development of efficient transient transfection systems for introducing antisense oligonucleotides into human epithelial skin cells. *Horm. Res.,* 54, 306–11, 2000.

[67] Bielinska, A. U., et al., Application of membrane-based dendrimer/DNA complexes for solid phase transfection *in vitro* and *in vivo*. *Biomaterials,* 21, 877–87, 2000.

[68] Wissing, S. A. and Müller, R. H., The influence of solid lipid nanoparticles on skin hydration and viscoelasticity: *in vivo* study. *Eur. J. Pharm. Biopharm.,* 56, 67–72, 2003.

[69] Schreurs, R., et al., Estrogenic activity of UV filters determined by an *in vitro* reporter gene assay and an *in vivo* transgenic zebrafish assay. *Arch. Toxicol.,* 76, 257–61, 2002.

[70] Schlumpf, M., et al., *In vitro* and *in vivo* estrogenicity of UV screens. *Environ. Health Perspect.,* 109, 239–44, 2001.