NANOSTRUCTURED LIPID CARRIER (NLC) A MODERN APPROACH FOR TOPICAL DELIVERY: A REVIEW

Dilip K. Patel1*, Surendra Tripathy1, Suresh K. Nair1, Roohi Kesharwani1

1Chandra Shekhar Singh College of Pharmacy, Kausambi, Allahabad, U.P. INDIA.

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 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 NLC, the technologies to produce the final topical formulation are described, especially the production of highly concentrated lipid nanoparticle dispersions. 30–80% lipid content.

Keywords: Nanostructured lipid carriers, NLC, Nanoparticle, Topical delivery.

INTRODUCTION
Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) are the two main types of lipid nanoparticles. The research activities in SLN and NLC in the last two decades focused mainly on pharmaceutical non dermal administration routes, i.e. parenteral peroral, ocular and pulmonary administration. During the last 5 years SLN and NLC have been intensively investigated for dermal application because of many positive features that have been reported after their application to the skin. Due to the lipid matrix, the small particle size and related adhesive properties, the residence time of SLN and NLC on the skin is prolonged2,3,5. The development of SLN overcame many problems related to ‘traditional’
nanoparticulate carrier technologies which limited the use (e.g. liposomes, i.v. emulsions) or even prevented the introduction to the market (e.g. polymeric nanoparticles). SLN can be prepared from regulatory accepted excipients, these excipients are well tolerated, large scale production by high pressure homogenization is possible even using existing production lines for i.v. emulsions, and the technology and the product itself are low cost. Lipid nanoparticles with solid particle matrix are derived from o/w emulsions by simply replacing the liquid lipid (oil) by a solid lipid, i.e. being solid at body temperature. The first generation of solid lipid nanoparticles (SLN) was developed at the beginning of the nineties. They were produced from a solid lipid only. In the second generation technology of the nanostructured lipid carriers (NLC), the particles are produced by using a blend of a solid lipid with a liquid lipid, this blend also being solid at body temperature\(^2\).

WHY LIPID NANOPARTICLES?

- Better control over release kinetics of encapsulated compound
  - b. Melting can serve as trigger.
- Enhanced bioavailability of entrapped bioactive.
- Chemical protection of labile incorporated compounds.
- Much easier to manufacture than biopolymeric nanoparticles.
- No special solvents required.
- Wider range of base materials (lipids).
- Conventional emulsion manufacturing methods applicable.
- Raw materials essential the same as in emulsions.
- Very high long-term stability.
- Application versatility:
  - a. Can be subjected to commercial sterilization procedures.
  - b. Can be freeze-dried to produce powdered formulation.

ADVANTAGES OF SLN/NLC OVER CONVENTIONAL PARTICULATE CARRIERS

- Their small size and relatively narrow size distribution permits site-specific drug delivery.
- Controlled and Sustained release of active drug can be achieved.
- The incorporated drug is protected from the onslaughts of biochemical degradation.
- High drug payload.
Incorporation of lipophilic and hydrophilic drugs feasible
- Can be sterilized by autoclave or gamma radiation.
- Can be lyophilizes and spray dried.
- Do not generate any toxic metabolites.
- Relatively cheap and stable.
- Easy of industrial scale production by hot dispersion technique.
- Surface modification can be easily performed.

POTENTIAL PROBLEMS ASSOCIATED WITH SLN AND ITS PRODUCTION TECHNOLOGY

The review by Mehnert high lights these aspects\(^3\,^4\).
- Pay-load for a number of drugs too low
- Drug expulsion during storage (Figure. 1)
- High water content of SLN dispersions

Figure 1: Mechanism of drug expulsion during storage of SLN dispersions, transition to highly ordered lipid crystal\(^6\).

NLC have been developed to overcome the drawbacks associated with SLN. They are considered to be the second generation of lipid nanoparticles. Compared to SLN, NLC show a higher loading capacity for active compounds by creating a less ordered solid lipid matrix, i.e. by blending a liquid lipid with the solid lipid, a higher particle drug loading can be achieved. Therefore, the NLC have an increased drug loading capacity in comparison to SLN and the possibility of drug expulsion during storage is less\(^7,^8,^9,^10\).
THE NEW CONCEPT OF NLC

The three types of NLC can be summarized

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. 2, 3). Drug load in SLN is limited due to the formation of the lipid crystal. Drug expulsion is caused by an ongoing crystallization process towards a perfect crystal. Thus, by avoiding crystallization, 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. 4). 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.10

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 This NLC type uses the fact that for a number of drugs, the solubility in oils is higher than their solubility in solid lipids (fig. 5).

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**SLN:**

![Image of SLN structure](image1)

c.e.g. tristearin

**NLC:**

![Image of NLC structure](image2)

**Figure 2:** Perfect crystal in SLN comparable with a brick wall (upper) and structure with imperfections due to spacially very different molecules in NLC type 1.6
Figure 3: Crystallisation process during storage to perfect crystal in SLN (left) and unchanged remaining NLC I structure with imperfections.

Figure 4: Structureless type II of NLC the lipid solidifies in the solid but amorphous state.

Figure 5: Theoretical proposed structure of multiple type NLC (oil-in-solid fat-in-water, O/F/W).

PRODUCTION PROCESSES FOR SLN/NLC
1: High-Shear Homogenization and Ultrasound
High-shear homogenization and ultrasound were initially used for the production of lipid nanodispersions. Both methods are widespread and easy to handle. However, in many cases, bimodal size distributions are obtained with one population in the micrometer range. In
addition, metal contamination has to be considered if ultrasound is used. Ahlin et al. used a rotor-stator homogenizer to produce SLN/NLC by melt-emulsification. They investigated the influence of different process parameters including emulsification time, stirring rate, and cooling conditions on the particle size and the zeta potential. In most cases, average particle sizes in the range of 100 to 200 nm were obtained using stirring rates of 20,000 to 25,000 rpm for 8 to 10 min and controlled cooling with a stirring rate of 5,000 rpm\textsuperscript{11,12}.

2: High-Pressure Homogenization

HPH has emerged as a reliable and powerful technique for the preparation of SLN/NLC. High-pressure homogenizers push a liquid with high pressure (10 to 200 MPa) through a narrow gap (in the range of few microns). The fluid accelerates on a very short distance to very high velocity (over 1000 km/h). Very high-shear forces disrupt the particles down to the submicron range. Typical lipid contents range between 5 to 10\% of the fluid and represent no problem to the homogenizer. Even lipid concentrations up to 40\% have been homogenized to lipid nanodispersions. Two general approaches of the homogenization step, the hot and the cold homogenization techniques, can be used for the production of SLN/NLC. In both cases, a preparatory step involves incorporating the drug into the bulk lipid by dissolving or dispersing the drug in the lipid melt\textsuperscript{13,14}.

2.1: Hot Homogenization

Hot homogenization is carried out at temperatures above the melting point of the lipid and can therefore be regarded as the homogenization of an emulsion. A pre-emulsion of the drug-loaded lipid melt and the aqueous emulsifier phase (same temperature) is obtained by a high-shear mixing device (Ultra-Turrax). The quality of the pre-emulsion affects the quality of the final product to a large extent, and obtaining droplets in the size range of a few micrometers is desirable. HPH of the pre-emulsion is carried out at temperatures above the melting point of the lipid. In general, higher temperatures result in lower particle sizes because of the decreased viscosity of the inner phase\textsuperscript{15}. However, high temperatures may also increase the degradation rate of the drug and the carrier. Furthermore, many surfactants have decreased solubility’s and HLB values at a higher temperature, which might have a negative impact on homogenization efficacy. The homogenization step can be repeated several times. It should always be kept in mind that HPH increases the temperature of the sample (approximately 10\°C for 500 bar). In most cases, 3 to 5 homogenization cycles at 500 to 1500 bar are sufficient. Increasing the homogenization pressure or the number of cycles often results in an
increase of the particle size because of particle coalescence, which occurs as a result of the high kinetic energy of the particles. The primary product of the hot homogenization is a nanoemulsion resulting from the liquid state of the lipid. Solid particles are expected to be formed by the cooling of the sample to room temperature or below.

2.2: Cold Homogenization

Cold homogenization is carried out with the solid lipid and can therefore be regarded as a high-pressure milling of a lipid suspension. Effective temperature control and regulation is needed to ensure the unmolten state of the lipid because of the increase in temperature during homogenization\textsuperscript{16}. Cold homogenization has been developed to overcome the following three problems of the hot homogenization technique:

- Temperature-induced drug degradation
- Drug distribution into the aqueous phase during homogenization
- Complexity of the crystallization step of the nanoemulsion leading to several modifications or supercooled melts.

The first preparatory step is the same as in the hot homogenization procedure and includes the solubilization or dispersing of the drug in the melt of the bulk lipid. However, different steps follow. The drug-containing melt is cooled very rapidly (e.g., by means of dry ice or liquid nitrogen). The high cooling rate favors a homogeneous distribution of the drug within the lipid matrix. The solid, drug-containing lipid is milled by means of ball or mortar milling in the range of 50 to 100 nm. Low temperatures increase the fragility of the lipid and, therefore, favor particle disruption. The solid lipid microparticles are dispersed in a chilled emulsifier solution. The presuspension is subjected to HPH at or below room temperature. In general, compared with hot homogenization, larger particle sizes and a broader size distribution are observed in cold homogenized samples\textsuperscript{14}.

3: SLN/NLC Prepared by Solvent Emulsification/Evaporation

Sjostrom and Bergenstahl used a solvent emulsification/evaporation method to prepare solid lipid nanodispersions. The lipophilic material is dissolved in a water immiscible organic solvent (e.g., cyclohexane) that is emulsified in an aqueous phase to give an oil/water (o/w) emulsion. On evaporation of the solvent by reduced pressure, solid lipid Nanoparticle dispersion is formed. The mean diameter of the obtained particles was 25 nm, with cholesterol acetate as the model drug and using a lecithin/sodium glycocholate blend as the emulsifier. The reproducibility of these results was confirmed by Siekmann and Westesen,
who also prepared nanoparticles of tripalmitin by dissolving triglyceride in chloroform. Mean particle sizes of the final particles ranged from 30 to 100 nm, depending on the lecithin/cosurfactant blend. The advantage of this procedure over the cold homogenization process described before is the avoidance of any thermal stress. A clear disadvantage is the use of organic solvents.17,18

4: Solvent Injection Method
The production of polymeric nanoparticles by dilution of polymer solutions in water has been described by De Labouret19. The process also can be easily used for the production of lipid nanodispersions. A requirement is the solubility of the lipid in the polar organic solvent, which limits the application range of this procedure. A further disadvantage is the low concentration of the lipid nanoparticles (typically 1% or less). Higher amounts of the organic solvent increase the solubility of the lipid in the aqueous phase and lead to an increase in particle size resulting from Ostwald ripening. The main advantage of the method is the avoidance of thermal stress.20

5: Micro emulsion-Based SLN/NLC Preparations
SLN preparation techniques that are based on the dilution of microemulsions have been developed by Gasco21. It should be mentioned that there are different definitions and opinions about the structure and dynamics of microemulsion in the scientific community. An extended review has recently been published by Moulik and Paul22. Gasco and other scientists describe microemulsions as two-phase systems composed of an inner and outer phase (e.g., o/w microemulsions). Microemulsions are made by stirring at 65 to 70°C an optically transparent mixture that is typically composed of a low-melting fatty acid (e.g., stearic acid), an emulsifier (e.g., polysorbate 20, polysorbate 60, soy phosphatidylcholine, taurodeoxycholic acid sodium salt), coemulsifiers (e.g., butanol, sodium monooctylphosphate), and water. The hot microemulsion is dispersed in cold water (2 to 3°C) under stirring. Typical volume ratios of the hot microemulsion to cold water are in the range of 1:25 to 1:50. The dilution process is critically determined by the composition of the microemulsion. According to the literature Boltri Gasco21,23, the droplet structure is already contained in the microemulsion, and, therefore, no energy is required to achieve submicron particle sizes. In addition to the composition, the temperature gradient and the pH value are key parameters for the quality of the final lipid nanosuspension. High-temperature gradients facilitate rapid lipid crystallization.
and prevent aggregation. Because of the dilution step, achievable lipid contents are considerably lower compared with the HPH-based formulations.  

**NLC AS TOPICAL DRUG DELIVERY SYSTEMS**

Topical drug application has been introduced since long time to achieve several purposes on different levels (skin surface, epidermis, dermis and hypodermis). However, several problems have been reported with the conventional topical preparations e.g. low uptake due to the barrier function of the stratum corneum and absorption to the systemic circulation. A lot of research groups paid attention to the topical application of the SLN and NLC. Many features, which these carrier systems exhibit for dermal application of cosmetics and pharmaceutics, have been pointed out. SLN and NLC are composed of physiological and biodegradable lipids that show low toxicity. 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.

**TOPICAL BENEFIT OF NLC**

**Increase of skin occlusion:** The lipid film formation on the top of the skin and the subsequent occlusion effect was reported for lipid nanoparticles. By using very small lipid particles, which are produced from highly crystalline and low melting point lipids, the highest occlusion will be reached. Particles smaller than 400 nm containing at least 35% lipid of high crystallinity have been most effective. Souto et al. found a higher occlusive factor for SLN in comparison to NLC of the same lipid content. Comparing NLC with different oil content showed that an increase in oil content leads to a decrease of the occlusive factor.

**Increase of skin hydration and elasticity:** The reduction of trans epidermal water loss (TEWL) caused by occlusion leads to an increase in skin hydration after dermal application of SLN, NLC or formulations containing them. An *in vivo* study showed that the SLN-containing o/w cream increased the skin hydration significantly more than the conventional o/w cream. In this study the skin hydration effect after repetitive application of an o/w cream containing SLN and a conventional o/w cream was investigated for 28 days. A significant higher increase in skin hydration was found by Müller et al. for an NLC-containing cream compared to conventional cream.
Enhancement of skin permeation and drug targeting: The stratum corneum in healthy skin has typically a water content of 20% and provides relatively an effective barrier against percutaneous absorption of exogenous substances. Skin hydration after applying SLN or NLC leads to a reduction of corneocytes packing and an increase in the size of the corneocytes gaps. This will facilitate the percutaneous absorption and drug penetration to the deeper skin layers\textsuperscript{31}.

An increase of skin penetration was reported for coenzyme Q 10 (Q10)-loaded SLN compared to Q10 in liquid paraffin and isopropanol. The cumulative amounts of Q10 were determined performing a tape stripping test. After five strips the cumulative amount of Q10 was 1\%, 28\% and 53\% of the applied amount from the liquid paraffin, the isopropanol and the NLC formulation, respectively. Jenning \textit{et al.} showed that enhanced penetration of retinol with epidermal targeting of this active could be achieved by applying retinol-loaded NLC\textsuperscript{10,32}.

Ricci \textit{et al.} investigated the \textit{in vitro} penetration of indomethacin from NLC-containing gel and gel without NLC through the stratum corneum and epidermis. He also investigated the \textit{in vivo} indomethacin release by tape-stripping test and the \textit{in vivo} anti-inflammatory activity using the UV-B induced erythrema model. In this work 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 \textit{in vitro} permeation through the stratum corneum and epidermis from indomethacin-loaded NLC gel was less than from gel without NLC\textsuperscript{33}.

Improve benefit/risk ratio: Skin atrophy and systemic side effect occurred after applying conventional prednicarb paste cream could be avoided when this drug was formulated as lipid nanoparticle. Prednicarb paste uptake was enhanced and it was accumulated in the epidermis with a low concentration in the dermis\textsuperscript{31,34}.

Tretinoin loaded-SLN formulation was studied by Shah \textit{et al} concerning skin irritation. One of the major disadvantages associated with the topical application of tretinoin is the local skin irritation such as erythrema, peeling and burning as well as increased sensitivity to sunlight. In the \textit{in vitro} permeation studies through rat skin they found that SLN based tretinoin gel has a permeation profile comparable to that of the market tretinoin cream. But on the other hand, Draize patch test showed that SLN based tretinoin gel resulted in remarkably less erythremic
episodes compared to the currently marketed tretinoin cream and hence, a better benefit/risk ratio is expected for the formulations containing tretinoin-loaded SLN. Conclusively, applying SLN or NLC can enhance skin penetration of incorporated actives, promote the epidermal targeting and minimize the systemic side effects and therefore, the benefit/risk ratio is improved\textsuperscript{35}.

**Enhancement of chemical stability of chemically labile compounds:** Enhancement of chemical stability after incorporation into lipid nanocarriers was proven for many cosmetic actives, e.g. coenzyme Q 10, ascorbyl palmitate, and retinol (vitamin A)\textsuperscript{32,36,37}.

**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 (\textit{Code of Federal Regulations, Food and Drugs 21}).

**TOPICAL TREATMENT OF SKIN DISEASE: POTENTIAL AND PROBLEMS**

Topical treatment of skin diseases is very attractive, since systemic load of active pharmaceutical ingredients (API) and thus also systemic side effects are reduced as compared to parenteral or oral drug administration. Hence application of drugs (preparations of API) to the skin surface was and still is not only used for skin disease but also for local antirheumatic therapy to control gastrointestinal side effects of nonsteroidal anti-inflammatory drugs. Moreover, drug application to the skin surface avoids the major fluctuations of plasma levels typical for repeated administration of rapidly eliminated drugs while it also allows to circumvent the first passage of API through liver after intestinal absorption. Thus transdermal drug application has gained still increasing importance for systemic treatment, e.g. with drugs subject to extensive first-pass elimination such as glyceryl trinitrate or estrogens as well as for the sustained suppression of chronic pain\textsuperscript{38}.

**SKIN MORPHOLOGY: BARRIER AND RESERVOIR FUNCTION**

Drug molecules in contact with the skin surface can penetrate by either three potential pathways: through the sweat ducts, via the hair follicles and sebaceous glands (collectively called the shunt or appendageal route), or directly across the stratum corneum (Fig.7). The relative importance of the shunt or appendageal route versus transport across the stratum corneum has been debated by scientists over the years and is further complicated by the lack
of a suitable experimental model to permit separation of the three pathways. In vitro experiments tend to involve the use of hydrated skin or epidermal membranes so that appendages are closed by the swelling associated with hydration. Scheuplein and colleagues proposed that a follicular shunt route was responsible for the pre steady-state permeation of polar molecules and flux of large polar molecules or ions that have difficulty diffusing across the intact stratum corneum\textsuperscript{39,40,41}.

A molecule traversing via the transcellular route must partition into and diffuse through the keratinocyte, but in order to move to the next keratinocyte, the molecule must partition into and diffuse through the estimated lipid lamellae between each keratinocyte. This series of partitioning into and diffusing across multiple hydrophilic and hydrophobic domains is unfavorable for most drugs. Consequently,

The intercellular route is now considered to be the major pathway for permeation of most drugs across the stratum corneum.

Figure 6: Simplified representation of skin showing routes of penetration Traditionally it was thought that hydrophilic chemicals diffuse within the aqueous regions near the outer surface of intracellular keratin filaments (intracellular or transcellular route) whilst lipophilic chemicals diffuse through the lipid matrix between the filaments (intercellular route)\textsuperscript{39}. 
Figure 7: Diagrammatic representation of the stratum corneum and the intercellular and transcellular routes of penetration. 

The three main factors determining the transdermal permeation of drugs are the mobility of drug in the vehicle, release of drug from the vehicle, and permeation of drug into the skin. These factors affect either the thermodynamic activity that drives the drug into the skin or the permeability of drug in the skin, particularly stratum corneum. NLC based gel improve the transdermal delivery of several drugs over the conventional topical preparations such as emulsions, and gels. Among the physical properties that make NLC based gel attractive as transdermal drug delivery vehicles is their transparent nature, which means that the product is not only aesthetically pleasing, but allows easy visualization of any contamination. Significantly the small droplet size provides a large interfacial area for rapid drug release, and so the drug should exhibit an enhanced bioavailability, enabling a reduction in dose, more consistent temporal profiles of drug absorption, and the protection of drugs from the hostile environment of the body. In addition to increasing the rate of drug release, NLC can also be used as a reservoir and actually slow the release of drug and prolong its effect, thereby avoiding high concentrations in the blood. Whether a drug is rapidly or slowly released from NLC depends very much on the affinity of the drug for the NLC. Since NLC contain surfactants (co-surfactants) and other excipients, they may serve to increase the membrane permeation of drug by:

- Disrupting the intercellular bilayer lipid structure
- Interacting with the intracellular proteins of the stratum corneum
- Improving the partitioning of a drug into the stratum corneum.
Aceclofenac is a potent analgesic, antipyretic and anti-inflammatory agent has been approved for the treatment of various kinds of pain, osteoarthritis and rheumatoid arthritis. An arthritic condition demands a controlled release drug delivery system for a prolong period so that can satisfy the goals of the treatment like reduction of the pain and inflammation, slowing the disease progression and prevention of adverse reaction. The requirement for designing of a topical drug delivery system of aceclofenac, which could not only increase the presence of the drug locally, and for a prolonged period but also reduce the risk of systemic toxicity. NLC have been shown to exhibit a controlled release behavior for various active ingredients such as ascorbyl palmitate, clotrimazole and other antifungal agents. As an alternative route for aceclofenac transdermal administration eliminate these systemic side effects, which also offer many advantages, such as patient compliance and possibility of continuous and controlled drug absorption.

CONCLUSION
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, increased activities 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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