Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations

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Abstract

Solid lipid nanoparticles (SLN) were developed at the beginning of the 1990s as an alternative carrier system to emulsions, liposomes and polymeric nanoparticles. The paper reviews advantages—also potential limitations—of SLN for the use in topical cosmetic and pharmaceutical formulations. Features discussed include stabilisation of incorporated compounds, controlled release, occlusivity, film formation on skin including in vivo effects on the skin. As a novel type of lipid nanoparticles with solid matrix, the nanostructured lipid carriers (NLC) are presented, the structural specialities described and improvements discussed, for example, increase in loading capacity, physical and chemical long-term stability, triggered release and potentially supersaturated topical formulations. For both SLN and NLC, the technologies to produce the final topical formulation are described, especially the production of highly concentrated lipid nanoparticle dispersions >30–80% lipid content. Production issues also include clinical batch production, large scale production and regulatory aspects (e. g. status of excipients or proof of physical stability).
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Keywords: Occlusivity; Topical drug targeting; Triggered release; Sunscreens; Film formation; Stability; Supersaturation; Loading capacity; Large scale production

Contents

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1. Introduction

During the last 20 years there was only one novel carrier system which can be considered a major innovative contribution in the dermal area, the liposomes first introduced to the cosmetic market by Dior in 1986. After some years delay, liposomes appeared on the market in pharmaceutical products. Apart from technological benefits, the liposome as a novel carrier found broad attention among the public. There is quite a number of other formulation principles used during the last two decades, e.g. microemulsions, multiple emulsions and also solid particles (e. g. microsponge delivery system (MDS), thalaspheres). However, none of them found a broader application due to various reasons and none of them received comparable attention as the liposomes.

Compared to liposomes and emulsions, solid particles possess some advantages, e.g. protection of incorporated active compounds against chemical degradation and more flexibility in modulating the release of the compound. Advantages of liposomes and emulsions are that they are composed of well tolerated excipients and they can easily be produced on a large scale, the pre-requisite for a carrier to be introduced to the market. At the beginning of the 1990s, the advantages of solid particles, emulsions and liposomes were combined by the development of the 'solid lipid nanoparticles' (SLN). The SLN were realised by simply exchanging the liquid lipid (oil) of the emulsions by a solid lipid, which means lipids being solid at room temperature but also at body temperature. There are two basic production methods

for SLN, the high pressure homogenisation technique developed by Müller and Lucks [1] and the microemulsion technique invented by Gasco in Turin [2].

At the beginning of SLN research, there were basically only three research groups working on this topic, apart from the groups of Müller and Gasco, the group of Westesen in Braunschweig [3]. The SLN system found more attention which was clearly documented in the increase of research groups working in this area and the number of published papers, a first review being published in 1995 [4]. The increase in research groups working with SLN continued, which is documented in two major SLN reviews covering the last decade of SLN research in the last century [5,6]. However, the research activities in SLN of this last decade focussed almost exclusively on pharmaceutical applications, and within these pharmaceutical applications mainly on non-dermal administration routes, i.e. oral administration and parenteral injection. However, during the last 4 years, SLN were used in topical formulations, not only for pharmaceutical but also for cosmetic products. Apart from the benefits of SLN for topical delivery of active compounds, another reason was the recognition that the time-to-market is very short for these products. This paper reviews the research in topical delivery of the last 5 years; for non-dermal and pharmaceutical delivery readers are referred to the reviews cited above which still representing the state of the art [4–6]. The two major SLN reviews [4,5] contain a high number of relevant SLN references—references regarding basic mechanisms such as drug incorporation. For some basic aspects,

readers are referred to these reviews and the sections of them describing general basic principals.

2. Features of SLN

2.1. Regulatory status of excipients

One hurdle for a formulation to be introduced to the market is the use of excipients having no accepted status. For topical SLN, all excipients used in current topical cosmetic and dermal pharmaceutical products can be used. In addition, GRAS substances and substances with accepted GRAS status can be used [7]. This provides a broad variety of lipids and surfactants/polymers for the formulation of SLN dispersions. Another important point is that these excipients are normally used in similar concentrations as in marketed products. There is no need to use higher surfactant concentrations, avoiding the potential necessity to perform a tolerability study for the excipient.

2.2. Laboratory scale and large scale production

Production of SLN by high pressure homogenisation can be performed using either the hot or the cold homogenisation technique. For both techniques, the active compound is dissolved, solubilised or dispersed in the melted lipid. In the hot homogenisation method, the active compound containing lipid melt is dispersed in hot surfactant solution of the same temperature by high-speed stirring. The obtained pre-emulsion is then passed through a high pressure homogeniser. Typical production conditions are 500 bar and two or three homogenisation cycles. In the cold homogenisation method, the active compound containing lipid melt is cooled and, after solidification, the lipidic mass is ground to yield lipid microparticles. The lipid microparticles are dispersed in cold surfactant solution by stirring, yielding a macro-suspension. This suspension is passed through a high-pressure homogeniser, the microparticles are broken down to solid lipid nanoparticles. The particles stay in their solid state—it is practically a kind of wet-milling process.

Hot homogenisation is the most frequently applied technique; in general even temperature sensitive compounds can be processed because the exposure time to elevated temperatures is relatively short. The cold homogenisation technique is recommended for extremely temperature sensitive compounds and hydrophilic compounds, which might partition from the liquid lipid phase to the water phase during the hot homogenisation [8].

Laboratory scale production can be performed using piston-gap homogenisers; in the case of very expensive compounds, the use of an Avestin B3 is recommended having a batch volume as small as 3 ml dispersion [9]. The Micron Lab 40 can be considered as the standard machine for laboratory scale—the batch size is 40 ml in the discontinuous version and ≈ 200–500 ml in the continuous version [10].

The definition of medium scale and large scale batches depends, of course, on the product to be produced. For particles with highly active compounds, a size of 10 kg dispersion can already be a large scale batch. This batch size can be realised with a modified Micron Lab 60 system [11]; 10 kg dispersion can be produced within 20 min. The system was qualified and validated, which means it can also be used to produce clinical batches, a pre-requisite to perform any clinical study. For topical products, a large batch is usually in the range of 100 kg dispersion up to 1000 kg (1 tonne) dispersion. Such quantities can be easily realised using a Gaulin 5.5 (150 kg dispersion per hour) or a Rannie 118 going up to 2000 kg/h (APV Systems, Unna, Germany [12]). These homogenisers are used in the pharmaceutical industry; they are accepted in production lines even for parenterals which means there should be no regulatory problems with the production lines. At the same time, the machines can be bought from the shelf as they are low cost equipment.

2.3. Chemical stabilisation of incorporated ingredients

Similar to polymeric nanoparticles, incorporation of active compounds into the solid matrix of SLN can protect them against degradation—this was shown for the cosmetic compound coenzyme Q10 [13]. Coenzyme Q10 was incorporated in SLN composed of cetyl palmitate (10 and 20%), stabilised

with 1.2% Tego Care 450 as surfactant. As a control, a supercooled melt (SM) dispersion of the same drug concentration (2.4%) was prepared. The samples were stored at different temperatures (Fig. 1).

A much more sensitive cosmetic molecule is retinol. Under the influence of light and oxygen, it decomposes to a variety of structures, e.g. different epoxy-retinoids. The stabilisation effect of SLN on retinol was investigated using different lipids as matrix material and different surfactants and surfactant mixtures [15–17]. Firstly, the study revealed that the stabilisation effect differed between the lipids used, this indicates that for very sensitive molecules, the lipid has to be selected carefully. Unfavourable lipids (e. g. too acidic lipids) can lead to a less pronounced stabilisation. Secondly, different extents of stabilisation were observed as a function of surfactant. This was an indication that obviously a pronounced fraction of retinol was localised in the outer shell, which means at or close to the interface lipid/surfactant to water. This was confirmed when investigating the stabilisation effect on retinol using the optimal lipid and the optimal surfactant but preparing particles different in size. The smallest particles with the largest interface area had the most pronounced stabilisation effect. Obviously, retinol located in the surface layer and an optimal surfactant showed highest stability, which means the smallest particles providing the largest interfacial area for

retinol accommodation led to highest stabilisation (Fig. 2).

It was a somewhat unexpected result. However, it is in agreement with the different models of compound incorporation which have been developed for SLN.

2.4. Models for incorporation of active compounds into SLN

There are basically three different models for the incorporation of active ingredients into SLN [19]:

(I)   Homogeneous matrix model
(II) Drug-enriched shell model
(III) Drug-enriched core model.

The structure obtained is a function of the formulation composition (lipid, active compound, surfactant) and of the production conditions (hot vs. cold homogenisation).

A homogeneous matrix with molecularly dispersed drug or drug being present in amorphous clusters is thought to be mainly obtained when applying the cold homogenisation method and when incorporating very lipophilic drugs in SLN with the hot homogenisation method. In the cold homogenisation method, the bulk lipid contains the dissolved drug in molecularly dispersed form, mechanical
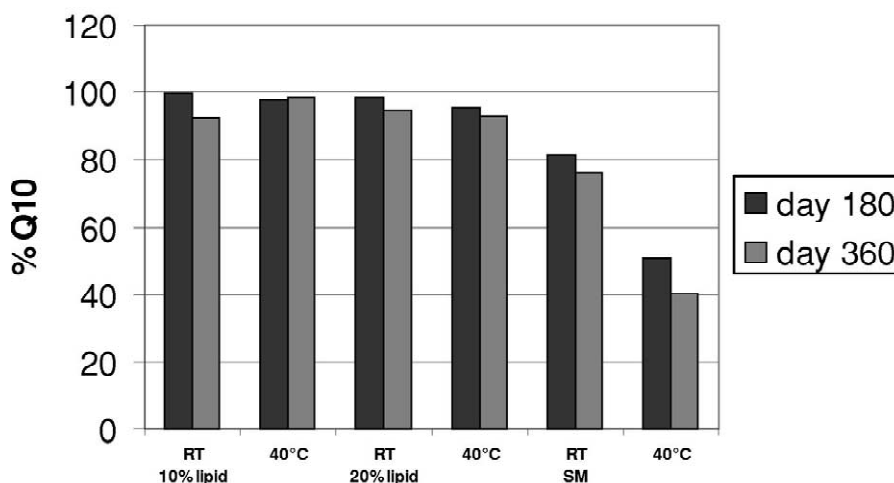

Fig. 1. Stability of coenzyme Q10 incorporated in SLN (10 and 20% lipid content) and as supercooled melt dispersion stored at different temperatures (with permission from Ref. [14]).
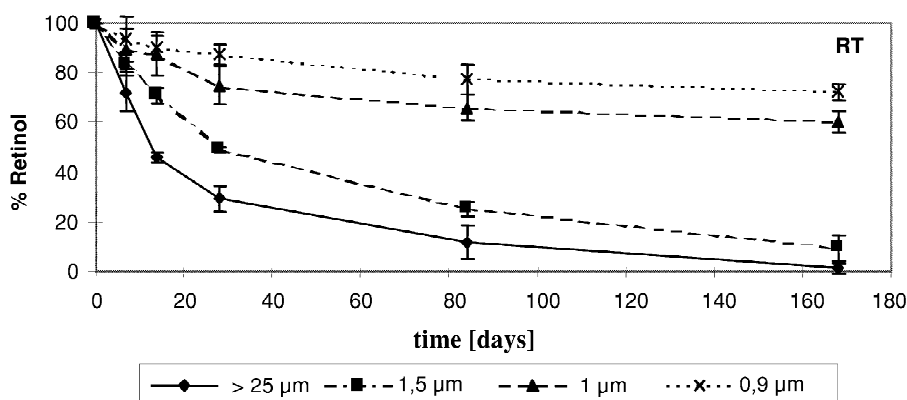
Fig. 2. Stabilisation effect of differently sized SLN being composed of optimal lipid and optimal surfactant. Sizes are given as laser diffractometry diameters 90% measured on day 1 (modified from Ref. [18]).

breaking by high pressure homogenisation leads to nanoparticles having the homogeneous matrix structure (Fig. 3, left). The same will happen when the oil droplet produced by the hot homogenisation method is being cooled, crystallises and no phase separation between lipid and drug occurs during this cooling process. This model is assumed to be valid for incorporation of, e.g. the drug prednisolone, which can show release from 1 day up to weeks [20].

An outer shell enriched with active compound can be obtained when phase separation occurs during the cooling process from the liquid oil droplet to the formation of a solid lipid nanoparticle. According to the TX diagram, the lipid can precipitate first forming a practically compound-free lipid core. At the same time, the concentration of active compound in the remaining liquid lipid increases continuously during the forming process of the lipid core. Finally, the compound-enriched shell crystallises comparable

to the eutecticum in the TX diagram. This model is assumed, for example, for coenzyme Q10 [13]—the enrichment leads to a very fast release. A fast release can be highly desired when application of SLN to the skin should increase the drug penetration, especially when using the occlusive effect of SLN at the same time.

A core enriched with active compound can be formed when the opposite occurs, which means the active compound starts precipitating first and the shell will have distinctly less drug (Fig. 3, right). This leads to a membrane controlled release governed by the Fick law of diffusion.

The three models presented each represent the ideal type. Of course, there can also be mixed types which can be considered as a fourth model.

From this, the structure of SLN formed clearly depends on the chemical nature of active compound and excipients and the interaction thereof. In addition, the structure can be influenced or determined by the production conditions (Section 2.5).

2.5. Release of active compounds from SLN

The effect of formulation parameters and production conditions on the release profile from SLN was intensively investigated by Mehnert, Müller and zur Mühlen [20–25]. For example, they investigated the release profile as a function of production temperature. It can be summarised that the release profiles were often biphasic—an initial burst release was followed by a prolonged release. The burst
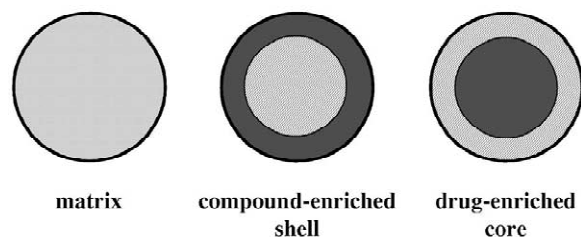

Fig. 3. Models of incorporation of active compounds into SLN: homogeneous matrix (left), compound-free core with compound-enriched outer shell (middle), drug-enriched core with lipid shell (right).

release was highest when producing at highest temperatures and applying the hot homogenisation method. It decreased with decreasing production temperature and was almost non-existent when applying the cold homogenisation method (Fig. 4).

The extent of burst release could also be controlled by the amount of surfactant used in the formulation. High surfactant concentration leads to high burst release, low surfactant concentration to minimisation of the burst (Fig. 4, *z*-axis). This was explained by redistribution effects of the active compound between the lipid and the water phase during the heating up process and subsequently the cooling down process after production of the hot oil in water emulsion during the hot homogenisation process. Heating the lipid/water mixture leads to an increased solubility of the active compound in the water phase, the compound partitions from the melted lipid droplet to the water phase. After homogenisation, the oil in water emulsion is cooled, the lipid core starts crystallising with still a relatively high amount of active compound in the water phase. Further cooling leads to supersaturation of the compound in the water phase, the compounds tries to partition back into the lipid phase; a solid core has

already started forming leaving only the liquid outer shell for compound accumulation (Fig. 5).

From this it can be summarised that the higher the solubility in the water phase during production, the more pronounced is the burst effect. The solubility increases with increasing production temperature and increasing surfactant concentration (the latter only when the surfactant solubilises the active compound). Consequently, little or no burst will be obtained when producing at low temperatures, low surfactant concentration or in surfactant-free medium.

2.6. In vitro occlusion of SLN

Small particles possess an adhesive effect. The adhesion increases with decreasing particle size. The adhesive forces between a flat surface and particle powders as a function of the particle size are well described and can be calculated [26]. Factors influencing adhesiveness are:

F_H = adhesion force
$\hbar\omega$ = Van der Waal's interaction energy
a = distance between adhesion partners
d = particle diameter
ϵ_0 = electric constant
ϵ = dielectric constant
U = contact potential of electric conductors
$\varphi_1 \varphi_2$ = surface charge density of adhesion partners

The adhesive forces can be calculated by:

Van der Waal's forces: $\quad F_H = \dfrac{\hbar\omega}{16\pi} \cdot \dfrac{d}{a}$

In case of a conductor: $\quad F_H = \dfrac{\pi}{2}\epsilon_0\epsilon U^2 \cdot \dfrac{d}{a}$

In case of an isolator: $\quad F_H = \dfrac{\pi}{2}\dfrac{\varphi_1\varphi_2}{\epsilon_0\,\epsilon} \cdot d^2$

The adhesive effect is claimed for small sized liposomes forming a film on the skin after application. The same was postulated for SLN some years ago. Intensive in vitro studies were performed to quantify the occlusivity of SLN in the form of the so-called 'occlusion factor'. First investigations were performed by de Vringer [27]. The in vitro model by
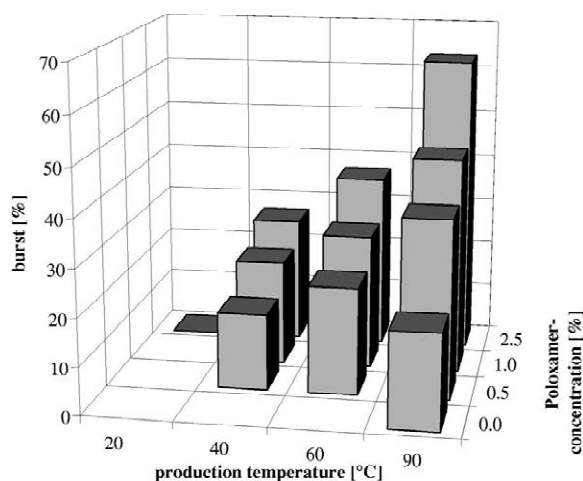
Fig. 4. Percentage of burst release of Compritol SLN containing 1% prednisolone and different amounts of Poloxamer 188 produced by the cold homogenisation method (left) and the hot homogenisation method at 40, 60, and 90 °C (modified from Ref. [25]).

during heating up

diffusion of drug into aqueous phase

cooling down

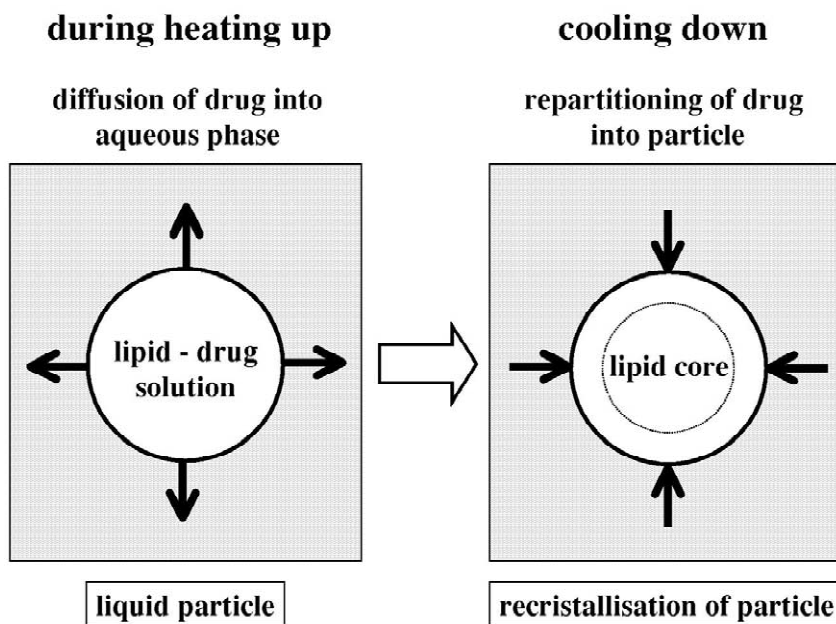### repartitioning of drug into particle

Fig. 5. Model of redistribution of active compound during the heating up and the cooling down phase of SLN production using the hot homogenisation technique; explanations in text (modified from Ref. [22]).

de Vringer consisted of a beaker of water covered by a filter paper. The formulation was spread in a definite amount of 200 mg on a filter surface of 18.8 cm^2; a reference control was a beaker with a filter only. An occlusion factor was calculated by the formula:

$$F = 100 \cdot ((A - B)/A)$$

where A = water loss without sample (reference) and B = water loss with sample.

From this, an occlusion factor of 0 means no occlusive effect compared to the reference; the maximum occlusion factor is 100.

De Vringer investigated only selected formulations; the first systematic occlusion study was performed by Wissing et al. [28], investigating the chemical nature of the lipid, crystallinity of the lipid matrix, and particle size. It could be found that highest occlusivity will be reached with:

1. Low melting lipids
2. Highly crystalline particles
3. Smallest particles

The study of particle size showed that one needs to have really small-sized nanoparticles; lipid microparticles have no or little effect. The study also showed the clear superiority of 200 nm SLN vs. 4 μm microparticles (Fig. 6).

SLN can be admixed to an already commercially available and established topical formulation, e.g. a cosmetic day cream. Admixing the SLN leads to an increase in occlusivity (Fig. 7) while still maintaining the 'light character' of the day cream and avoiding the glossiness of more occlusive night creams. This is a clear marketing advantage. However, having a highly occlusive night cream already, addition of SLN will have little or no effect. The smartness of the concept is that the occlusiveness of day creams can be improved by maintaining their typical day cream character.

Highly occlusive night creams might be produced by preparing creams composed of lipid nanoparticles only (see below NLC) and having the lipid particles at a very high concentration, e.g. 50–60% lipid. This is a field not yet explored.

A first model for the film formation by SLN on the skin was developed by Müller and Dingler [29]—a
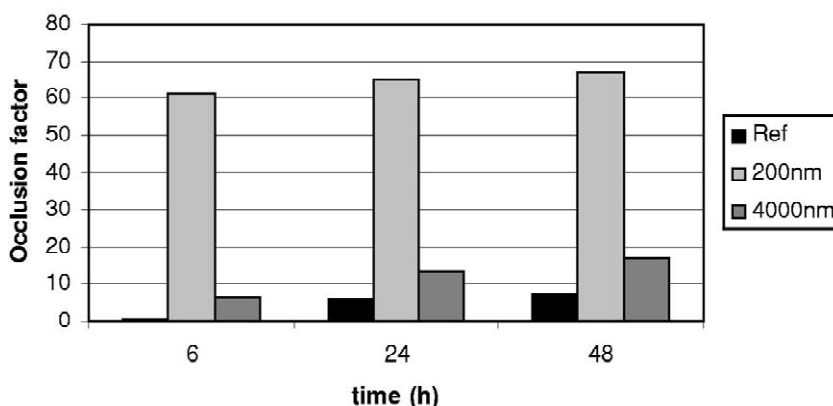
Fig. 6. Occlusivity of 200 nm SLN vs. 4 μm lipid particles as a function of time (modified from Ref. [9]).
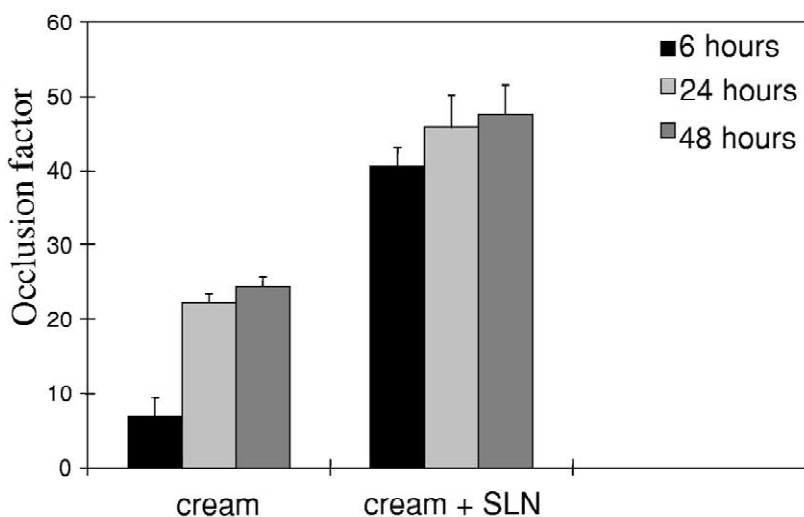

Fig. 7. Occlusion factor of a commercial o/w cream (left) and a cream with additional 4% SLN incorporated (right) as a function of time.

hexagonal packaging in a monolayer was assumed. Fig. 8 shows the difference for 2 μm lipid microparticles compared to 200 nm (note the figure shows correct size relations). In hexagonal packing, about 76% of the surface are covered, 24% are uncovered, meaning the uncovered surface is identical for both the microparticles and the nanoparticles. However, the 'holes' in between the microparticles are relatively large and favour the evaporation of

water hydrodynamically. In contrast, only tiny nano-sized pores exist in the monolayer of SLN. From the pore dimensions, evaporation of water is hydrodynamically unfavourable. The pores are reminiscent of the occurrence of capillary condensation in silica gel. Water condensates in the pores due to their small size and reduced vapour pressure (La Place equation), thus the pores in the SLN film would rather attract than lose water. Recent investigations by
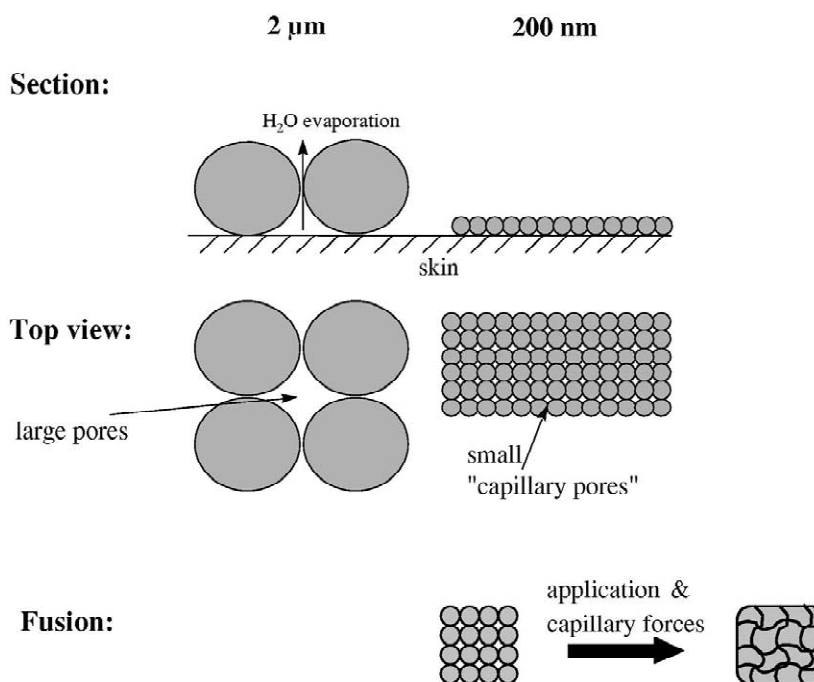
Fig. 8. Model of film formation on the skin for lipid 2-μm particles and lipid 200 nm particles shown as section (upper) and from the top (middle), and a new model of fusion of the nanoparticles to a pore-less film (lower).

electron microscopy showed that after evaporation of the water from an SLN dispersion, a continuous, pore-less film was formed (Fig. 8 lower, and Fig. 9), thus suggesting that the previous model might be in need of updating.

2.7. SLN in vivo: occlusion, elasticity and wrinkle depth

Until recently, to our knowledge no in vivo data about the effect of SLN on skin hydration and elasticity were reported. Of course, investigations were made by various companies; however, these results were kept secret for obvious reasons. One in vivo study was performed with 25 volunteers in which a commercial cosmetic formulation was applied to the left lower arm of each volunteer; the commercial formulation with 4% SLN particles was applied to the right lower arm twice daily for 4 weeks. Skin hydration was measured as a function of time using the Corneometer CM 825 and elasticity

was quantified with the Cutometer SEM 575. Addition of SLN to the established commercial formulation could increase skin hydration by 32% while the pure commercial formulation increased skin hydration by 24% (Fig. 10) [31].

Little or no increase in elasticity was observed. However, this was attributed to the young age of the volunteers—25 years on average. If skin is still highly elastic, there is no room for further improvement. At present, a study is being performed on older volunteers.

The effect on wrinkle depth was studied comparing an established formulation effective in wrinkle treatment with the same formulation having additionally SLN added. It should be noted that the difference found cannot be considered significant; however, from the tendency, the formulation with SLN was more effective (wrinkle depth of untreated control 100%, 95.9% with established cream after 1 week of treatment, and a reduction to 89.7% when treating with cream having SLN added [5]).
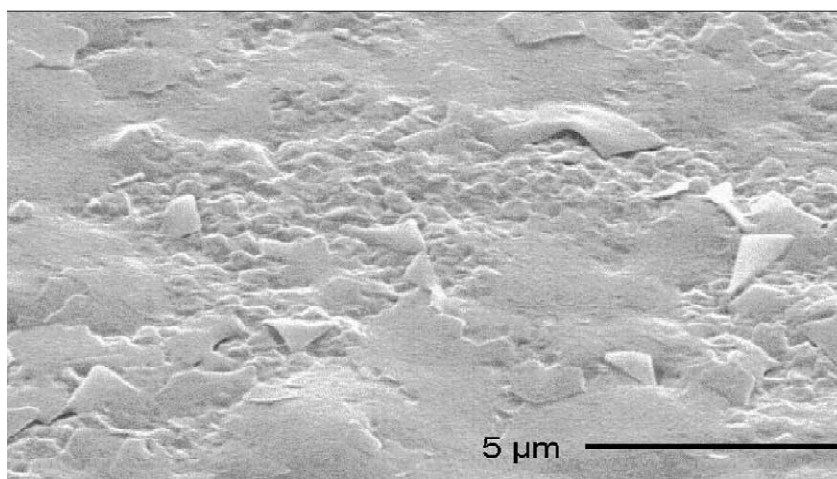
Fig. 9. Electron micrograph of an air-dried SLN dispersion (from Ref. [30]).
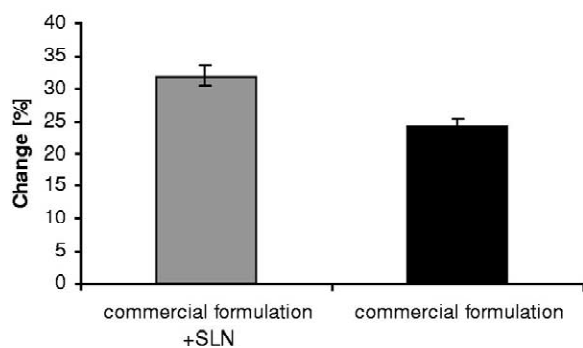

Fig. 10. Skin hydration of a commercial formulation (right) and the commercial formulation after addition of 4% SLN (left) (from Ref. [31]).

To detect adhesive SLN on human skin, a Tesa strip test has been performed [14,32]. The strip was analysed using electron microscopy at different magnifications, showing the presence of SLN at the largest magnification (Fig. 11). From this, the SLN seem to stick to the skin surface, explaining film formation and measured increasing skin hydration.

From these data, the SLN appears as a promising system for skin care.

2.8. Penetration of active compounds into the skin

The penetration of active compounds into human skin was studied using the Tesa stripping test—

investigated compounds included coenzyme Q10 [13,14] and retinol [15–18]. The coenzyme Q10 was dissolved in isopropanol, in liquid paraffin or applied as an aqueous SLN dispersion. Fig. 12 shows the cumulative amount of the compound in the strips as a function of the strip number. SLN proved to be most efficient in promoting penetration into the stratum corneum.

For cosmetic products, it is important that the active compounds stay in the skin, penetrate sufficiently deep but not too deep leading to systemic availability. Penetration should be sufficient to lead to a cosmetic effect but not a pharmaceutic effect. Penetration studies with drugs in pharmaceutical dermal formulations revealed that the degree of penetration obviously depends on the chemical composition of the formulation. Film formation properties and resulting skin hydration, but also the interaction of the SLN lipids and surfactants with the skin lipids (M. Schäfer-Korting, Berlin, personal communication), are considered as factors affecting the degree of penetration.

2.9. Skin penetration of drugs

The results obtained with prednicarbate formulations were very interesting. Depending on the composition of the SLN, different penetration profiles were obtained [33]. In the optimised formulation, a therapeutically desired enrichment in the upper
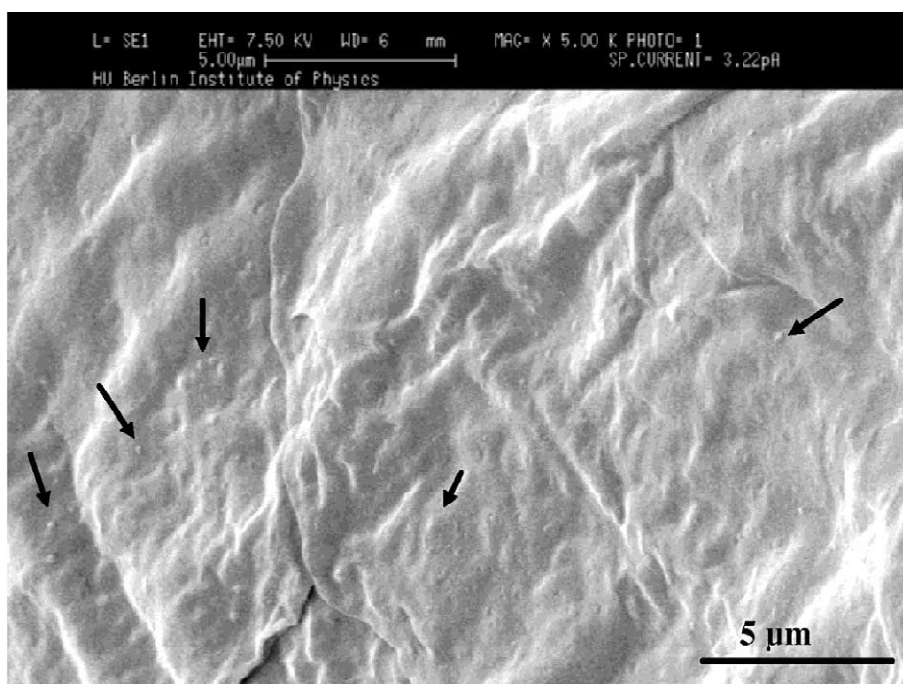
Fig. 11. Electron micrograph of the first skin strip after application of cetylpalmitate SLN dispersion (magnification 1:50 000) (from Ref. [14]).
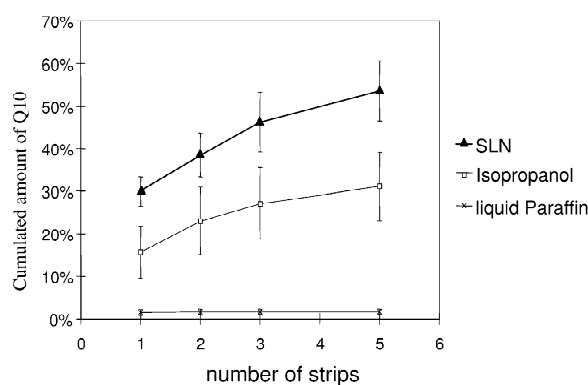

Fig. 12. Penetration of coenzyme Q10 from different formulations into the skin: isopropanol, liquid paraffin and aqueous SLN dispersion (modified from Ref. [14]).

layers of the skin was obtained (targeting effect), simultaneously minimising the systemic uptake (Fig. 13).

In vitro skin penetration studies were also performed in NLC (Section 3.4) using retinol as the compound of interest for cosmetics and pharmaceuticals [15–18]. The studies revealed a different penetration profile compared to the nanoemulsion used as reference. Initially, the concentrations were lower (due to prolonged release from particle), after a 24-h period, higher retinol levels were found in the residual skin.

2.10. Controlled release of cosmetic compounds: perfumes and insect repellents

The perfume Allure was incorporated in SLN and the release studied compared to a nanoemulsion of identical lipid content and surfactant composition. The initial release was similar, most likely due to perfume present in the outer shell of the SLN. During the follow-up period to 8 h, release from the SLN was delayed. This opens the prospect of developing longer lasting perfume formulations based on the prolonged release of the perfume from the solid lipid matrix [34].

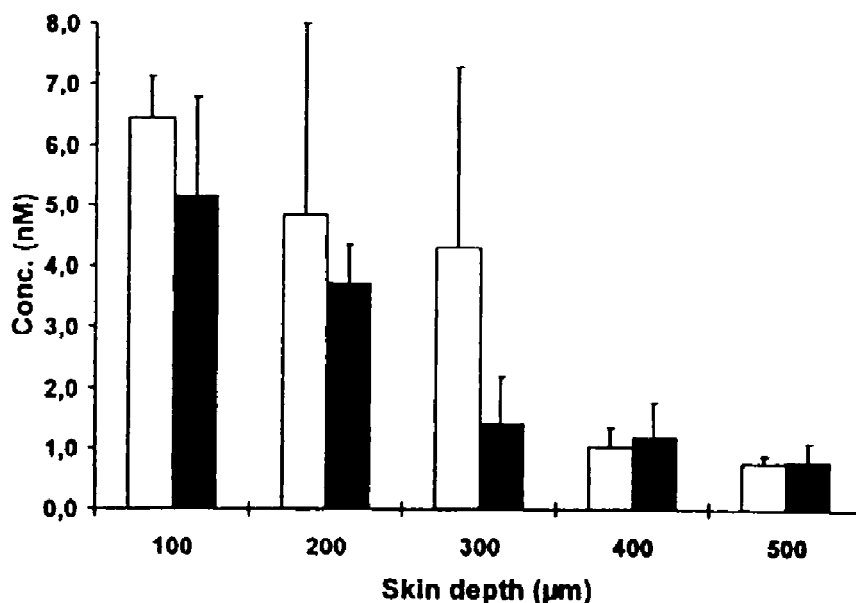Prolonged release is also desired for insect re-

Fig. 13. Distribution of prednicarbate (PC) and its metabolites in human skin after 24 h. The drug was applied as SLN dispersion (open bars) or standard cream (black bars). The PC mean values are given ±S.D ($n=3$) (from Ref. [33]).

pellents while simultaneously the releasing carrier should stick firmly to the skin. From the required adhesiveness, SLN are a suitable carrier system. The insect repellent DEET (*N*,*N*-diethyltoluamide) was incorporated in different SLN formulations in a screening procedure. A loading of 10% (calculated on particle matrix mass) could be achieved in stearic acid SLN stabilised with Tween 80 as surfactant. The particles were physically long-term stable after incorporation into a ready-to-use gel [35,36]

2.11. SLN as novel UV sunscreen system

Due to the reduction of the ozone layer, there is an increasing need of effective UV protection systems with simultaneously minimised side-effects. The two basic UV protection systems are molecular UV blockers (sunscreens) and particulate compounds such as titanium dioxide. Side-effects of molecular blockers are photoallergies and phototoxic effects; as an alternative, particulate blockers are used. Due to their particulate character, it was hoped that they would not show side-effects as the molecular blockers—the mechanism of protection is simply scatter-

ing of UV rays. However, there are indications that the very small titanium dioxide particles (e. g. 5–20 nm) penetrate into the skin and can interact with the immune system [37]. Surprisingly, it was discovered, that highly crystalline solid lipid nanoparticles can also act as particulate UV blockers by scattering the light efficiently. Fig. 14 shows an UV scan of a 10% cetyl palmitate SLN dispersion versus an o/w nanoemulsion of identical lipid content and surfactant concentration.

To enhance the UV protection by SLN further, a molecular sunscreen was incorporated into the solid lipid matrix. Incorporation was performed in a way that the release was prolonged, i.e. very little release within the application time of 6–8 h. As shown for prednisolone, release by diffusion can take place over several weeks [20]. The fixation of the molecular sunscreen inside the solid matrix minimises side effects due to penetration of the molecular sunscreen into the skin. When measuring the UV absorption, it was surprisingly found that incorporation of the molecular sunscreen into the SLN matrix led to a synergistic protective effect, i.e. the measured UV absorption was higher than the theoretically calcu-
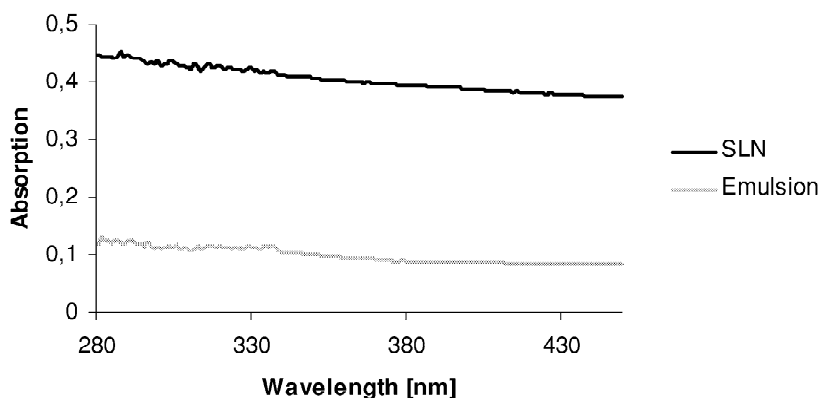
Fig. 14. UV scan (absorption vs. wavelength) of placebo SLN (upper) vs. an emulsion (lower).

lated values from the single effects of the molecular sunscreen and the particle dispersion itself (Fig. 15). This means the total amount of molecular sunscreen in the formulation can be reduced, thus further minimising the side effects in addition to the already achieved reduction by firm incorporation of the sunscreen into the particle matrix.

In vitro release studies were performed to compare the release of the sunscreen from o/w nanoemulsions and from SLN dispersions. A membrane-free release model was used, i.e. putting an oil phase above the aqueous nanoemulsion or aqueous SLN dispersion in a test tube. After 4 h, 6.5% of sunscreen were released from the nanoemulsion, however only 3.1% of the incorporated amount from the SLN dispersion [38]. In this in vitro test, a membrane-free model was used; thus in vivo, even less uptake is expected due to the membrane function of the stratum corneum. Stripping tests on human skin were performed confirming this. The concentrations found on the strips were 2-fold higher for the nanoemulsion compared to the SLN dispersion (6.2 vs. 3.2%) [38].
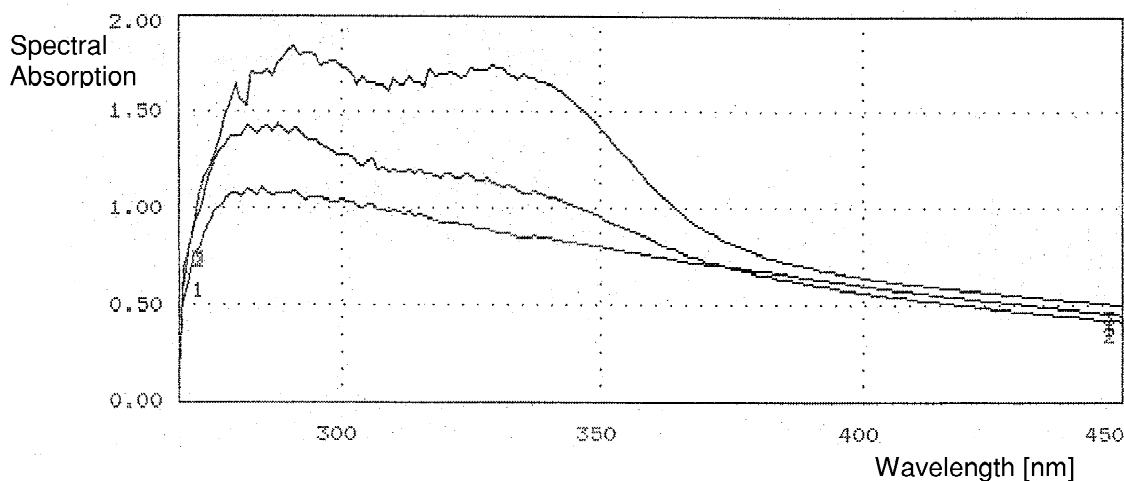

Fig. 15. UV scan (absorption vs. wavelength) of an UV-protective SLN dispersion (upper) vs. a placebo SLN dispersion (lower); theoretical curve calculated from the single UV absorption of molecular sunscreen and SLN dispersion (middle) (modified from Ref. [30]).

3. Nanostructured lipid carriers (NLC): the new generation of lipid nanoparticles with solid matrix

3.1. Potential problems associated with SLN and its production technology

The section above described the advantages and manifold applications of lipid nanoparticles possessing a solid matrix. These advantages are valid for the 'old' SLN system but also for the novel NLC carrier system. However, the NLC system minimises or avoids some potential problems associated with SLN. The review by Mehnert and Mäder [6] highlights these aspects:

1. Pay-load for a number of drugs too low
2. Drug expulsion during storage
3. High water content of SLN dispersions

The SLN are prepared from solid lipids or blends of solid lipids. After preparation by the hot homogenisation technique, the particles crystallise, at least partially, in higher energy modifications α and 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 thus leading to drug expulsion (Fig. 16).

The creation of a less ordered solid lipid matrix is the pre-requisite for a sufficiently high drug-load. In general, the drug can be located in between the chains of the fatty acids or in between the lipid layers and also in imperfections (e. g. amorphous drug clusters). In case of spacially very similar lipid molecules, especially when mono acid highly

Fig. 16. Mechanism of drug expulsion during storage of SLN dispersions; transition to highly ordered lipid crystal (with permission from Ref. [39]).

purified glycerides such as tristearin are used, drug load is very limited and drug expulsion occurs within hours or a few days due to the formation of the perfect β modification [40].

According to the SLN patent, the lipid concentration in the dispersion ranges from 0.1 to 30%. The limitation was set to 30% because, according to the patent, bicoherent creams were formed in the homogenisation process above 30%. The resulting water content of 99.9 to 70% can potentially create problems when incorporating the SLN dispersion into a conventional cream (Section 4). One might not achieve the desired percentage of solid lipid particle mass in the cream. In addition, for the preparation of creams and pastes consisting only of lipid particles, water needs to be removed. That means there was also a need to improve the production process, i.e. to reduce the water content.

3.2. The new concept of NLC

For the production of NLC, spacially very different lipid molecules are mixed, i.e. blending solid lipids with liquid lipids (oils). The resulting matrix of the lipid particles shows a melting point depression compared to the original solid lipid but the matrix is still solid at body temperature. Depending on the way of production and the composition of the lipid blend, different types of NLC are obtained. The basic idea is that by giving the lipid matrix a certain nanostructure, the pay-load for active compounds is increased and expulsion of the compound during storage is avoided.

Fig. 17 shows the three different types of NLC compared to the more or less highly ordered matrix of SLN. The three types of NLC can be summarised:

1. The imperfect type
2. The amorphous type
3. The multiple type

A potential problem in SLN is the formation of a perfect crystal, which can be compared to a dense 'brick wall'. Using different molecules, i.e. different 'stones' to build the matrix or 'wall', leaves enough imperfections to accommodate the drug (Fig. 18).

Drug load in SLN is limited due to the formation of the lipid crystal. Drug expulsion is caused by an
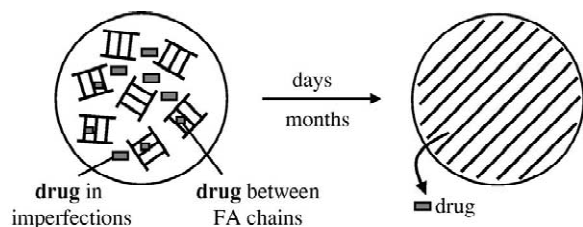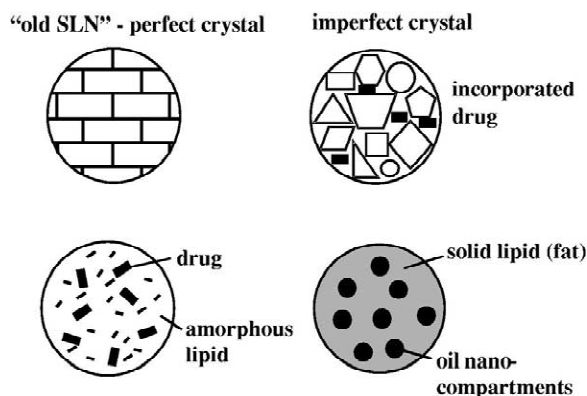
Fig. 17. The three types of NLC compared to the relatively ordered matrix of SLN (upper left), NLC types: imperfect type (upper right), amorphous type (lower left), multiple type (lower right) (with permission from Ref. [39]).

ongoing crystallisation process towards a perfect crystal. Thus, by avoiding crystallisation, one can avoid these obstacles—which is realised in the NLC type 2. The lipid matrix is solid but not crystalline—it is in an amorphous state (Fig. 17, lower left). This can be achieved by mixing special lipids, e.g. hydroxyoctacosanylhydroxystearate with isopropylmyristate. The solid character of the particles was proven by NMR measurements and the lack of crystallinity by DSC analysis [18,41,42].

The third type of NLC is a multiple system, being comparable to w/o/w emulsions. In this case it is an oil-in-solid lipid-in-water dispersion. The solid lipid matrix contains tiny liquid oil nanocompartments (Fig. 17 lower right). This NLC type uses the fact

that for a number of drugs, the solubility in oils is higher than their solubility in solid lipids.

A classical example is retinol. The oil nanocompartments are incorporated into the solid matrix; they contain a higher amount of active compound but release is still controlled by the surrounding solid lipid barrier. In the SLN system composed of only the solid lipid Compritol 888 ATO, the retinol payload was just 1% calculated on the total lipidic mass (lipid + retinol). In the NLC multiple type system, a concentration of 5% retinol could be incorporated and firmly included during long-term storage [18].

The multiple type NLC are produced by mixing a solid lipid with a higher amount of liquid oil. At low concentrations of oil, the oil molecules are distributed within the solid lipid matrix (no oily nanocompartments are formed). When increasing the oil concentration, the solubility of the oil molecules in the solid lipid is exceeded, phase separation occurs and oily nanocompartments are formed. This occurs during the cooling process after production of the particles by the hot homogenisation method. At high temperature, complete miscibility occurs between the melted solid lipid and the oil. During the cooling-down process, the solubility of the oil in the solid lipid compound is exceeded, the oil precipitates in the form of fine droplets being incorporated into the solid lipid matrix. The liquid lipid needs to be chosen so that the melted lipid and liquid lipid are miscible at the production temperature of the lipid particles. During the cooling process, phase separation occurs due to the miscibility gap. Ideally, in
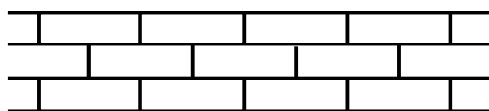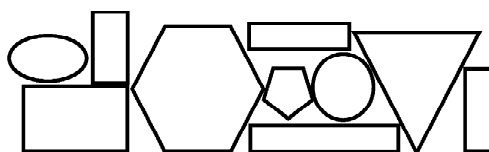
Fig. 18. Perfect crystal in SLN comparable with a brick wall (upper) and structure with imperfections due to spacially very different molecules in NLC type 1 (lower) (with permission from Ref. [39]).

the solid state of the particles, the liquid lipid should only be soluble in the solid lipid matrix to a low degree. Nanocompartments are only found when the liquid lipid is used in such a high concentration that it is well above its solubility in the solid lipid at room temperature. The differences in the solubility of molecules, such as retinol, between the solid and the liquid lipid can be explained by differences in the state of order between a solid and liquid lipid (in the solid matrix with higher order there is less space for accommodation of drugs). In addition, solubility differences in the two lipids are also caused by the molecular structure.

The presence of liquid Miglyol 812 above a certain concentration in the matrix was proven by DSC measurements. Solidification peaks of different modifications of Miglyol 812 were found in the range between −24.5 and −40 °C. The absence of two different particle populations, i.e. solid lipid nanoparticles and separate oil droplets, was also demonstrated. The oil is associated with the solid lipid as shown by NMR and ESR measurements [42–45]. This supports the stated theory in Fig. 17 (lower right). The distribution of the nanocompartments throughout the solid lipid matrix might not be even in any case. Due to a relatively fast compound release in some cases, it is suggested that these nanocompartments can also be eventually localised in the outer shell of the solid particles. In the case where nanocompartments are localised in the outer shell of the solid particles, theoretically it cannot be excluded that some might be transferred to the water phase, and then potentially being solubilised by the

surfactants. However, this is difficult to prove. It could definitely be shown that there are no nanoparticles of solid lipid and separate oil droplets of an emulsion.

In the first exploratory phase of developing the NLC, publications still appeared using the term SLN for this particle type and making no clear differentiation [41,42]. However, these papers describe in detail and also prove the existence of the separate liquid lipid phase in the solid particle matrix. This explains why for some properties of NLC these papers are cited.

3.3. Creation of supersaturated systems with NLC

A potential disadvantage of SLN is the transform to the more perfect β modification leading to drug expulsion. If this transform process can be controlled, it can be used to trigger the release of drugs in a controlled way (Fig. 19).

The principle of supersaturation is one mechanism exploited in topical microemulsions to increase drug penetration into the skin and also to finally achieve availability in the tissue underneath. Microemulsions saturated with drug are applied to the skin; water from the skin diffuses into the microemulsion increasing its water content. In microemulsions with high water content, the saturation solubility of the drug is lower, i.e. water uptake by the microemulsion leads to supersaturation of the drug [46]. The drug wants to leave the microemulsion system—in an in vitro situation, drug crystals would be formed, while
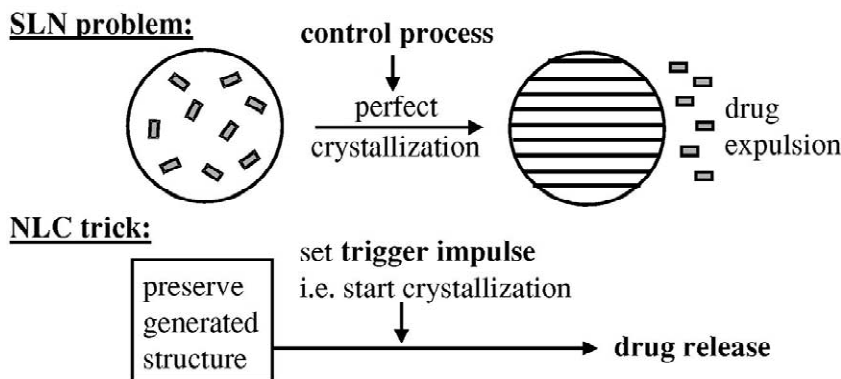

Fig. 19. Triggered release of active compounds by controlling the transform from α and β' to β.

in vivo the only way to leave the microemulsion is to penetrate into the skin.

Similar supersaturation can be created by incorporating drug-loaded NLC in traditional o/w emulsions saturated with drug. During storage on the shelf, the drug remains in the NLC, the NLC structure is preserved accommodating the drug. Application to the skin leads to an increase in temperature and water loss, this initiates the transition to more stable lipid modifications in the NLC leading to drug expulsion. The drug is being expelled into the emulsion being already saturated with drug and thus leading to supersaturation (Fig. 20). At present, studies are being performed to develop this system for topical cyclosporine delivery.

The increase in ordered structure was nicely shown for NLC type 3 (multiple type) with incorporated retinol. During this transition process even the Miglyol 812 molecules in the liquid nanocompartments contained a more ordered structure as shown by NMR measurements (reduced molecular mobility) [42]. From this, it appears feasible to create supersaturated systems similar to microemulsions while simultaneously avoiding the undesired high surfactant concentration of microemulsion systems.

3.4. In vitro penetration into skin

Intensive in vitro penetration studies were performed by Jenning and co-workers [16,18] with lipid nanoparticles loaded with retinol. The particles were produced by mixing retinol-containing Miglyol 812 with melted Compritol 888 ATO and were stabilised by Miranol ultra C32 as surfactant. The studies were performed using the Franz cell model and porcine skin. Preparation of the particles with this relatively high amount of oil led to the formation of oil nanocompartments, which means the solid lipid nanoparticle investigated was in fact an NLC type 3. The flux of retinol from lipid particle dispersion was compared to the flux of retinol from an o/w nanoemulsion which served as control (identical composition, solid lipid replaced by Miglyol 812). The flux of retinol from the nanoemulsion system remained unchanged during the investigated period. Due to increased order and increased expulsion of drug the flux increased from the nanoparticle dispersion (Fig. 21).

Drug penetration into the different layers of the skin was also investigated for the lipid nanoparticle dispersions versus the reference nanoemulsion (Fig. 22). Based on the differences in the flux, different concentrations in the skin layers were obtained; the particle dispersion showed higher concentration values in deeper layers at the end of the investigated time period. In the investigations with retinol, simple aqueous lipid particle dispersions were compared with an o/w nanoemulsion—the systems were not saturated. For the above described supersaturation effect, NLC need to be dispersed in an already saturated emulsion. However, the data by Jenning and co-workers [16,17] show the increase in the
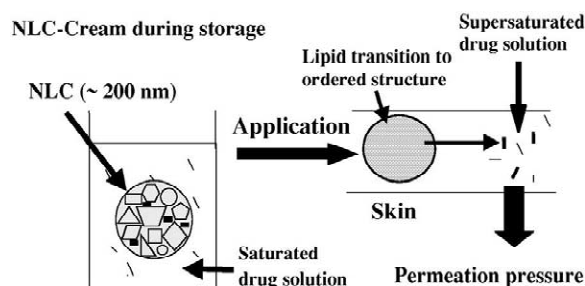

Fig. 20. Triggered drug release and supersaturation effect. Drug-loaded NLC are incorporated into an o/w cream. On the shelf the drug stays inside the NLC (left); after application to the skin, the increase in temperature and water loss initiate transition to higher ordered structure in the lipid particle, drug is being expelled, supersaturation occurs in the oil-in-water phase of the emulsion, thus increasing the thermodynamic activity and leading to increased penetration of drug into the skin.
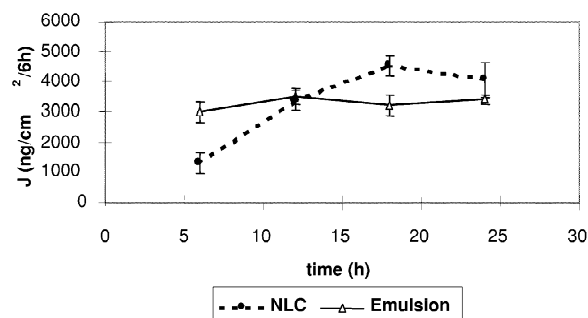

Fig. 21. In vitro release of retinol. Due to an increase in crystalline order, the flux increases from the lipid nanoparticle dispersion, the flux remains unchanged from the reference emulsion (from Ref. [15]).
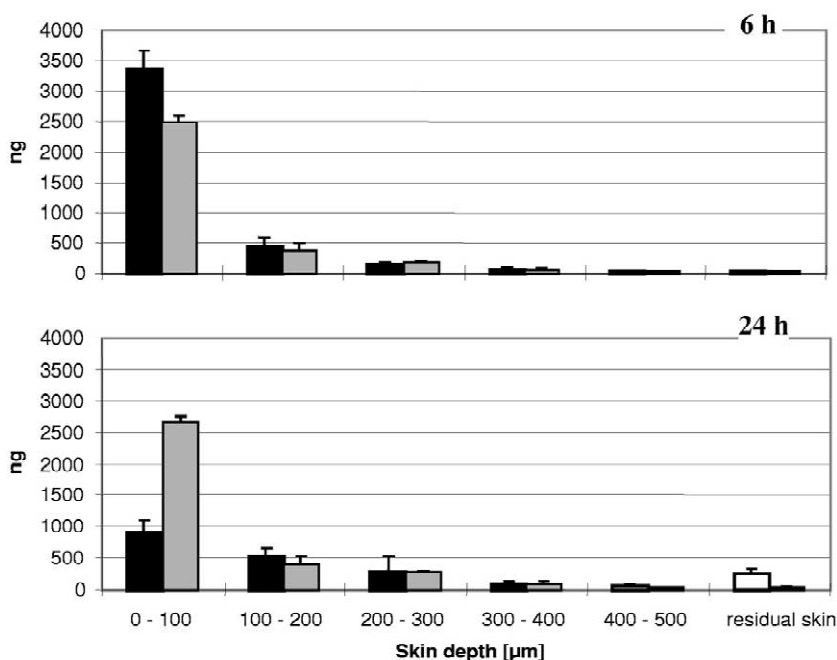
Fig. 22. Distribution of retinol in porcine skin at 6 h (upper graph) and 24 h (lower graph). The drug was applied as NLC dispersion (black bars) or nanoemulsion (gray bars). The retinol mean values are given $\pm$S.D. ($n = 3$) in the individual skin slices (from Ref. [16]).

order of the lipid matrix leading to an increased drug expulsion/flux and subsequently to the observed higher concentration values in some skin layers at the end of the experimental period.

3.5. Novel production technology applicable to NLC and SLN

SLN are produced by high pressure homogenisation of o/w emulsions at elevated temperature (solid lipid is melted) or homogenisation of lipid particle suspensions. For both, a maximum lipid concentration of 30% is claimed in the SLN patent [1]. As mentioned above, the reason was that the cream-like systems produced by the homogeniser were thought to be bicoherent cream structures. However, it could be shown that even when homogenising 50% lipid concentration and higher, the homogenisation product consisted of definite nanoparticles [47–49]. Due to the high particle concentration, the particles form a pearl-like network comparable to aerosol gels for example. This is one of the reasons for the high viscosity of the systems produced. Dilution of the system with water destroys this structure leading to separate, freely diffusible lipid nanoparticles. Fig. 23 shows an EM graph of 35% lipid nanoparticle dispersion, clearly visible is the dense packing of the particles and the small distances between them [49]. Dilution of the system with water to yield a dispersion with 10% lipid concentration is shown in Fig. 23 (left). Definite particles are obtained identical to an SLN dispersion produced with 10% lipid.

Of course, the homogenisation of a lipid concentration above 30% can not only be used for NLC; the same production technology is applicable to produce highly concentrated SLN. A study was performed to investigate the effect of increasing lipid concentration on the obtained particle size; production conditions were kept constant (500 bar, three homogenisation cycles). In each case, nanoparticles were obtained; however, the mean PCS diameter increased from approximately 180 to 280 nm (Table 1).

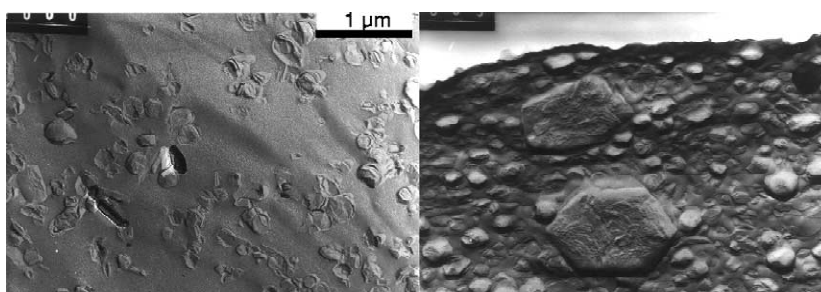The observed increase in particle size is logical

Fig. 23. EM graph of 35% lipid nanoparticle dispersion (right) and of this system after dilution with water to a 10% lipid concentration (left). The mean PCS particle size was 260 nm (bar = 1 μm) [49].

Table 1
Increase of PCS diameter as a function of the lipid concentration in the lipid nanoparticle dispersion (total solid: lipid and emulsifier) (from Ref. [48])

Lipid content (%)	Total solid (%)	PCS size [nm]	Polydispersity index
20	25	180	0.128
30	35	208	0.072
35	40	266	0.210
40	45	283	0.244

and was therefore expected. Three homogenisation cycles at given pressure provide the same amount of dispersion energy for each system; however, the mass to be dispersed increased from 20 to 35 g in a 100-g dispersion. That means less dispersion energy is available per unit lipid, thus leading to larger particle sizes after the three homogenisation cycles. To compensate for this, a higher number of homogenisation cycles needs to be applied for the higher concentrated dispersions to provide the same amount of dispersion energy per unit lipid (i.e. reaching the same small size if required).

Depending on the lipid and type of stabiliser used, lipid dispersions above 50% can be very viscous. Due to the high viscosity, these dispersions cannot be processed with normal homogenisers having no additional features. Most of the homogenisers only process freely flowable systems. For cream- or paste-like systems, additional features are necessary, e.g. pressurising the feeding container to move the mixture to the homogeniser or using a piston-cylinder system (available for homogenisers from Stanstead, UK to process more viscous goods). Therefore, a two-step production method is used to produce, for example, 80% NLC dispersions.

In the two-step production method, first a stock nanoparticle dispersion, with e.g. 60% lipid concentration, is produced by high pressure homogenisation. For better illustration of the process, let us assume we start with 100 g nanoparticle dispersion having 60% lipid, i.e. 60 g lipid and ≈40 g water (ignoring the surfactant). In this mixture, another small amount of lipid, e.g. 10 g is dispersed by high speed stirring, that means that the 10-g are dispersed in the present 40 g water phase. This leads to a dispersion now having 70 g lipid and still 40 g water phase (Fig. 24). In the next step, again 10 g lipid are added and dispersed in the 40-g water leading to a dispersion with now 80 g lipid and still 40 g water phase; again 10 g lipid are added and so on leading to a gradual increase in the total lipid concentration of the system. Of course, the high speed stirring is less effective than high pressure homogenisation thus leading to particles in the nanometer but also low micrometer range (a few μm). However, limited contamination with lipid microparticles appears acceptable in such cases when extremely high concentrated lipid particle dispersions are desired. Of course, the very high concentrated lipid particle dispersions are not paste-like anymore, after cooling to room temperature, they are relatively solid and can be cut with a knife. Such highly concentrated dispersions are of less interest for topical administra-
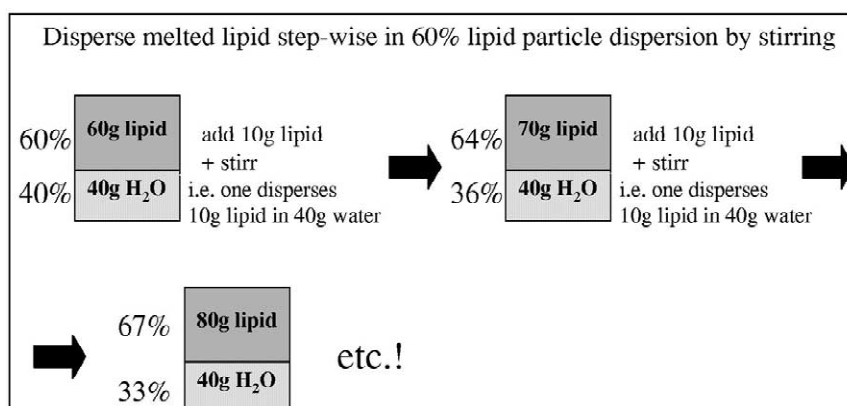
Fig. 24. Scheme for the process of highly concentrated nanoparticle dispersions. A stock dispersion with 60% lipid content is produced by high pressure homogenisation; in subsequent steps small amounts of lipid are added stepwise and each time dispersed by high speed stirring thus increasing the total lipid content from one step to the next (explanation in text).

tion (but they can be used for sticks). They are most interesting for the filling of hard gelatine capsules when using a non-aqueous medium as outer phase for the SLN or NLC dispersion.

3.6. Improved physical stability of highly concentrated lipid nanoparticle dispersions

Identical to any other suspension, the SLN dispersions can aggregate in case of a suboptimal choice of stabiliser type and concentration during long-term storage. Of course, one could try to improve the physical stability by exchanging the surfactant. However, for some applications and administration routes it might be highly desirable just to keep the surfactants used—in case of particles for i.v. injection you use only surfactants accepted for i.v. use. Surprisingly, but only at first glance, it was found that the higher concentrated particle dispersions showed sufficient physical stability during storage whereas low concentrated ones aggregated. Lipid nanoparticle dispersions with increasing lipid content were produced. The higher concentrated were practically unchanged in size during storage avoiding the problems of lower concentrated dispersions (Fig. 25).

The explanation is that in the low concentrated particle dispersions, the particles are freely diffusible—can collide and aggregate. In the highly concentrated dispersions, the particles are fixed in the
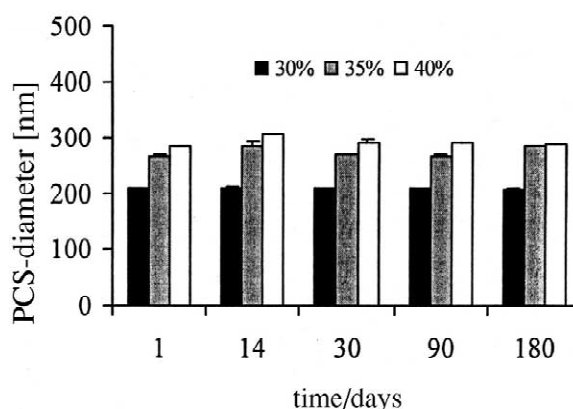
Fig. 25. PCS diameters of differently concentrated cetyl palmitate SLN dispersions during 6 month of storage at room temperature (composition of SLN: 30, 35, or 40% cetyl palmitate; 5% sucrose ester, water).

pearl-like network—diffusion and subsequent aggregation is reduced (Fig. 26).

3.7. Rheological performance of concentrated lipid nanoparticle dispersions

The basic advantage of concentrated NLC dispersions or SLN dispersions produced after the method described above (Section 3.6) is the consistency ranging from soft creams to highly viscous pastes. A topical formulation of desired consistency can be produced in a one-step production process, there is

Fig. 26. Stabilisation effect in highly concentrated lipid particle dispersions. Freely diffusible nanoparticles in low concentrated dispersion can collide and aggregate (upper); in 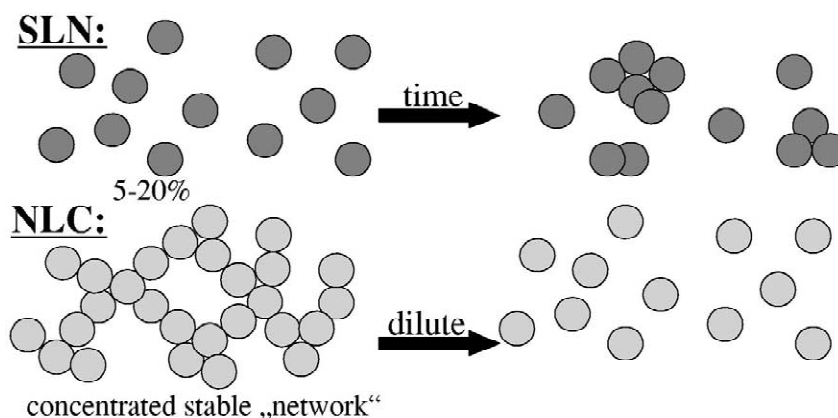highly concentrated dispersions the particles are fixed in a network, while dilution with water releases non-aggregated definite nanoparticles.

no need to perform a subsequent gel formation anymore or to admix the particle dispersions to a cream. In addition, the highly concentrated dispersions offer the possibility to produce topical formulations of desired viscosity from nanoparticles only. This overcomes potential problems with the loading capacity for active compounds (see below).

The viscous and elastic properties of such dispersions are important for their application to the skin and, especially in cosmetics, for the subjective feeling of the customer when using the product. The dispersions were intensively characterised rheologically and compared to standard ointments of the German Pharmacopoeia (e.g. Unguentum emulsificans aquosum). Both systems showed similar viscous and elastic properties (Fig. 27)—the desired rheological profile can be produced by an appropriate selection of the particle concentration. This is of high importance for cosmetic products because these products need to create a 'nice application feeling' when applied by the customer.

4. Formulation of cosmetic products with SLN and NLC

The formulation of topical products is identical for both nanoparticles, SLN and NLC. There are basically three approaches to formulate products:

1. Incorporation of SLN/NLC in existing products
2. Production of SLN/NLC containing gels by addition of viscosity enhancers to the aqueous phase of the dispersions
3. Direct production of the final product containing only nanoparticles in a one-step process using the production process of highly concentrated dispersion (Section 3.6)
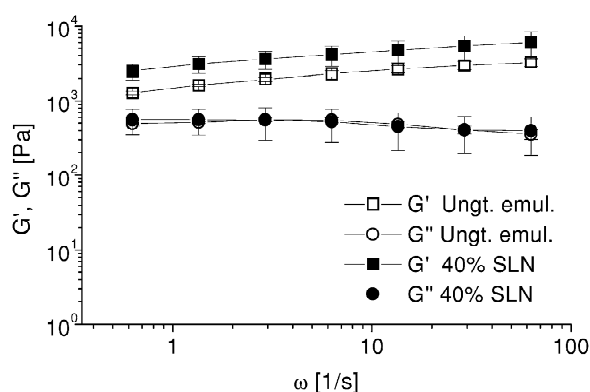
Fig. 27. Storage (G') (squares) and loss (G'') modulus (circles) of unguentum emulsificans aquosum (empty symbols) and 40% SLN dispersion (filled symbols) as a function of the radial frequency (ω) at a stress amplitude of 5 Pa (modified from Ref. [48]).

The first approach exploits the benefits of an already established product and combines them with the additional advantages of lipid particles having a solid matrix, e.g. protection of chemically labile com-

pounds or occlusion effect on skin/skin hydration. To do this, a part of the water in the traditional formulation is replaced by aqueous lipid nanoparticle dispersion. To avoid an undesired increase in viscosity of the product, it might sometimes be necessary to slightly reduce the lipid content of the traditional formulation. To admix the lipid particle dispersion, for example into a cream, there are two ways to do this:

(a) Production of the cream with reduced water content and subsequent admixing of the aqueous lipid nanoparticles
(b) Producing the cream as before but having initially replaced a part of the water in the formulation by the aqueous lipid nanoparticle dispersion

When suggesting method (b), the question arises to which extent the lipid nanoparticles are physically stable during the production process of the cream. From experience, the lipid nanoparticles are sufficiently stabilised to avoid coalescence with each other or coalescence with oil droplets. Depending on the production temperature of the cream and the melting point of the lipid matrix of the nanoparticles, they might melt during the production process of the cream, but at the end of the production process they re-crystallise during the cooling of the product.

When admixing lipid nanoparticles loaded with active compounds to an existing product, problems with the loading capacity might occur. This is especially valid when admixing relatively low concentrated SLN dispersions. Only a certain percentage of the aqueous SLN dispersion can be admixed. This aqueous SLN dispersion contains only a certain percentage of lipid mass, the lipid mass again contains only a certain percentage of active compound. This is less or no problem when admixing highly concentrated lipid nanoparticle dispersions.

In regards to achieving the desired loading capacity, optimal solutions are topical formulations consisting of lipid nanoparticles only, that means transferring aqueous particle dispersions into gels (method 2) or directly producing highly concentrated particle dispersions with the desired consistency. To illustrate this, the production of 100 g product containing 0.1% retinol is briefly discussed. When the product consists of a 20% lipid nanoparticle gel (20 g lipid particles in 100 g gel), the lipid mass needs to contain 0.5% retinol. This can easily be achieved with the old SLN system allowing incorporation of up to 1% retinol in the lipid matrix. If only 10 g of a 20% lipid nanoparticle dispersion (=2 g lipid particles) are admixed to 90 g cream, the lipid mass of the nanoparticles requires a 10-times higher loading, i.e. 5% retinol. This is only achievable by an NLC system

5. Regulatory aspects of lipid nanoparticles in topical formulations

There are variations from country to country, but basically, a pharmaceutical or cosmetic producer has to face the problem of proving the physical and chemical stability of the carrier system used. For liposomes, for example, this can require having a limitation in the formation of lysolecithin and also to prove quantitatively the existence of liposomes (e.g. when liposomes are contained in an o/w cream, facing the possibility that the liposomes dissolve by fusion with the oil droplets). To avoid problems with proof of the physical stability of liposomes, the manufacturer can use lecithin as excipient without specifying the existence of liposomes as active principle [50]. In Japan, there are the same regulations for cosmetics as for pharmaceuticals; the physical stability of liposomes needs to be proven not only qualitatively but also quantitatively. This is a problem when placing liposomal products on the Japanese market. There is no such problem with solid lipid nanoparticles (i.e. SLN or NLC)—their quantitative existence can easily be proven by DSC via the melting peak. An unchanged melting enthalpy and shape of the melting peak proves the physical stability (Fig. 28).

6. Perspectives

Both the SLN and NLC are attractive carriers for topical cosmetic and pharmaceutical products. They possess the potential to develop as the new generation of carrier systems after the liposomes. The SLN themselves represent a solid technology; however, the NLC are a smarter system. It is expected
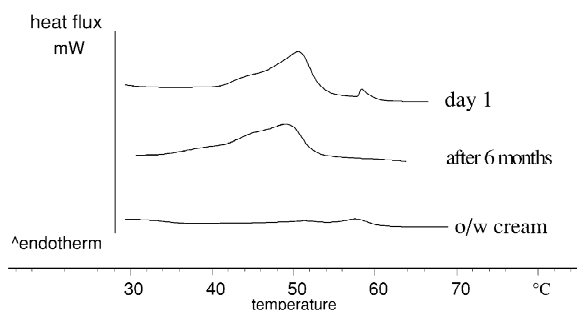
Fig. 28. DSC thermogram of o/w cream after incorporation of SLN into the cream (day of production = day 1) and after 6 months of storage. The enthalpy values were 16.74 J/g at the day of production and 16.27 J/g after 6 months (from Ref. [29]).

that they will be introduced not only into cosmetic but also into pharmaceutical formulations; however, due to lower regulatory hurdles the first product on the market will be a cosmetic product.

However, despite the fact that the use of lipid particles for topical administration is very promising and a highly attractive application area, further basic research needs to be done.

For example, it is highly desirable to have a much better understanding of the reasons for formation of certain lipid modifications, the effect of surfactants used on these modifications, and their transition during storage. Interesting work on these effects has recently been published [51].

Also, a better understanding is needed of how lipid nanoparticles modify drug penetration into the skin, how lipid particles interact with the lipids of the stratum corneum, and how they then affect drug penetration. Definitely, more human studies need to be done to have 'real life' data. Apart from application of lipid nanoparticles to the skin, future research should also consider mucosal applications. To achieve these goals, more research groups need to focus on this area, as has happened for oral and parenteral administration of lipid nanoparticles.

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