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Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products

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ABSTRACT

Solid lipid nanoparticles (SLN) are distinguishable from nanostructured lipid carriers (NLC) by the composition of the solid particle matrix. Both are an alternative carrier system to liposomes and emulsions. This review paper focuses on lipid nanoparticles for dermal application. Production of lipid nanoparticles and final products containing lipid nanoparticles is feasible by well-established production methods. SLN and NLC exhibit many features for dermal application of cosmetics and pharmaceutics, i.e. controlled release of actives, drug targeting, occlusion and associated with it penetration enhancement and increase of skin hydration. Due to the production of lipid nanoparticles from physiological and/or biodegradable lipids, this carrier system exhibits an excellent tolerability. The lipid nanoparticles are a "nanosafe" carrier. Furthermore, an overview of the cosmetic products currently on the market is given and the improvement of the benefit/risk ratio of the topical therapy is shown.

Contents

1. Introduction

At the beginning of the 1990s there were only the research groups of Müller (Berlin, Germany), Gasco (Turin, Italy) and Westesen (Braunschweig, Germany) working on lipid nanoparticles. Currently more than 20 research groups are working on lipid nanoparticles world wide, estimated by the published articles. This proves the increasing interest in the field of lipid nanoparticles. Lipid nanoparticles have been investigated for various pharmaceutical applications, e.g. parenteral (Wissing et al., 2004a; Blasi et al., 2007; Bondi et al., 2007; Brioschi et al., 2007), peroral (Müller et al., 2006; Martins et al., 2007; Sarmento et al., 2007; Yuan et al., 2007), dermal (Müller et al., 2002; Priano et al., 2007), ocular (Ugazio et al., 2002; Attama and Müller-Goymann, 2008) and

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pulmonary (Xiang et al., 2007; Liu et al., 2008) administration. Moreover, since the last decade, they have been studied intensively for dermal application, both in pharmaceutical and cosmetic uses. This review paper provides an overview of the ongoing research in cosmetic and pharmaceutical dermal preparations containing SLN or NLC. The production technology of these lipid nanoparticles and preparation of lipid nanoparticles containing products for dermal application is presented. Furthermore, the excellent tolerability of these carriers for dermal application is outlined and discussed based on available data.

2. What exactly are lipid nanoparticles?

Solid lipid nanoparticles (SLN) were developed at the beginning of the 1990s as an alternative carrier system to emulsions, liposomes and polymeric nanoparticles. SLN are produced by replacing the liquid lipid (oil) of an o/w emulsion by a solid lipid or a blend of solid lipids, i.e. the lipid particle matrix being solid at both room and body temperature (Lucks and Müller, 1991). SLN are composed of 0.1% (w/w) to 30% (w/w) solid lipid dispersed in an aqueous medium and if necessary stabilized with preferably 0.5% (w/w) to 5% (w/w) surfactant. The incorporation of cosmetic and pharmaceutical actives is feasible. The mean particle size of SLN is in the submicron rage, ranging from about 40 to 1000 nm (Lucks and Müller, 1991).

In the second generation of the lipid nanoparticle technology, the particles are produced using blends of solid lipids and liquid lipids (oils). To obtain the blends for the particles matrix, solid lipids are mixed with liquid lipids (oils), preferably in a ratio of 70:30 up to a ratio of 99.9:0.1. Due to the oil in these mixtures a melting point depression compared to the pure solid lipid is observed, but the blends obtained are also solid at body temperature (Müller and Olbrich, 2000b). This second generation of nanoparticles is called nanostructured lipid carriers (NLC). The overall solid content of NLC could be increased up to 95% (Müller et al., 1999). These second generation of submicron particles can be loaded with cosmetic and pharmaceutical actives as well.

NLC were developed to overcome some potential limitations associated with SLN. Compared to SLN, NLC show a higher loading capacity for a number of active compounds, a lower water content of the particle suspension and avoid/minimize potential expulsion of active compounds during storage (Mehnert and Mäder, 2001).

SLN are produced from solid lipids only and after preparation at least a part of the particles crystallizes in a higher energy modification (α or β'). During storage, these modifications can transform to the low energy, more ordered β modification. Due to its high degree of order, the number of imperfections in the crystal lattice is reduced leading to drug expulsion. By creating a less ordered solid lipid matrix, i.e. by blending a solid lipid with a liquid lipid, a higher active load of the particles can be achieved. In general, the drug (or cosmetic active) can be located in between the fatty acid chains or in between the lipid layers and also in imperfections of the lipid matrix (e.g. amorphous drug clusters). In case of very similar lipid molecules, especially when highly purified monoacid glycerides are used, the drug loading is very limited and drug expulsion occurs within a short time due to the formation of the well ordered β modification (Fig. 1). Therefore, the production of NLC yields to an increase of the loading capacity of the active compounds in the particles and also avoids or minimizes the expulsion of the active compound during storage. SLN are dispersions having typically water contents of 70–99.9% which might lead to problems regarding the SLN content in a potential final topical formulation. NLC concentrates with higher lipid content can be produced, which simplifies the incorporation into a final product. An NLC concentrate (e.g. 40–50% solid contend (w/w)) is simply admixed to a dermal formulation produced with a reduced amount of water.

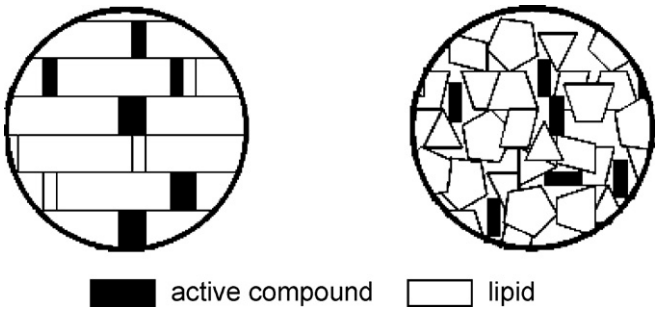

Fig. 1. Formation of an almost perfect crystalline structure in SLN (left) by identically shaped molecules similar to a brick wall with limited loading capacity for actives. Formation of a solid particle matrix of NLC (right) with many imperfections comparable to building a wall from very differently shaped stones, the increased number of imperfections leads to an increased loading capacity for active compounds (modified after Müller et al. (2002)).

3. Production and incorporation into creams

Many different techniques for the production of lipid nanoparticles have been described in the literature. These methods are high pressure homogenization (Liedtke et al., 2000; Mehnert and Mäder, 2001; Wissing et al., 2004a), microemulsion technique (Gasco, 1993, 1997; Priano et al., 2007), emulsification-solvent evaporation (Sjöström and Bergenstahl, 1992), emulsification-solvent diffusion method (Hu et al., 2002; Trotta et al., 2003), solvent injection (or solvent displacement) method (Schubert and Müller-Goymann, 2003), phase inversion (Heurtault et al., 2002), multiple emulsion technique (Garcý-Fuentes et al., 2002), ultrasonication (Pietkiewicz and Sznitowska, 2004; Puglia et al., 2008) and membrane contractor technique (Charcosset et al., 2005; El-Harati et al., 2006).

However, high pressure homogenization technique has many advantages compared to the other methods, e.g. easy scale up, avoidance of organic solvents and short production time. High pressure homogenizers are widely used in many industries including the pharmaceutical industry, e.g. for the production of emulsions for parenteral nutrition. Therefore, no regulatory problems exist for the production of topical pharmaceutical and cosmetic preparations using this production technique. It can be considered as being industrially the most feasible one.

Lipid nanoparticles can be produced by either the hot or cold high pressure homogenization technique. Fig. 2 shows schematically the steps of these two methods. The active compound is dissolved or dispersed in melted solid lipid for SLN or in a mixture of liquid lipid (oil) and melted solid lipid for NLC. In the hot homogenization method the lipid melt containing the active compound is dispersed in a hot surfactant solution of the same temperature (5–10 °C above the melting point of the solid lipid or lipid blend) by high speed stirring. The obtained emulsion (generally called pre-emulsion) is then passed through a high pressure homogenizer adjusted to the same temperature generally applying three cycles at 500 bar or two cycles at 800 bar. In the cold homogenization method, the active containing lipid melt is cooled down. After solidification the mass is crushed and ground to obtain lipid microparticles. The lipid microparticles are then dispersed in a cold surfactant solution yielding a cold pre-suspension of micronized lipid particles. This suspension is passed through a high pressure homogenizer at room temperature applying typically 5–10 cycles at 1500 bar.

For the production of lipid nanoparticles by high pressure homogenization there are many machines available on the mar-

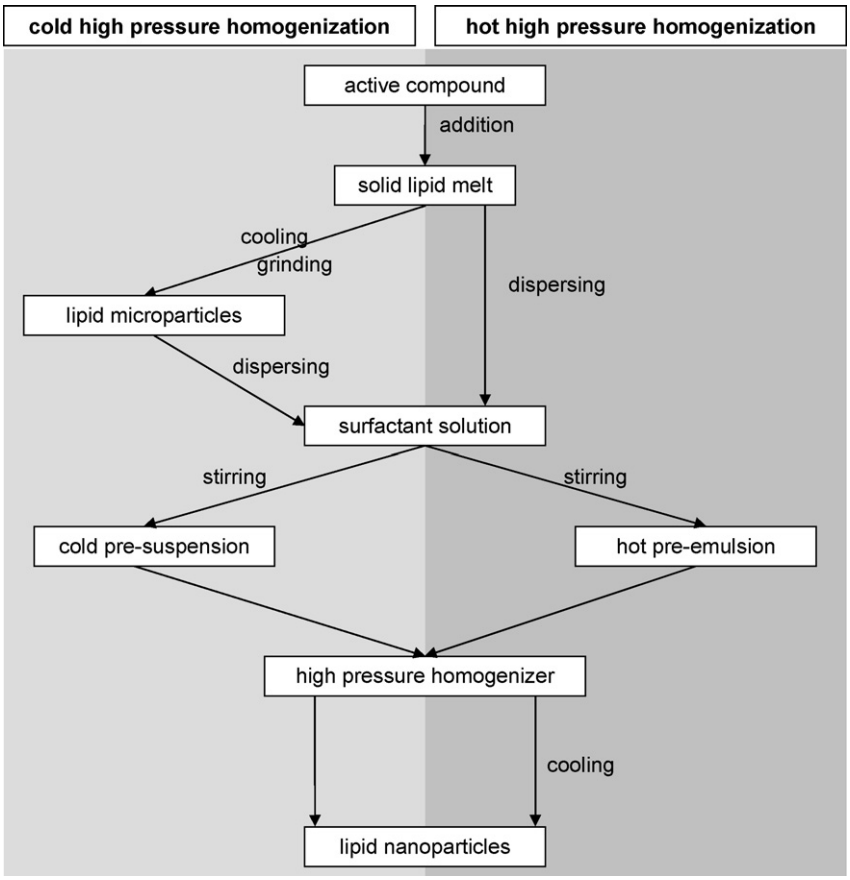

Fig. 2. Production process of lipid nanoparticles using cold (light gray background) and hot (dark gray background) high pressure homogenization technique.

ket. For some technical reasons, e.g. temperature control and cost of large-scale equipment, piston-gap homogenizers are preferred over jet-stream homogenizers (Müller et al., 2005). Piston-gap homogenizers are available with different production capacities. Therefore, production on laboratory scale up to large scale is possible with the same dispersion principle. With a minimum batch size of 3 ml an EmulsiFlex-B3 (Avestin, Ottawa, Canada) can be used for the laboratory scale production with limited new chemical entities or very expensive active materials. The Micron LAB 40 (APV Deutschland GmbH, Unna, Germany) is a laboratory scale high pressure homogenizer with a maximum batch size of 40 ml if operated discontinuously and a batch size range from 200 to 1000 ml when modified to work continuously. Another laboratory scale homogenizer is the Panda (tabletop homogenizer) (Niro Soavi, Lübeck, Germany). It is used for feasibility testing and process development. The batch size ranges from 500 ml to 2 l. Medium scale batches (up to 10 l) can be produced using a Micron LAB 60 (APV Deutschland GmbH, Unna, Germany). Examples of high pressure homogenizers for large scale production are the Gaulin 5.5 (APV Deutschland GmbH, Unna, Germany) and the Rannie 118 (APV Deutschland GmbH, Unna, Germany). These machines have a homogenization capacity of 150 and 2000 l/h, respectively (also depending on the pressure applied) (Gohla and Dingler, 2001; Müller et al., 2002).

In general the formulation of topical products containing SLN or NLC is identical for both of them. Products can be obtained by admixing SLN/NLC to existing products, addition of viscosity enhancers to the aqueous phase of SLN/NLC to obtain a gel or the direct production of a final product containing only nanoparticles in a one-step process.

Addition of SLN or NLC to an existing product, e.g. cream or lotion, is realized by replacing a part of the water phase with concentrated SLN or NLC dispersion. To maintain the lipid content of the original cream or lotion, the lipid content of the original formulation can be reduced about the amount of incorporated lipid from the lipid nanoparticles (Müller et al., 2002). The creams and lotions are produced using the established way of production, cooled to about 30 °C, and the concentrated lipid nanoparticles suspension is then admixed applying gentle stirring.

Instabilities of lipid nanoparticles in cosmetic or pharmaceutical creams or lotions containing oil droplets that might occur are aggregation or dissolution. The presence of solid lipid in such formulations can be proven by differential scanning calorimetry (DSC) (Müller and Dingler, 1998). The particle size can be determined using photon correlation spectroscopy (PCS) or laser diffractometry (LD) (Pardeike and Müller, 2007b).

Hydrogel formulations (xanthan gum, hydroxyethylcellulose 4000, Carbopol 943 and chitosan) containing SLN or NLC were investigated regarding the physical stability of the lipid nanoparticles. For both lipid nanocarriers a good physical stability was reported (Shahgaldian et al., 2003; Souto et al., 2004b).

Using high lipid concentrations a final product can be produced in one step. These particle dispersions have a relatively high consistency; they are cream like or almost solid. By PCS, LD and electron microscopy the existence of intact particles can be proven (Lippacher et al., 2001; Radtke and Müller, 2001).

Comparing lipid nanoparticle formulations for dermal application in the pharmaceutical and the cosmetics field, the technological aspects are similar if not identical (e.g. incorporation

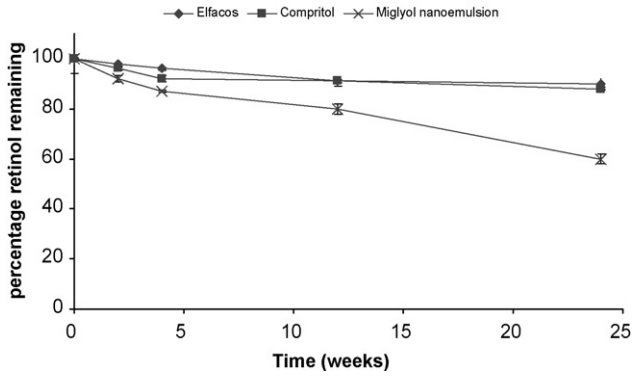

Fig. 3. The %age remaining retinol in the lipid nanoparticles (solid lipid Elfacos or Compritol) and Miglyol nanoemulsion with 0.5% (w/w) retinol loading stored at room temperature for 6 months (10% lipid, 1.5% Miranol 32 as stabilizer) ($n = 3$) (modified after Jenning (1999)).

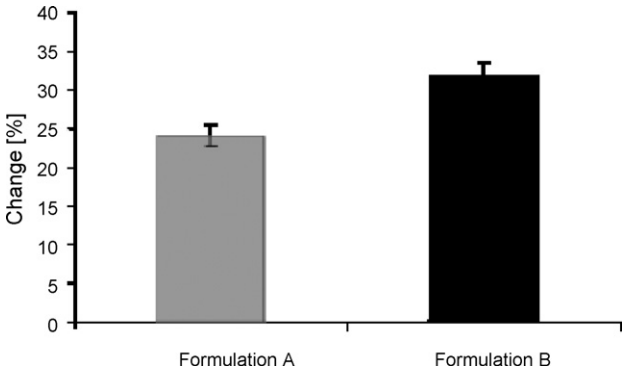

Fig. 5. Increase in skin hydration after application of formulation A (cream without SLN) and formulation B (cream with SLN) for 28 days (with permission from Wissing and Müller (2003b)).

of actives into particles, incorporation of particles into cream, stability in cream, etc.). However, the time for product development and market introduction for cosmetic products is much shorter due to the more complex regulations for the development of a pharmaceutical product. Therefore, analogously to liposomes, the first lipid nanoparticles product on the market was a cosmetic product.

4. Science-based cosmetics: formulations and products

Both NLC and SLN have many features that are advantageous for dermal application. They are colloidal carriers providing controlled release profiles for many substances. They are composed of physiological and biodegradable lipids exhibiting low toxicity and low cytotoxicity, that means an excellent tolerability. The small size ensures a close contact to the stratum corneum and can increase the amount of drug penetrated into the skin. Due to the occlusive properties of lipid nanoparticles, an increased skin hydration effect is observed. Furthermore, lipid nanoparticles are able to enhance the chemical stability of compounds sensitive to light, oxidation and hydrolysis. Enhancement of chemical stability after incorporation into lipid nanocarriers was proven for many cosmetic actives, e.g. coenzyme Q10 (Dingler, 1998; Puglia et al., 2006), ascorbyl palmitate (Teeranachaideekul et al., 2007a), tocopherol (vitamin E) (Dingler, 1998) and retinol (vitamin A) (Fig. 3) (Jenning, 1999; Jenning and Gohla, 2001; Jee et al., 2006).

Film formation on the skin and subsequent occlusion effect was reported for lipid nanoparticles. It was found by Wissing et al., that the highest occlusion will be reached using low melting lipids, highly crystalline particles and very small particles. Nanoparticles have been found to be 15-folds more occlusive than microparticles (de Vringer, 1992, 1999). Particles smaller than 400 nm containing at least 35% lipid of high cystallinity have been most effective (Fig. 4) (Wissing et al., 2001a; Wissing and Müller, 2002a). Consequently Souto et al., found a higher occlusive factor for SLN in comparison to NLC of the same lipid content (Souto et al., 2004a). Comparing NLC with different oil content led to the conclusion that an increase in oil content leads to a decrease of the occlusive factor (Teeranachaideekul et al., 2008).

Due to the reduced water loss caused by occlusion, the skin hydration is increased after dermal application of SLN or NLC or lipid nanoparticles-containing formulations. Wissing and Müller performed an *in vivo* study investigating the skin hydration effect after repetitive application of an o/w cream containing SLN and a conventional o/w cream for 28 days. The SLN containing o/w cream increased the skin hydration significantly more than conventional o/w cream (Wissing and Müller, 2003b) (Fig. 5). A significant higher increase in skin hydration was found by Pardeike and Müller for an NLC-containing cream compared to conventional cream (Pardeike and Müller, 2006).

In healthy skin the stratum corneum has typically a water content of 20% and provides a relative effective barrier against percutaneous absorption of exogenous substances. Skin occlusion can increase the stratum corneum hydration and therefore influence percutanious absorption (Zhai and Maibach, 2001). For cosmetic products it is important that the cosmetic active is not systemically absorbed. But a certain penetration into the skin is needed to obtain a cosmetic effect. In general lipid nanoparticles do not penetrate the horny layer (Schäfer-Korting et al., 2007) but a follicular uptake by the hair follicles has been reported for particulate systems (Lademann et al., 2007). Improved dermal uptake of active compounds loaded to lipid nanoparticles might also result from an increased contact surface of the active compound with the corneocytes, a rapid or a steady release and surfactants (Schäfer-Korting

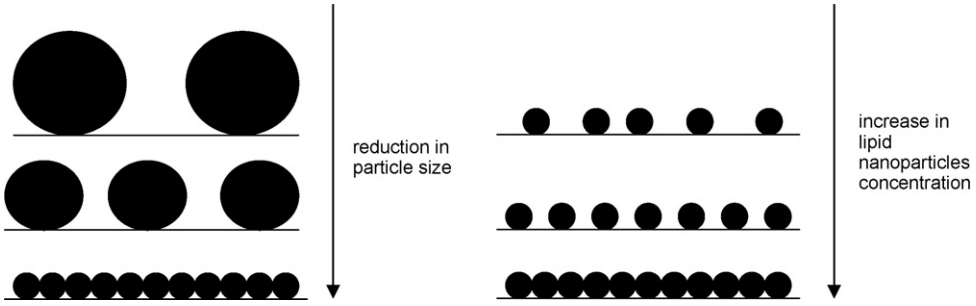

Fig. 4. The occlusion factor of lipid nanoparticles depends on various factors: at identical lipid content, reducing the particle size leads to an increase in particle number, the film becomes denser (left) and therefore the occlusion factor increases. At a given particle size, increasing the lipid concentration increases particle number and density of the film (right) which also leads to a higher occlusion factor (with permission from Müller et al. (2007a)).

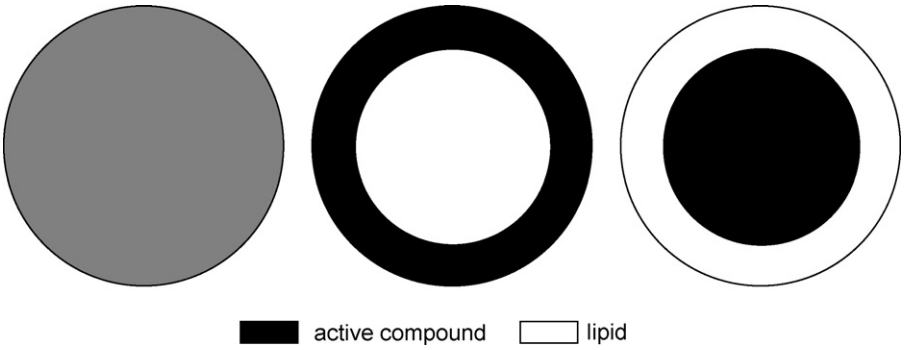

Fig. 6. Models of incorporated actives in lipid nanoparticles, homogeneous matrix (left), active-free lipid core with active-enriched shell (middle) and active-enriched core with active-free lipid shell (right) (modified after Müller et al. (2000b)).

et al., 2007). The distribution of the active compound within the lipid particles can vary considerably (Fig. 6) and hence its influence on the percutanious uptake (Müller et al., 2000b; Wissing et al., 2004b). Therefore, each system has to be studied separately considering its influence on percutanious absorption (Sivaramakrishnan et al., 2004; Lombardi Borgia et al., 2005; Stecova et al., 2007). Burst release as well as sustained release has been reported for SLN and NLC dispersions (Müller et al., 2000a). For dermal application both features are of interest. Burst release might improve the penetration of active compounds. Sustained release becomes important for active ingredients that are irritating at high concentrations or to supply the skin over a prolonged period of time with an active compound (e.g. antimycotics).

Comparing the release profiles of retinol-loaded SLN and nanoemulsion as well as retinol-loaded SLN and nanoemulsion incorporated in xanthan gum hydrogel or o/w cream, it was found that the SLN formulations showed a controlled release over the first 6 h. After longer periods (12–24 h) the release rate increased and even exceeded the release rate of the formulations containing retinol-loaded nanoemulsions (Jenning et al., 2000b). In *in vitro* penetration studies using porcine skin retinol-loaded SLN and retinol-loaded nanoemuslion were compared. High concentrations of retinol were found in the upper skin layers following the application of SLN preparation, whereas the deeper regions showed very low retinol levels. Therefore, a localization effect in upper skin layers was suggested (Jenning et al., 2000a).

Teeranachaideekul et al. compared the release profile of Q10-loaded NLC and nanoemulsion. The Q10-loaded NLC exhibit a biphasic release pattern. NLC provided a fast initial release followed by slow release while the Q10-loaded nanoemulsion showed a constant release over the time (Teeranachaideekul et al., 2007b).

An increase of skin penetration was reported for coenzyme Q10-loaded SLN and NLC compared to nanoemulsion and liquid paraffin or isopropanol respectively, performing a tape stripping test (Dingler, 1998; Pardeike and Müller, 2007a). Using the fluorescence dye Nile red as a marker, it could be shown by Teeranachaideekul et al. that the penetration depth and the amount penetrated into the skin depends on the oil content used in NLC (Teeranachaideekul et al., 2008). It was found that the degree of epidermal targeting depends on the oil content, and associated with the oil content, the occlusive factor.

A prolonged release is of interest for perfumes as well as for perfumes incorporated into cosmetic products. Wissing et al. found that SLN loaded with the perfume Allure (Chanel) yield a prolonged release of the perfume from the solid lipid matrix of SLN. Comparing the release of the perfume from an emulsion and SLN, after 6 h 100% of the perfume were released from the emulsion but only 75% was released from the SLN (Wissing et al., 2000a). Müller et al. showed a prolonged release of the perfume Kenzo from NLC compared to emulsion and conventional shampoo (Müller et al., 2007b). Hommoss et al. studied the effect of changing the solid lipid of the perfume-loaded lipid nanoparticles on the release profile of the incorporated perfume (Hommoss and Müller, 2006). It could be concluded that by selecting a solid lipid that can enclose the perfume in its solid matrix a controlled perfume released can be achieved (Fig. 7). Furthermore, it was found that the release of perfume depends on the lipid matrix composition, the perfume load and the surfactant type (Hommoss et al., 2007a).

A prolonged release is also desired for insect repellents. With SLN and NLC a prolonged release can be achieved. The natural insect repellent lemon oil and the synthetic insect repellent DEET (N,N-diethyltoluamide) were successfully incorporated into SLN (Iscan et al., 2005). A prolonged release of the insect repellent from SLN could be shown (Fig. 8) (Wissing et al., 2000b; Wissing, 2002).

It was found by Wissing et al. that SLN can act as a physical UV blocker themselves and are able to improve the UV protection in combination with organic sunscreens such as 2-hydroxy-4-methoxy benzophenone which allows a reduction of the concentration of the UV absorber (Wissing and Müller, 2001b; Müller et al., 2002; Wissing and Müller, 2002b). These findings were confirmed by Song and Lui comparing UV absorption properties of 3,4,5-trimethoxybenzochitin-loaded SLN and SLN free system (Song and Liu, 2005). Comparing SLN to a conventional emulsion, the amount of molecular sunscreen can be reduced by 50% in the SLN formulation maintaining the protective level of the conventional emulsion (Wissing and Müller, 2003a). Furthermore, a significant increase in SPF up to about 50 was reported after the encapsulation of titanium dioxide into NLC (Villalobos-Hernandez

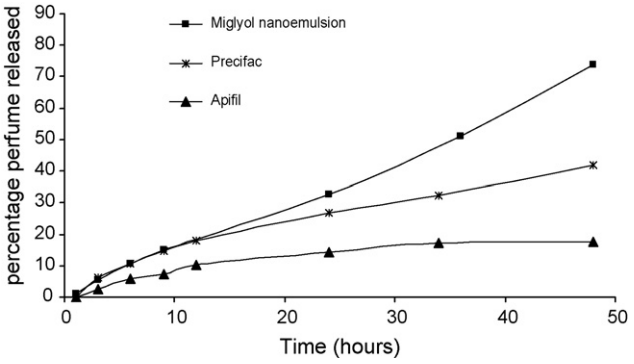

Fig. 7. A prolonged release can be seen for the perfume CA from two different lipid nanoparticle formulations (Preifac, Apifil) compared to a fast releasing nanoemulsion (modified after Hommoss and Müller (2006)).

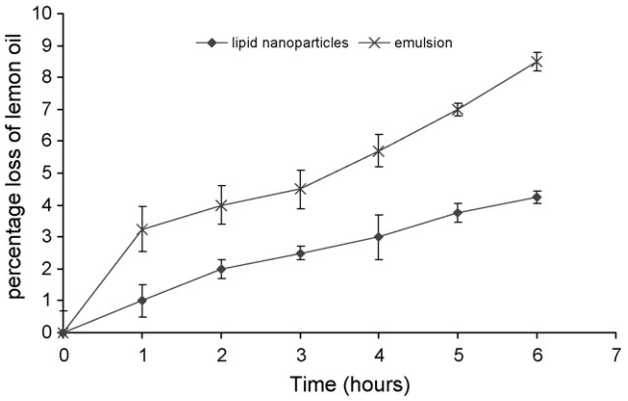

Fig. 8. Evaporation profile of lemon oil upon storage at 32 °C for 6 h. A prolonged release of the insect repellent DEET was observed from lipid nanoparticles compared to emulsion (modified after Wissing et al. (2000b)).

and Müller-Goymann, 2005). Encapsulation of inorganic sunscreens into NLC is therefore a promising approach to obtain well tolerable sunscreens with high SPF.

The positive features of lipid nanoparticles leaded to the market introduction of a number of cosmetic products. Table 1 provides an overview of cosmetic products containing lipid nanoparticles (NLC) currently on the market.

Investigations on Cutanova Cream NanoRepair Q10 (Dr. Rimpler GmbH, Wedemark, Germany), being the first lipid nanoparticles-based cosmetic product introduced to the market in 2005, have proven that the NLC containing cream is superior with regard to skin hydration in comparison to a conventional o/w cream having the same composition (Pardeike and Müller, 2006). Fig. 9 shows the increase in skin hydration after repetitive application of both creams for 42 days by 30 female volunteers. The evaluation of the occlusive factor of the NLC dispersion used in the final product and an o/w emulsion (obtained by replacing the solid lipid by Miglyol 812), at the same concentration level as in the final product, showed a five-times higher occlusive factor for the NLC dispersion than for the o/w emulsion (Pardeike and Müller, 2007a). Furthermore, the

Table 1
Examples of cosmetic products currently on the market containing lipid nanoparticles.

Product name	Producer/distibuter	Market introduction	Main active ingredients
Cutanova Cream Nano Repair Q10	Dr. Rimpler	10/2005	Q 10, polypeptide, hibiscus extract, ginger extract, ketosugar
Intensive Serum NanoRepair Q10		10/2005	Q 10, polypeptide, mafane extract
Cutanova Cream NanoVital Q10		06/2006	Q 10, TiO_2, polypeptide, ursolic acid, oleanolic acid, sunflower seed extract
SURMER Crème Legère Nano-Protection	Isabelle Lancray	11/2006	Kukuinut oil, Monoi Tiare Tahiti®, pseudopeptide, milk extract from coconut, wild indigo, noni extract
SURMER Crème Riche Nano-Restructurante			Kukuinut oil, Monoi Tiare Tahiti®, pseudopeptide, milk extract from coconut, wild indigo, noni extract
SURMER Elixir du Beauté Nano-Vitalisant			Kukuinut oil, Monoi Tiare Tahiti®, pseudopeptide, milk extract from coconut, wild indigo, noni extract
SURMER Masque Crème Nano-Hydratant			Kukuinut oil, Monoi Tiare Tahiti®, pseudopeptide, milk extract from coconut, wild indigo, noni extract
NanoLipid Restore CLR	Chemisches Laboratorium	04/2006	Black currant seed oil containing ω-3 and ω-6 unsaturated fatty acids
Nanolipid Q10 CLR	Dr. Kurt Richter, (CLR)	07/2006	Coenzyme Q10 and black currant seed oil
Nanolipid Basic CLR		07/2006	Caprylic/capric triglycerides
NanoLipid Repair CLR		02/2007	Black currant seed oil and manuka oil
IOPE SuperVital Cream Serum Eye cream Extra moist softener Extra moist emulsion	Amore Pacific	09/2006	Coenzyme Q10, ω-3 und ω-6 unsaturated fatty acids
NLC Deep Effect Eye Serum	Beate Johnen	12/2006	Coenzyme Q10, highly active oligo saccharides
NLC Deep Effect Repair Cream			Q10, TiO_2, highly active oligo saccharides
NLC Deep Effect Reconstruction Cream			Q10, acetyl hexapeptide-3, micronized plant collagen, high active oligosaccharides in polysaccharide matrix
NLC Deep Effect Reconstruction Serum			
Regenerationscreme Intensiv	Scholl	6/2007	Macadamia ternifolia seed oil, avocado oil, urea, black currant seed oil
Swiss Cellular White Illuminating Eye Essence	La prairie	1/2007	Glycoprotiens, panax ginseng root extract, equisetum arvense extract, Camellia sinensis leaf extract, viola tricolor extract
Swiss Cellular White Intensive Ampoules		1/2007	Glycoprotiens, panax ginseng root extract, equisetum arvense extract, Camellia sinensis leaf extract, viola tricolor extract
SURMER Creme Contour Des Yeux Nano-Remodelante	Isabelle Lancray	03/2008	Kukuinut oil, Monoi Tiare Tahiti®, pseudopeptide, hydrolized wheet protein
Olivenöl Anti Falten Pflegekonzentrat	Dr. Theiss	02/2008	Olea europaea oil, panthenol, acacia senegal, tocopheryl acetate
Olivenöl Augenpflegebalsam			Olea Europaea oil, prunus amygdalus dulcis oil, hydrolized milk protein, tocopheryl acetate, rhodiola rosea root extract, caffeine

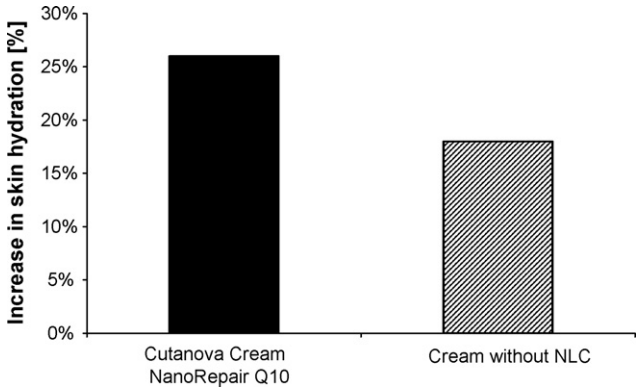

Fig. 9. The increase in human skin hydration after application of Cutanova Cream NanoRepair Q10 and a cream having the same composition but replacing the NLC by o/w emulsion for 42 day (modified after Müller et al. (2007c)).

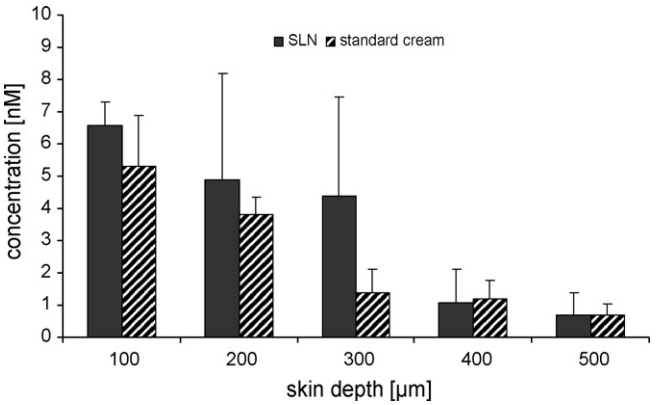

Fig. 11. Distribution of prednicarbate and its metabolites in human skin after 24 h. Mean value ± S.D. ($n = 3$) (modified after Santos Maia et al. (2000)).

penetration of coenzyme Q10 into the stratum corneum from the NLC used in Cutanova Cream NanoRepair Q10 was evaluated by tape stripping test on human female volunteers and compared with an o/w emulsion having the same coenzyme Q10 and lipid content. The cumulative amount of coenzyme Q10 found in nine tape strips was 21% for the NLC dispersion and 16% for the o/w emulsion, related to the applied amount of coenzyme Q10 (Pardeike and Müller, 2007a).

NanoLipid Restore CLR (Chemisches Laboratorium Dr. Kurt Richter, Berlin, Germany) is a semi-finished cosmetic product based on lipid nanoparticles. The easily oxidized black currant seed oil (BCO) is incorporated in NLC. NLC are able to protect BCO against oxidation, which enhances the stability of the final product. This was proven by performing an oxidation stress test comparing NanoLipid Restore CLR with a reference nanoemulsion (containing Miglyol 812) (Petersen et al., 2006). It was found, that the BCO is better stabilized against oxidation in the NanoLipid Restore CLR than in the reference emulsion (Fig. 10). The stability of coenzyme Q10 in NanoLipid Restore CLR was also assessed and results showed that the NLC lead to an enhanced stability of coenzyme Q10 (Hommoss et al., 2007b). NanoLipid Restore CLR was used in the prestigious cosmetic product line IOPE (Amore Pacific, Seoul, South Korea) introduced to the market in September 2006.

In the cosmetic products line Surmer (Dr. Rimpler GmbH, Wedemark, Germany) the lipid nanoparticles were used for their occlusive properties. It was possible to increase the occlusion of a day cream without changing its light character, i.e. achieving higher occlusive properties without having the glossy skin appearance associated with the high occlusive night creams.

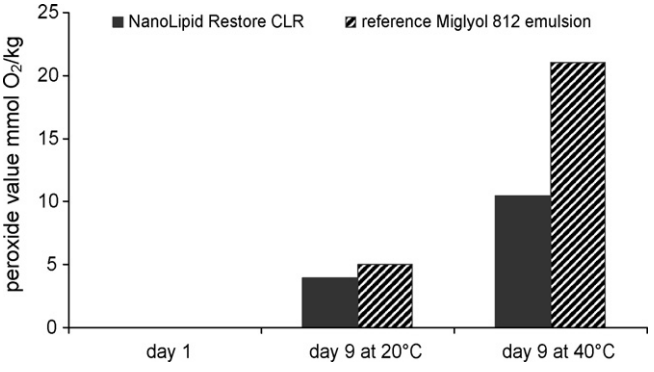

Fig. 10. The peroxide value results of the oxidative stress test for NanoLipid Restore CLR and the reference emulsion at day 1 and after 9 days. The BCO in the NLC-based formula was better stabilized against peroxidation (modified after Hommoss et al. (2007b)).

5. Pharmaceutical formulations and benefits

Topical treatment of skin diseases has the advantage that high drug levels can be achieved at the site of disease and systemic side effects can be reduced compared to oral or parenteral drug administration. Topical drug administration is still a challenge in pharmaceutics due to the difficulties in controlling and determining the exact amount of drug that reaches the different skin layers. The drugs and the vehicles physicochemical properties are considered to be the main features responsible for the drug differential distribution in the skin. Lipid nanoparticles have been investigated to improve the treatment of skin diseases such as atopic eczema, psoriasis, acne, skin mycosis and inflammations. Apart from the treatment of skin diseases by topical application, e.g. gastrointestinal side effects of non-steroidal anti-inflammatory drugs can be decreased by topical antirheumatic therapy. Drugs under investigations for dermal application using lipid nanoparticles at the present are for instance glucocorticoids, retinoids, non-steroidal anti-inflammatory drugs, COX-2 inhibitors and antimycotics. It was show that it is possible to enhance the percutaneous absorption with lipid nanoparticles. These carriers may even allow drug targeting to the skin or even to its substructures. Thus they might have the potential to improve the benefit/risk ratio of topical drug therapy (Schäfer-Korting et al., 2007).

5.1. Topical glucocorticoids

Topical corticosteroids are the first-line therapy of acute exacerbations of atopic dermatitis and contact dermatitis. Prednicarbate is superior to the halogenated glucocorticoids because of an improved benefit/risk ratio. However, at the present the separation of desired anti-inflammatory effects and undesired antiproliferative effects is still not satisfying. Therefore, lipid nanoparticles were investigated as a delivery system for prednicarbate. Santos et al. reported an improved extent of prednicarbate uptake by human skin *in vitro*, if applied as SLN dispersion or cream containing prednicarbate-loaded SLN (Fig. 11) (Santos Maia et al., 2000). The authors found that a prednicarbate targeting to the epidermis occurred (Santos Maia et al., 2002). This is particular relevant because prednicarbate in the dermis is responsible for the induction of irreversible skin atrophy while the inflammatory process is most pronounced within the epidermis (Schäfer-Korting et al., 2007). Therefore, a better benefit/risk ratio is expected for the application of pretnicarbate in SLN containing topical formulations.

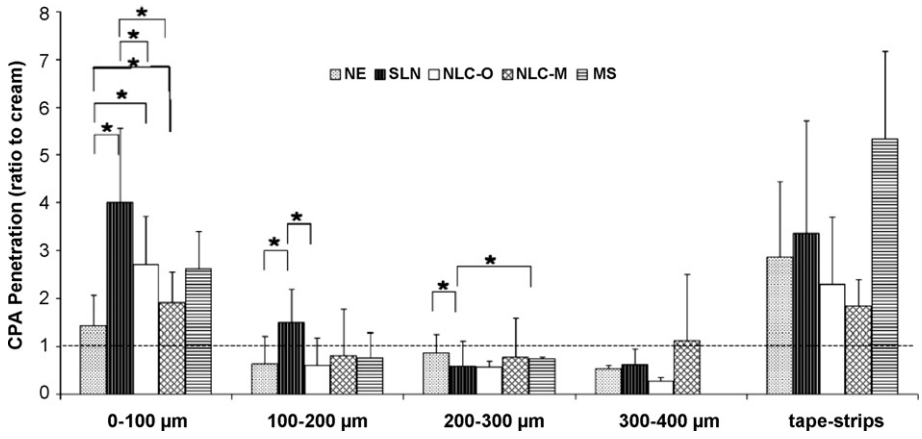

Fig. 12. 0.05% Cyproterone acetate containing dispersion of drug carriers (NE = nanoemulsion, $n = 3$ donors, SLN $n = 7$ donors, NLC-O = NLC containing olic acid, $n = 5$ donors, NLC-M = NLC containing Miglyol, $n = 3$ donors, MS = microspheres, $n = 2$ donors) were applied to cryoconserved human skin for 6 h and the penetration into human skin was investigated. Particulate dispersions were tested in parallel to o/w cream ($n = 7$ donors) using 2–4 skin tissue specimen per donor for each preparation. Except for NE, cyproterone acetate amounts in the first layer exceed amounts following the cream, moreover penetration ratios of the layers 0–100 and 100–200 mm differed for all preparations except for NLC-M ($p \leq 0.05$). (*) Differences in CPA-ratios (over cream) between the particulate systems ($p \leq 0.05$) (with permission from Stecova et al. (2007)).

5.2. Antiandrogen

The oral application of cyproterone acetate can be used to reduce sebum secretion rate and acne lesions. For female patients a combination of cyproterone acetate and ethinyl estradiol is given to exclude teratogenic effects of cyproternoe acetate (feminization of the male fetus). In male patients loss of libido, gynecomastia, vasomotor flushing and loss of bone mineral density can be observed as cyproterone acetate side effects, which is acceptable when used for metastatic prostate cancer but not for acne treatment. To avoid systemic antiandrogen effects a topical application is preferable (Iraji et al., 2006). Application of cyproterone acetate-loaded SLN increased the skin penetration at least four-folds over the uptake from cream and emulsion (Fig. 12), whereas the drug amount found in the dermis was low for all preparations. No difference was seen in the penetration profiles into intact and stripped skin. Cyproterone acetate-loaded SLN enhanced skin absorption resulting in therapeutic drug levels within the target tissue while reducing systemic side effects compared to the oral administration (Stecova et al., 2007).

5.3. Vitamin A derivates

Tretinoin, a metabolite of vitamin A is used for topical treatment of various proliferateive and inflammatory skin diseases such as psoriasis, acne, photo aging, epidermotropic T-cell lymphomas and epithelial skin cancer. One of the major disadvantages associated with the topical application of tretinoin is local skin irritation such as erythrema, peeling and burning as well as increased sensitivity to sunlight. To overcome this problems tretinoin was incorporated into SLN by Shah et al. (Shah et al., 2007). *In vitro* permeation studies through rat skin indicated that SLN-based tretinoin gel has a permeation profile comparable to that of the market tretinoin cream. Furthermore, Draize patch test showed that SLN-based tretinoin gel resulted in remarkably less erythremic episodes compared to the currently marketed tretinoin cream (Fig. 13). Therefore, also for formulations containing tretinoin-loaded SLN a better benefit/risk ratio is expected.

Isotretinoin, a derivate of retinoic acid, is used for the treatment of severe acne and other dermatological diseases. The marketed products such as isotretinoin cream show significant skin irritation and systemic absorption, which is associated with side effects (Queille-Roussel et al., 2001). Penetration through and permeation into rat skin of isotretinoin was investigated from SLN and ethanolic solution. For the isotretinoin-loaded SLN in comparison to ethanolic solution, no penetration through rat skin was found. Therefore, isotretionin-loaded SLN formulation can avoid systemic uptake of the drug. For the SLN a high accumulative amount of isotretinoin was found in the skin, showing a skin targeting effect (Liu et al., 2007).

5.4. PUVA-therapy

PUVA-therapy involves the application of a psoralen in combination with long-wavelength UV light (UV-A). It is used for the treatment of skin diseases like psoriasis, mycosis fungoides and vitiligo. Fang et al. studied the permeation of psoralens from SLN, NLC, emulsion and aqueous suspension through nude mice skin with and without introduction of hyperproliferative skin by repeated tape stripping (Fang et al., 2008). The permeation of psoralens increased in the order 8-methoxypsoralen > 5-methoxypsoralen > 4,5,8-trimethylpsoralen for all formulations tested. If was found that the drug flux through nude mice skin was highest for the NLC formulations while SLN were not able to improve the skin permeation over the aqueous suspension. The flux of the emulsion was the lowest. No difference was found in the permeation behavior of 8-methoxypsoralen through hyperproliferative skin and normal nude mice skin while the permeation through hyperproliferative skin was significantly reduced for the other formulations.

5.5. Non-steroidal anti-inflammatory drugs

The non-steroidal anti-inflammatory drugs celecoxib and valecoxib acting by selective inhibition of COX-2 have been investigated for dermal application using NLC-based delivery systems. Celecoxib is widely used for the treatment of rheumatoid arthritis, osteoarthritis, acute pain, familial adenomatous polyposis and primary dysmenorrheal. Furthermore, topical formulations of COX-2 inhibitors have been developed for the treatment of COX-2 mediated skin diseases like inflammation, pain and nociception, skin tumors, injury and wounds (Lee et al., 2003). Joshi et al. compared a NLC-based gel of celecoxib with a micellar gel with the same composition regarding the *in vitro* skin penetration using rat skin and the pharmacodynamic efficiency by Aerosil induced rat paw edema (Joshi and Patravale, 2008). The *in vitro* permeation of cele-

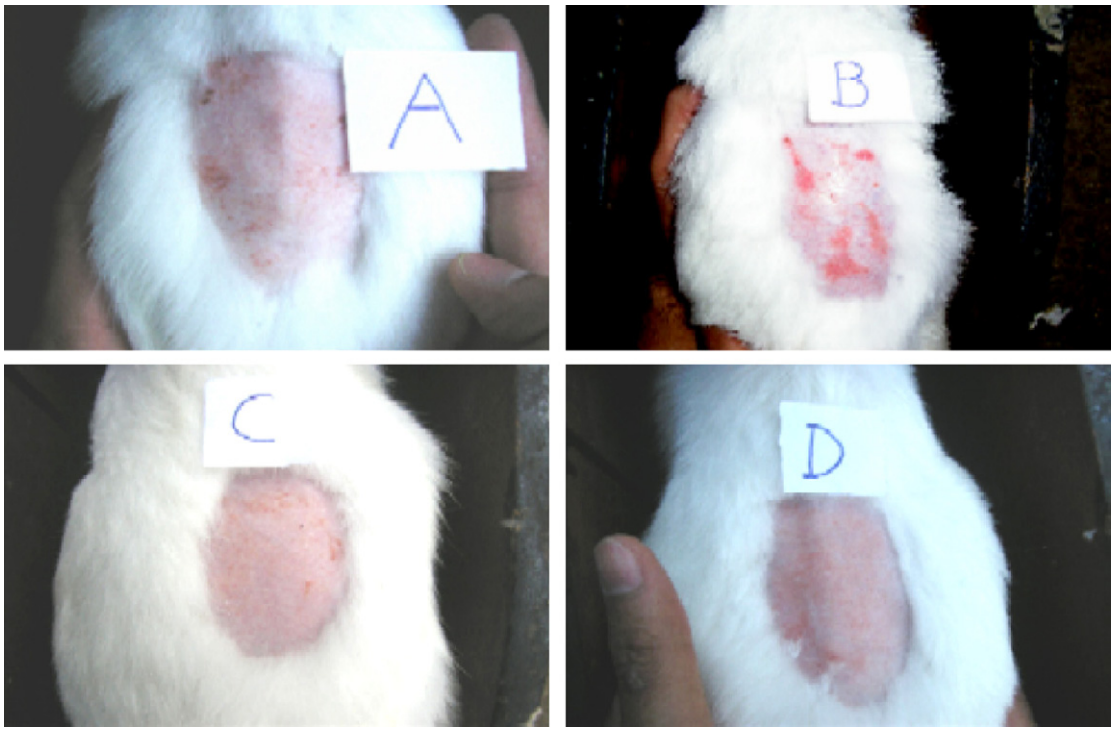

Fig. 13. Pictures of Draize skin irritation studies carried out on New Zealand rabbits 24 h after application of (A) control (no application); (B) marketed formulation (Retino-A® cream); (C) SLN-based gel without tretinoin; (D) SLN-based gel containing tretinoin (0.05%, w/w). The Marketed tretinoin cream clearly shows erythemal lesions, which are not visible in SLN based tretinoin gel (with permission from Shah et al. (2007)).

coxib from NLC gel was less than the permetration from the micellar based gel, which confirms findings about nanoparticles leading to a drug deposit in the skin resulting in sustained release. The *in vivo* comparison of the percentage edema inhibition produced by NLC and micellar gel showed a significant higher inhibition after application of the NLC based gel up to 24 h (Fig. 14) (Joshi and Patravale, 2008).

Valdecoxib is used for the treatment of inflammation and arthritis. The topical marketed valdecoxib formulation contains 56% alcohol, which may have a drying effect on the skin after repetitive application. Therefore, an alcohol free delivery system with faster onset and prolonged action was targeted by developing a gel based on valdecoxib-loaded NLC. Valdecoxib-loaded NLC incorporated into Carbopol gel were investigated regarding *in vitro* release, skin irritation using the Draize patch test and efficacy using Aerosil-induced rat paw edema model and compared to a market product (Joshi and Patravale, 2006). *In vitro* the NLC gel showed burst release followed by steady release while the market formulation showed 100% release within 1 h. In the Draize patch test the NLC containing gel showed no skin irritation while the market gel showed slight irritation after 48 h. The NLC-based gel showed prolonged activity up to 24 h while the activity of the market gel was much shorter. This indicates better skin tolerability and longer activity of the NLC-formulation compared to the marketed formulation.

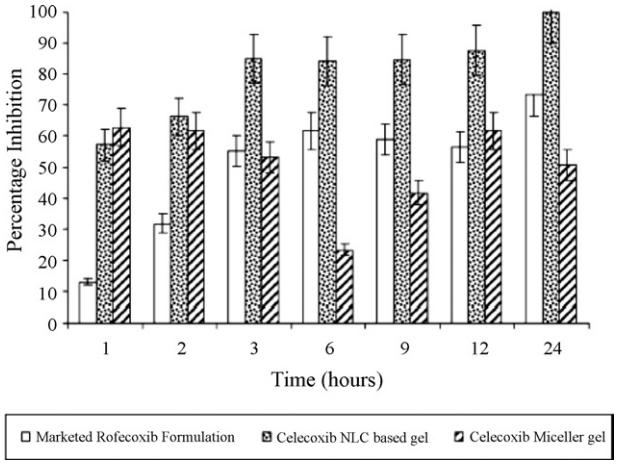

Fig. 14. *In vivo* comparison of the percentage inhibition observed after application of NLC-based celecoxib gel, micellar gel and marketed gel in the aerosil-induced rat paw edema method (with permission from Joshi and Patravale (2008)).

Indomethacin is one of the most potent non-steroidal anti-inflammatory drugs, widely used topically for the treatment of dermatitis and rheumatic diseases. Ricci et al. investigated the *in vitro* penetration of indomethacin from NLC containing gel and gel without NLC through the stratum corneum and epidermis, the *in vivo* indomethacin release by tape-stripping test and the *in vivo* anti-inflammatory activity using the UV-B induced erythrema model (Ricci et al., 2005). It was found, that the anti-inflammatory effect following the topical application of indomethacin was more prolonged with indomethacin-loaded NLC gel. In the tape stripping test higher amounts of indomethacin were found in the stratum corneum after application of the indomethacin-loaded NLC gel. The *in vitro* permeation through the stratum corneum and epidermis from indomethacin-loaded NLC gel was less than from gel without NLC.

Ketoprofen and naproxen are non-steroidal anti-inflammatory drugs used for the treatment of musculoskeletal disorders, e.g. rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. Puglia et al. prepared ketoprofen-loaded NLC and naproxen-loaded NLC which were incorporated into gels and compared to reference gels containing ketoprofen or naproxen solution, respectively. The *in vitro* percutaneous absorption, the *in vivo* active localization in the stratum corneum and the anti-inflammatory effect were

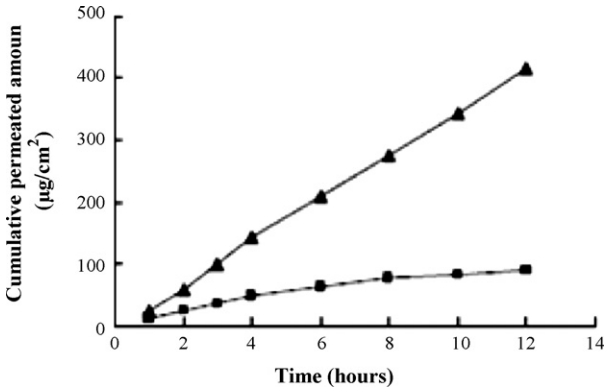

Fig. 15. Permeation profiles of flurbiprofen through rat skin from NLC (▲) and saturated physiological saline (■) (with permission from Han et al. (2008)).

studied. NLC were able to reduce the drug penetration through excised human skin while it was found by tape-striping test that the drug permeation and drug accumulation in the horny layer was increased. Furthermore, a prolonged anti-inflammatory effect could be shown for drug-loaded NLC compared to drug solution (Puglia et al., 2008).

Flurbiprofen, another non-steroidal anti-inflammatory drug, is used for the treatment of gout, osteoarthritis, rheumatoid arthritis and sunburn. In order to avoid irritation of the gastrointestinal tract which might occur after oral administration of flurbiprofen, drug administration via the skin is targeted. Han et al. investigated the physical stability and the permeated amount of flurbiprofen from NLC through rat skin. The authors found that the investigated flurbiprofen containing NLC formulation was stable over the observation period. Comparing the permeation of flurbiprofen through rat skin after 12 h a 4.5-folds increased permeation was reported from the NLC formulation compared to phosphate buffered saline (Fig. 15) (Han et al., 2008). Therefore, NLC might be promising as a delivery system for transdermal delivery of flurbiprofen.

The non-steroidal agent ketorolac, with powerful analgesic and low anti-inflammatory activity, is widely used for the treatment of moderate and severe pain. Puglia et al. studied the *in vitro* delivery of ketorolac through human epidermis (Puglia et al., 2006). The formulations under investigation were Carbopol 934P gel containing an alcoholic solution of ketorolac, alcoholic solutions of ketorolac prodrugs (polyoxyethylene glycol ester derivates with different amounts of polyovxethylene units), ketorolac-loaded NLC and blank NLC together with free ketorolac. The authors found that the penetration of the ester prodrugs was significantly enhanced apart from the pentaethylene glycol ester, the ester with the longest ethylene glycol unit tested. The NLC-containing formulations gave the lowest ketorolac permeation rate among the formulations tested. However, NLC seemed appropriate for sustained release due to the possible formulation of a drug reservoir in the skin.

5.6. Traditional Chinese medicine

Triptolide is a purified compound of a traditional Chinese medicine, showing anti-inflamatory, immunosuppressive, antifertility and anti-neoplastic activity. The transdermal delivery and anti-inflammatory activity was evaluated by Mei et al. using triptolide-loaded SLN and microemulsion. SLN were found to increase the triptolide penetration into the skin as well as the anti-inflammatory activity (Mei et al., 2003). This strategy improved the bioavailability at the site of action, reduces the required dose and the dose-dependent side effects like irritation and stinging.

5.7. Antimycotics

SLN and NLC have been investigated for topical delivery of antifungals like clotrimazole and ketoconazol (Souto et al., 2004a; Souto, 2005; Souto and Müller, 2006). Ketoconazol is widely used in topical formulations for the treatment of human mycotic infections. Adverse effects like severe irritation, pruritus and stinging have been reported. Up to now it could be shown by Souto and Müller that stable lipid nanoparticles for topical delivery of ketoconazol can be prepared (Souto and Müller, 2005). Further investigations regarding the skin permeation, skin penetration and reduction of adverse effects need to be done.

For clotrimazole it was found, that SLN and NLC can be potential topical delivery systems. Good stability over a storage time of 3 months was reported. NLC loaded with clotrimazole showed a faster drug release than SLN loaded with clotrimazole. Furthermore, it was found, that the release rate of clotrimazole depends on the drug concentration. A fast release was reported using low drug concentrations while a higher drug concentration prolonged the release (Souto et al., 2004a).

Sanna et al. investigated econazole nitrate-loaded SLN incorporated into hydrogels for topical application (Sanna et al., 2007). In an *ex vivo* permeation test using porcine stratum corneum the authors showed controlled drug release properties of SLN whereby the release rate depended upon the lipid content of the nanoparticles. In *in vivo* tape-stripping tests it was found that SLN promote a rapid penetration of econazol nitrate through the stratum corneum after 1 h and improve the penetration of the drug into deeper skin layers after 3 h of application compared to reference gel.

5.8. Podophyllotoxin

Podophyllotoxin is used for the treatment of genital warts and for the inhibition of growth of epithelial cells infected by human papilloma virus in the epidermis. Commercially available podophyllotoxin tinctures and creams can lead to systemic absorption which results in severe side effects. Chen et al. compared podophyllotoxin-loaded SLN with podophyllotoxin tincture with regards to skin permeation, skin penetration and epidermal targeting effect (Chen et al., 2006). It was found that podophyllotoxin was able to permeate porcine skin from the tincture while no permeation was found for drug-loaded SLN. For one SLN-formulation an increased penetration into porcine skin up to 3.48-times over tincture was reported. Furthermore, it was found, that podophyllotoxin was located in the epidermis and hair follicles when applied as SLN-formulation. No drug was found in the dermis after SLN application while podophyllotoxin after tincture application was distributed in each layer of the skin. Therefore, a localization effect in the epidermis was suggested and a reduction in systemic side effects is expected after application of podophyllotoxin using a formulation containing SLN.

6. Lipid nanoparticles: a “nanosave” carrier

During the last decade there was an increasing hype about nanotechnology in almost any discipline, ranging from computer technology via products of daily life (e.g. coating of clothes, supratex) to cosmetic and pharmaceutical formulations and products. Nanotechnology seemed to open unexpected perspectives (what indeed many nanotechnology products are doing!). Within this enthusiasm about nanotechnology, the potential “dark side” of each technology was forgotten. In the last years there was an increasing awareness about potential toxicity of nanosized materials. This considers that limited experience is available how nanosized materials interact with the body. Due to their nanosize nanoparticles have

completely different possibilities to interact with cells in the body, they can use "infiltration routes" and utilize certain mechanisms, which are not accessible by micrometer material. For example, injected polymeric microparticles with a size around 50 μm cannot be phagocytosed by immune cells. This is completely different when having material in the range of few micrometers or in the nanorange. Internalization of such material can cause release of certain cytokines (Schöler et al., 2000a) and also cause death of cells. Smith and Hunneyball described already in the 1980s the cytotoxicity of small polymeric microparticles made from poly(lactide acid) (Smith and Hunneyball, 1985). The intention was to treat arthritis in joints by injecting prednisolone-loaded polymeric microparticles. In cell cultures they discovered that the amount of the polymeric microparticles required for administration of an efficient dose led to the death of cells.

Schroeder et al. determined the acute effects of polystyrene microspheres with a size of 3, 8, 15 and 25 μm upon intravenous administration to beagle dogs. Four weeks after application they found that all 25 and 15 μm particles and the major part of the 8 μm particles were accumulated in the lung. Traces of the 8 μm particles could also be found in the liver, spleen, heart and kidneys. The main part of the 3 μm particles was found in the liver, followed by spleen and lung. Traces of these particles were detectable in the kidneys and the heard (Schroeder et al., 1978a). The histological evaluation of the tissue samples showed no evidence of damage that could be attributed to the presence of microspheres in the organs (Schroeder et al., 1978b). These results were confirmed by Kanke et al. Furthermore, these authors found that large microspheres (12 μm) are not phagocytized within 4 weeks after intravascular administration to beagle dogs whereas smaller sphere sizes are phagocytized, implying a size limit for phagocytosis (Kanke et al., 1980).

However, nowadays the discussion about nanotoxicity seems to go into a wrong direction. At the beginning we had the equation "nano = good", nowadays it appears that we are rather moving towards a witch hunt for everything which is nano. It is the time to essentially assess the benefits and risks on scientific, neutral and emotionless basis.

The most important factor for potential nanotoxicity is the lack of biodegradability of many nanomaterials. After generation of these nanomaterials, they will stay for ever and pollute the environment and, considering the circle in the environment, finally end up in the human body. Examples for such materials are Fullerenes and carbon nanotubes. At the beginning the Fullerenes were even considered to be used as "hollow spheres" for transporting drugs to their target sites, but it was completely forgotten that they cannot be biodegraded. The same is valid for carbon nanotubes. To illustrate the potential dangers of such non-biodegradable nanomaterial, it is simply referred to the clarification plants for sewage water. Clearing of the water from suspended particulate material is performed by sedimentation process. After sedimentation of the particles, the water supernatant is being removed. However, nanoparticles will never sediment, in a normal clarification plant they will stay in the water forever, and of course with the water they will finally end up in the drinking water for humans. It might be that in the future for clearing water from nanomaterial, special processes have to be developed, e.g. further development of controlled flocculation by addition of flocculants (however many flocculants are also toxicologically dubious).

The outstanding advantage of lipid nanoparticles is their easy and complete biodegradation. Lipids are natural materials, glycerides are easily degraded by natural processes such as enzymes. The time for degrading lipid nanoparticles is depending on the nature of the lipid and the stabilizers used (Olbrich and Müller, 1998; Olbrich, 2002). The degradation products, fatty acids and glycerol, are natural compounds present in the human body (at least when selecting lipid matrix materials which are composed of fatty acids being present in the body, e.g. using fatty acids from emulsions for parenteral nutrition).

To judge about the safety or toxicity of carriers systems like lipid nanoparticles it is important to perform studies comparing the toxicity of lipid nanoparticles with other nanoparticulate carrier systems and to compare lipid nanoparticles composed of different excipients with each other.

Nanoparticles can cause cytotoxicity by adherence of the particle to the cell membrane, degradation and subsequent release of cytotoxic degradation products (Lherm et al., 1992). Another mechanism is the internalization of nanoparticles by cells, intracellular degradation and subsequent toxic effects inside the cell. There are quite a number of different cell culture studies looking at the viability of cells evaluating either the damage of the cell membrane, e.g. by neutral red uptake or LDH release or other factors like activity of the mitochondrial succinate dehydrogenases of living cells (MTT test).

It was shown by Müller et al. that the phagocytic uptake of SLN is low applying them to human granulocytes. At 2.5% SLN concentration no toxic effects were observed on these cells. That shows, that SLN do not exhibit any extracellular toxic effect. Comparing the internalization effect of SLN and PLA/GA nanoparticles by human granulocytes, it was found that the effect is similar for both nanoparticles. Nevertheless, the toxicity of SLN was much lower. Comparing the toxicity of SLN composed of Compritol and cetylpalmitate the effect was similar for both systems indicating a good tolerability (Müller et al., 1997a).

Schöler et al. found that the cytotoxicity of SLN assessed by MTT test on murine peritoneal macrophages is concentration dependent and influenced by the lipid matrix. SLN composed of stearic acid or dimethyl-dioctadecylammonium bromide showed toxic effects at concentrations of 0.01%, whereas SLN composed of triglycerides, cetylpalmitate and paraffin did not exhibit major cytotoxic effects at the same concentration (Schöler et al., 2002). Furthermore, it was found that the size of SLN did not affect the cytotoxicity (Schöler et al., 2001, 2002).

Müller et al. analyzed the influence of nanoparticulate carrier, the lipid matrix of SLN and surfactants on the cytotoxicity using HL60 cells (Müller et al., 1997b). It was found that SLN show lower toxicity compared to polyalkylcyanoacrylate and PLA/GA nanoparticles. The nature of the lipid (Dynasan 114, Compritol ATO 888) had no influence on the viability of HL60 cells. In a concentration range of 0.015–1.5% SLN no significant difference in viability was found compared to reference cells. Distinct differences were found for surfactants (Poloxamer 407, Tween 80, Soya lecithin and sodium dodecyl sulfate). The cell damaging effect of surfactants depends on the status of the molecules, i.e. free in solution or bounded to surfaces. Binding of the surfactant to the SLN surface was found to reduce the toxicity. The same effect was observed by Olbrich et al. using RAW 264.7 cells (Olbrich et al., 2004). This was confirmed by Kristl et al., assessing the effect on cell viability of the surfactant Tyloxapol in solution and an SLN-dispersion stabilized with the same surfactant using Jurkat and HEK 293 cells. It was found, that both the solution of Tyloxapol and the SLN showed an initial unfavorable effect, while longer incubation periods result in cell recovery after application of Tyloxapol stabilized SLN whereas Tyloxapol solution caused cell death (Kristl et al., 2008).

In another study comparing the cytotoxicity of magnetite-loaded polylactide (PLA) nanoparticles, polylactide/glycolide (PLA/GA) nanoparticles and SLN using human granulocytes, the effective concentration to reduce the cell viability in the MTT test to 50% was 0.38% for high molecular weight PLA, 0.30% for low molecular weight PLA, 0.15% for PLA/GA and over 10% for SLN. That means SLN were the least cytotoxic formulation (Müller et al., 1996).

Weyenberg et al. investigated the influence of SLN formulated using different lipids and different surfactants on cell viability of J774 macrophages, mouse 3T3 fibroblasts and HaCaT keratinocytes using the MTT test (Weyenberg et al., 2007). The surfactant had a big impact on the toxicity of SLN. SLN formulated with lecithin, sodium taurocholate, phosphatidylserine and polysorbate 80 did not affect the viability of the three cell lines while the cell viability was significantly reduced by stearylamine. SLN formulated with stearic acid were toxic for all cell lines exhibiting the most toxic effect on macrophages. Viability of >90% was observed when semi-synthetic glycerides or hard fat was used to formulate SLN.

Membrane damage and MTT reduction are relatively good parameters to assess cytotoxicity. However, it is more appropriate and more sensitive to look at the release of cytokines.

Schöler et al. showed that the interaction of Compritol and cetylpalmitate SLN with murine peritoneal macrophages does not cause stimulation of pro-inflammatory cytokines (IL-6, IL-12 and TNF-α) responses. At higher concentration levels of SLN a significant decrease in IL-6 production caused by cytotoxic effects of SLN was observed (Schöler et al., 2000a). This was confirmed in other studies, where it was also found that the size of SLN did not influence the cytokine production (Schöler et al., 2001, 2002).

Furthermore, it was found by Schöler et al. that SLN preserved with thiomersal did neither cause an increase in cytotoxic effects on the murine peritoneal macrophages nor led to secretion of proinflammatory cytokines by these cells compared to unpreserved SLN (Schöler et al., 2000b).

Comparing all these data, and considering the biodegradability without toxic degradation products, the lipid nanoparticles are really very well tolerated at the cellular level.

In general mucosal surfaces can be considered as being more sensitive towards toxic effects compared to the skin, being protected by the stratum corneum and having in general the function to protect against the environment. Recently the pulmonary cytotoxicity of lipid nanoparticles was investigated. Nassimi et al. compared the *in vitro* cytotoxicity on human alveolar epithelial cancer cell line (A549) by MTT test and neutral red assay and the *ex vivo* toxicity on murine precision cut lung slices. SLN reduced the cell viability in a concentration dependent manner. The *in vitro* toxic dose of SLN was approximately 2000 μg/ml. While the *ex vivo* toxic dose was 500 μg/ml. For efficient pharmaceutical drug delivery lower concentrations are expected to be needed (Nassimi et al., 2008). Therefore, the lipid nanoparticles are a promising new drug delivery system for the lungs. Considering the good tolerability of the lipid nanoparticles in the lung at the simultaneously higher sensitivity of the alveolar epithelial cells, especially when administering the lipid nanoparticles to the normal skin, they will be very well tolerated and are safe for dermal application.

For topically applied nanoparticles a frequently discussed question is if and to which extent the nanoparticles might penetrate deeply into the epidermis, maybe reaching dermis and finally the systemic circulation. At present there is an increased concern that very small nanoparticles might penetrate via the hydrophilic channels (about 50 nm sized) into the skin. This is especially valid for example for titanium dioxide nanoparticles used in sunscreens which have sizes about 10–20 nm. Of course the quantity will be definitely very low but the titanium dioxide nanoparticles are not biodegradable. In addition even low particle quantities can interact with the immune system. Regarding this one will be on the safe side with lipid nanoparticles because typically the size is around 200 nm and therefore well above the 50 nm. Even if smaller lipid nanoparticles should be present in the formulation, the material can be biodegraded. In addition the theoretical potential amounts in the skin are so low that they are few dimensions below the concentrations which caused an effect on cytokine production and release. Even assuming that some lipid nanoparticles might arrive in the systemic blood circulation, they will be very well tolerated. Based on their composition they are rather a kind of parenteral nutrition. In the 1990s *in vivo* studies showed that intravenously injected lipid nanoparticle suspensions were very well tolerated (Weyhers, 1995; Weyhers et al., 1995).

Weyhers et al. performed i.v. injections of SLN composed of either Compritol or cetylpalmiate in mice. The administered dose was extremely high, 400 μg SLN dispersion with lipid content of 10%, six times bolus injection within 20 days. This does correspondents to 100 g of lipid given six-fold to men (75 kg) in a bolus injection. Using SLN as a drug carrier the does of lipid would be distinctly lower (e.g. single does of 1 g lipid, corresponding to 10 ml of 10% SLN dispersion). For the cetylpalmitate SLN no pathological effects were obtained even in this high concentration. SLN composed of Compritol led to an accumulation of the lipid in liver and spleen and subsequently to pathological alterations which were partially reversible within 6 weeks after i.v. administration. These side effects were attributed to the slow degradation of the Compritol matrix. Administration of Compritol SLN in a lower dose (200 μl SLN dispersion, lipid content 2.5%) led to a good tolerability. All in all this *in vivo* study of SLN indicated a good tolerability of this carrier system (Weyhers et al., 2005).

The tolerability of lipid nanoparticles was also investigated intramuscularly. They were injected i.m. and the tissue reactions were compared with non-biodegradable aluminum hydroxide (Müller et al., 2000b). Also these data prove good tolerability of the lipid nanoparticles.

The use of solid lipid matrices is known in pharmacy for many years, e.g. drug release from lipid pellets. Solid lipid mircoparticles were introduced by Speiser et al. (Speiser, 1990; Eldem et al., 1991). In the next step the size of the particles was further reduced to the nanosize yielding the lipid nanoparticles, the SLN and as a further development of the SLN the NLC. For topical applied lipid nanoparticles all excipients, which used in the current topical cosmetic and dermal pharmaceutical products, can be used. In addition, GRAS substances and substances with accepted GRAS status can be used. The choice of the lipid matrix and surfactant is essential in order to formulate an optimal safe and stable formulation (Weyenberg et al., 2007).

Despite nanoparticles being biodegradable and leading to non-toxic degradation products, the particle itself might interact in an undesired way with the body prior to its degradation. Lipid nanoparticles were investigated intensively regarding their effects on skin when the cosmetic products were introduced to the market. In Europe such products undergo certain tests. Animal tests like the Draize skin irritation test or Draize rabbit eye test are prohibited for cosmetic products in Europe (Verordnung über kosmetische Mittel, 2008). To ensure the safety of cosmetic products alternative tests are performed, e.g. the Epikutan test, the human patch test, the EPISKIN test, the HET-CAM test and cell culture tests (Spielmann, 1992; Prinsen, 1999; ZEBET, 2001; Liebsch et al., 2004; Hartung, 2007; Vinardell and Mitjans, 2008). That means, thanks to the cosmetic products, broad skin irritation studies have been successfully performed prior to placing lipid nanoparticle products on the market.

To evaluate the skin irritation potential and the eye irritation potential of SLN and dendritic core-multishell nanoparticles the EPISKIN test and HET-CAM test were performed by Küchler et al. (2008). No irritation potential according to EU classification system R38 was found for both carriers with the EPISKIN test. In the HET-CAM test no eye irritation potential was found for both SLN and dentritic core-multishell nanoparticles.

The evaluation of cell viability by MTT test on human fibroblasts and keratinocytes after application of SLN and SLN containing

prednicarbate showed a viability >90%. Cell viability evaluated by MTT test after application of prednicarbate containing SLN to reconstructed epidermis was 94.5% (Santos Maia et al., 2000). This shows a good tolerability of the carrier system by the skin.

It can be summarized that it might be difficult to find a nanoparticulate delivery system for which so many regulatorily accepted excipients (lipids, surfactants and stabilizers) are available than for the lipid nanoparticles. In addition a priori they are made from materials which are natural compounds in the body or are composed of glycerides being made from natural compounds in the body (fatty acids and glycerol). On top they are easily degraded by processes available in the body. In addition the dermal administration route further reduces the risks of cytotoxicity or systemic toxicity. The excellent tolerability of the lipid nanoparticles is supported by many available cosmetic dermal products already being introduced to the market fulfilling the regulatory requirements towards tolerability and nanotoxicity. From this it appears justified to call the lipid nanoparticles a "nanosave" carrier.

7. Conclusion and perspectives

SLN and NLC are very well-tolerated carrier systems for dermal application. The production of these carrier systems as well as of lipid nanoparticle containing topical formulations is feasible in laboratory and on large scale. The physical stability of the SLN and NLC in dermal products was proven for various formulations and can be assessed using well-established methods.

Many features of SLN and NLC that are advantageous for dermal application of cosmetic and pharmaceutical products have been reported, e.g. occlusive properties, increase in skin hydration, modified release, increase of skin penetration associated with a targeting effect and avoidance of systemic uptake.

The first two cosmetic products containing lipid nanoparticles were introduced to the market in 2005. Within 3 years after the introduction of the first products about 30 cosmetic products containing lipid nanoarticles are in the market nowadays.

SLN and NLC have shown many advantages for dermal delivery of drugs but unfortunately up to now there are no pharmaceutical products on the market containing lipid nanoparticles. It could be shown already for various drugs that topical formulations containing lipid nanoparticles can enhance the penetration into the skin increasing treatment efficiency, target the epidermis, reduce systemic absorption and side effects. Furthermore, an increased activity as well as prolonged activity was reported while the benefit/risk ratio was increased for many drugs. Due to the superior performance of lipid nanoparticles containing topical formulations compared to market formulations, the market introduction of pharmaceutical topical formulations is expected in the near future.

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