International Journal for Pharmaceutical Research Scholars (IJPRS)
ISSN No: 2277-7873
RESEARCH ARTICLE
V-1, I-3, 2012

Nanostructured Lipid Carrier: The Second Generation of Solid Lipid Nanoparticle

Sahu MK*[1], Soni GC[1], Prajapati SK[1]

[1]*Institute of Pharmacy, Bundelkhand University, Jhansi - 284128. U.P. India*

Manuscript No: IJPRS/V1/I3/00177, Received On: 26/09/2012, Accepted On: 05/10/2012

ABSTRACT

Since the beginning of the 1990's the lipid nanoparticles were getting a growing interest from the pharmaceutical technology research groups worldwide. SLN was referred to as alternative carrier system to traditional colloidal system such as liposomes, emulsions and polymeric nanoparticles due to its exceptional stability, scaling-up potential and biocompatible components. A new generation of nano structured lipid carriers (NLCs) consisting of a lipid matrix with a special nanostructure has been developed. This nanostructure improves drug loading and firmly incorporates the drug during storage. The present article discusses the nanostructured lipid carrier as a novel drug delivery system.

KEYWORDS

Nanoparticles, lipid matrix, Nano structured lipid carriers.

INTRODUCTION

The term colloid is broadly applicable to systems consisting of at least 2 components; one dispersed in the other as fine particles in any state of matter.

As pharmaceutical carriers, colloidal drug delivery systems can be subdivided into:

- Polymer systems (micelles, dendrimers, etc),
- Self-assembled lipid systems (liposomes, emulsions, solid lipid nanoparticles, etc),
- Drug nanoparticle systems and pro-colloidal systems (self-emulsifying oral delivery systems and liquid crystalline systems).

Colloidal drug carrier systems offer many advantages as drug delivery vehicles including capability of increasing bioavailability of poorly soluble drugs, provide protection for sensitive active compounds and facilitate controlled release of drugs[1].

Solid lipid nanoparticles (SLN) combining the advantages of colloidal carriers, had attracted increased attention as a drug delivery system when it was introduced in 1991[1]. Since then, SLN is referred to as alternative carrier system to traditional colloidal system such as liposomes, emulsions and polymeric nanoparticles due to its exceptional stability, scaling-up potential and biocompatible components[2]. SLN are composed of 0.1% (w/w) to 30% (w/w) solid lipid dispersed in an aqueous medium and if necessary stabilized with preferably 0.5% (w/w) to 5% (w/w) surfactant. The mean particle size of SLN is in the submicron rage, ranging from about 40 nm to 1000 nm[3]. However, the disadvantages of SLN include tendency for particle growth, unpredictable gelation tendency, unexpected dynamics of polymorphic transitions and inherently low incorporation capacities due to the crystalline structure of solid lipids[4,5].

Nanostructured lipid carriers (NLC) are the second generation SLN composed of solid lipid matrix which are incorporated with liquid lipids[6]. To obtain the blends for the particles matrix, solid lipids are mixed with liquid lipids, preferably in a ratio of 70:30 up to a ratio of 99.9:0.1. Because of the oil presence in these mixtures, a melting point depression compared

***Address for Correspondence:**
Mukesh K. Sahu
Department of Pharmaceutics,
Institute of Pharmacy, Bundelkhand University,
Jhansi - 284128. U.P., India.
E-Mail Id: sahu.mukesh5@gmail.com

to the pure solid lipid is observed, but the blends obtained are also solid at room and body temperatures[7]. The overall solid content of NLC could be increased up to 95%[1].

Among the nanostructured lipid carriers that contain solid lipids together with liquid oils are,Miglyol®, _-tocopherol, etc[8]. The presence of liquid lipids with different fatty acid C-chains produces NLC with less organized crystalline structure and therefore provides better loading capacity for drug accommodation[9]. Liquid lipids are better solubilizers of drugs than solid lipids.

These carriers are composed of physiological and biodegradable lipids exhibiting low systemic toxicity and low cytotoxicity[10]. Most of the used lipids have an approved status or are excipients used in commercially available topical cosmetic or pharmaceutical preparations. The small size of the lipid particles ensures close contact to stratum corneum and can increase the amount of drug penetrating into mucosa or skin. Due to their solid lipid matrix, a controlled release from these carriers is possible. This becomes an important tool when it is necessary to supply the drug over prolonged period of time, to reduce systemic absorption, and when drug produces irritation in high concentrations.As a result of film formation after topical application, occlusive properties have also been reported for SLN [11,12,13].SLN and NLC have been shown to exhibit a controlled release behavior for various active ingredients such as ascorbyl palmitate[14], clotrimazole[15], ketoconazole[16], suncreens[12,13]and other antifungal agents[17].

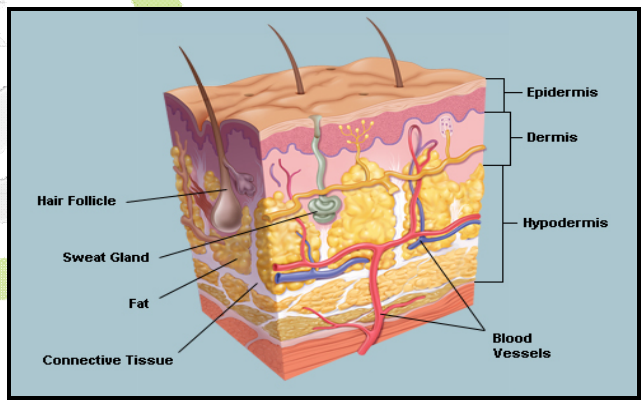
Types of NLC

Different methods have been proposed for creating NLCs:

- Imperfect type NLCs: These are produced by mixing solid lipids with small amounts of oil (liquid lipids) to improve the drug loading capacity.
- Multiple type NLCs: When mixed with large amount of oil, the lipid was found to solubilize certain drugs which were not soluble otherwise.
- Amorphous NLC: It was created to reduce drug expulsion by mixing special lipids like hydroxyl octacosanylhydroxystearate and isopropyl myristate.

Skin: Anatomy and Physiology

Skin contains an uppermost layer, epidermis which has morphologically distinct regions; basal layer, spiny layer, stratum granulosum and upper most stratum corneum, it consists of highly cornified (dead) cells embedded in a continuous matrix of lipid membranous sheets. These extracellular membranes are unique in their compositions and are composed of ceramides, cholesterol and free fatty acids. The human skin surface is known to contain, on an average, 10-70 hair follicles and 200-250 sweat ducts on every square centimeters of the skin area. It is one of the most readily accessible organs of the human body. In the thick epidermis of the palms of the hand and soles of the feet, there are five typical layers (strata).



The skin comprises the stratum corneum, stratum lucidum, stratum granulosum, stratumspinosum and stratum basale. The strata spinosum and basale together are known as the stratum germinativum once they generate new cells. In parts of the body other than the palms and soles, only the stratum corneum and stratum germinativum are regularly present.

In the stratum germinativum, the basal layer is composed of basal cells, which are nucleated, columnar and about 6 μm wide, with their long axis at right angles to the dermoepidermal junction, connected by cytoplasmic intercellular bridges.

Advantages of SLN and 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[18].

Increase of Skin Occlusion

The lipid film formation on the top of the skin and the subsequent occlusion effect was reported for lipid nanoparticles[19,20,21]. 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 cystallinity have been most effective[22]. Souto et al. found a higher occlusive factor for SLN in comparison to NLC of the same lipid content[23]. Comparing NLC with different oil content showed that an increase in oil content leads to a decrease of the occlusive factor[24].

Increase of Skin Hydration and Elasticity

The reduction of transepidermal 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[22]. A significant higher increase in skin hydration was found by Müller et al. for an NLC-containing cream compared to conventional cream[25].

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 percutanious absorption and drug penetration to the deeper skin layers[26,27].

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 SLN formulation, respectively. Similar results were achieved by another study for Q10-loaded NLC[28]. Another tap stripping test study showed that the tocopherol-loaded SLN formulation enhances the tocopherol penetration into the skin. Jenning et al. showed that enhanced penetration of retinol with epidermal targeting of this active could be achieved by applying retinol-loaded NLC[29]. Application of antiandrogen drug cyproterone acetate-loaded SLN increased the skin penetration at least four folds over the uptake from the conventional cream and emulsion[30]. SLN were found to increase the triptolide penetration into the skin as well as the anti-inflammatory activity. This strategy improved the bioavailability at the site of action, reduces the required dose and the

dose-dependent side effects like irritation and stinging[31].

Chen et al. compared podophyllotoxin-loaded SLN with podophyllotoxin tincture with regards to skin permeation, skin penetration and epidermal targeting effect. The podophyllotoxin permeated porcine skin from the tincture while no permeation was found for drug-loaded SLN. For one SLN formulation an increased penetration into porcine skin up to about four times over the tincture was reported. Furthermore, it was found that podophyllotoxin was located in the epidermis and hair follicles when applied as SLN formulation. No drug was found in the dermis after SLN application while podophyllotoxin after tincture application was distributed in each layer of the skin. Therefore, a localization effect in the epidermis was suggested and a reduction in systemic side effects is expected after application of podophyllotoxin using a formulation containing SLN[32]. Liu et al. found that epidermal targeting of isotretinoin could also be achieved using SLN[33].

Ricci et al. investigated the in vitro penetration of indomethacin from NLC-containing gel and gel without NLC through the stratum corneum and epidermis. He also investigated the in vivo indomethacin release by tape-stripping test and the 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 in vitro permeation through the stratum corneum and epidermis from indomethacin-loaded NLC gel was less than from gel without NLC[34].

Joshi et al. compared an NLC based gel of the nonsteroidal anti-inflammatory drug celecoxib with a micellar gel of the same composition regarding the in vitro skin penetration using rat skin and the pharmacodynamic efficiency by Aerosil induced rat paw edema. The in vitro permeation of celecoxib from NLC gel was less than the permeation from the micellar based gel. This confirms former findings about nanoparticles leading to a drug deposit in the skin resulting in sustained release. The in vivo comparison of the percentage edema inhibition produced by NLC and micellar gels showed a significant higher inhibition after application of the NLC based gel up to 24 hrs[35].

Improve Benefit/Risk Ratio

Skin atrophy and systemic side effect occurred after applying conventional prednicarbate cream could be avoided when this drug was formulated as SLN. Predinicarbate uptake was enhanced and it was accumulated in the epidermis with a low concentration in the dermis[36,37].

In another study Joshi et al. compared a valdecoxib-loaded NLC carbopol gel with a valdecoxib market product. The NLC containing gel showed no skin irritation while the market gel showed slight irritation after 48 hrs. Moreover, the NLC based gel showed prolonged activity up to 24 hrs while the activity of the market gel was shorter. This indicates a better skin tolerability and a longer activity of the NLC formulation compared to the marketed formulation[38].

Tretinoin loaded-SLN formulation was studied by Shah 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 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[39].

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.

Enhancement of UV Blocking Activity

Some side effects of organic UV blockers were reported due to the penetration of these compounds into the skin causing skin irritation and allergic reaction. This penetration can be reduced by incorporating these compounds in lipid nanoparticles. It was found that incorporating benzophenone in SLN not only improves the UV blocking activity evaluated using in vitro photoprotection assay but also reduces the absorption of the benzophenone into the skin in comparison to a conventional nanoemulsion. Improving the UV blocking activity allows the reduction of the concentration of the UV blocker while maintaining the protective level of the conventional formulation[40,41,42,43]. These findings were confirmed by Song and Lui comparing UV absorption properties of 3,4,5-trimethoxybenzochitin-loaded SLN and SLN free system[44].

Furthermore, a significant increase in SPF up to about 50 was reported after the encapsulation of titanium dioxide into NLC. Encapsulation of inorganic sunscreens into NLC is therefore a promising approach to obtain well tolerable sunscreens with high SPF[45].

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[46], tocopherol (vitamin E) and retinol (vitamin A)[47,48].

METHODS OF PREPARATION OF NANOSTRUCTURED LIPID CARRIER

Solvent Emulsification/Evaporation Technique

Sjostrom and Bergenstahl described a production method to prepare nanoparticle dispersions by precipitation in o/w emulsions[55]. The lipophilic material is dissolved in a water-immiscible organic solvent (e.g. cyclohexane) that is emulsified in an aqueous phase. Upon evaporation of the solvent nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium. The mean diameter of the obtained particles was 25 nm with cholesterol acetate as model drug and by using a lecithin/ sodium glycocholate blend as emulsifier. The reproducibility of these results is confirmed by Westesen[57].

High Shear Homogenization and Ultrasound

High shear homogenization and ultrasound are dispersing techniques which were initially used for the production of solid lipid Nano dispersions[49]. Both methods are widespread and easy to handle. However, dispersion quality is often compromised by the presence of micro particles. Furthermore, metal contamination has to be considered if ultrasound is used.

High Pressure Homogenization

High pressure homogenization (HPH) has emerged as a reliable and powerful technique for the preparation of NLC. Homogenizers of different sizes are commercially available from several manufacturers at reasonable prices.High pressure homogenizers push a liquid with high pressure (100–2000 bar) through a narrow gap (in the range of a few microns). The fluid accelerates on a very short distance to very high velocity (over 1000 km/h). Very high shear stress and cavitation forces disrupt the particles down to the submicron range. Two general approaches of the homogenization step, the hot and the cold homogenization techniques, can be used for the production of NLC[50,51,52].

Micro Emulsion Based NLC preparations

Gasco and co-workers developed SLN preparation techniques which are based on the dilution of micro emulsions[53]. It should be mentioned that there are different opinions in the scientific community about the structure and dynamics of micro emulsion. An extended review has recently been published by Moulik and Paul[54].

Gasco and other scientists understand micro emulsions as two-phase systems composed of an

inner and outer phase (e.g. o/w-micro emulsions). They are made by stirring an optically transparent mixture at 65–70^0 C which is typically composed of a low melting fatty acid (e.g. stearic acid), an emulsifier (e.g. polysorbate 20, polysorbate 60, soyaphosphatidylcholine, taurodeoxycholic acid sodium salt), coemulsifiers (e.g. butanol, sodium monooctylphosphate) and water. The hot microemulsion is dispersed in cold water (2–3^0 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 determined by the composition of the microemulsion.

Solvent Diffusion Technique

NLC can be prepared by solvent diffusion method in an aqueous system as reported by Hu et al.[55]. Weighed solid lipid was melted and liquid lipid was added to it in a water bath at 55^0 C. Then the drug was added to it. 5 ml of mixed organic solvent of ethanol and acetone (1:1, v/v) was also added to it.

The resultant organic solution was quickly dispersed into 20 ml of aqueous solution of Tween80 (1% (W/V)) and Poloxamer188 (1% (W/V)) at room temperature (25^0 C) under mechanical agitation with 3000 rpm for 30min until NLC suspensions were obtained. Prepared NLCs were placed in a vacuum desiccator for 24 h at room temperature to evaporate the residual organic solvent[56,57].

Principles of Drug Release from NLC

The effect of formulation parameters and production conditions on the release profile from SLN was intensively investigated by Mehnert, Muller and zur Muhlen[58,59,60,61]. 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 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[62].

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, when oil in water emulsion is cooled, a relatively high amount of active compound is incorporated in the core. Cooling further causes super saturation of compound in water phase. The compound now tries to partition back into the lipid phase, where a solid core has already started forming leaving only the liquid outer shell for compound accumulation (Fig. 1.2).

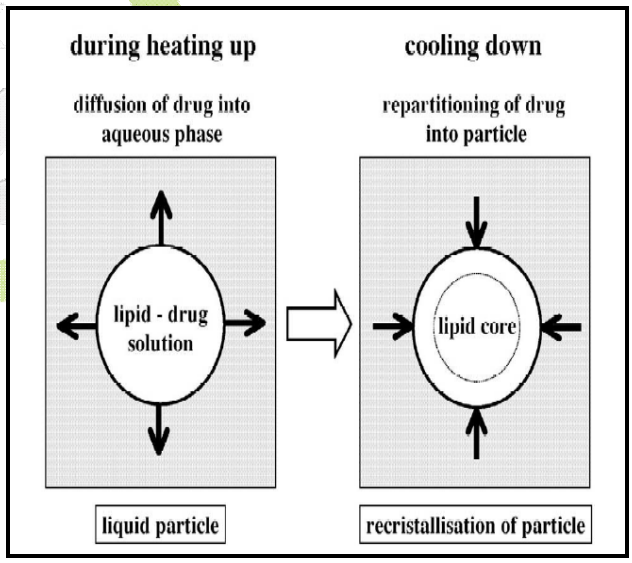


Figure 1.2: Model of redistribution of active compound during the heating up and the cooling down phase of SLN production using the hot homogenisation technique; explanations in text (modified from Ref.[59]).

From this it can be summarised that the higher the solubility in the water phase during production, the more pronounced is the burst effect. Thus, little or no burst will be obtained when producing at low temperatures, low

surfactant concentration or in surfactant-free medium.

In-vitro Occlusion of NLC

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[63]. The in vitro model by 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: (Equation 1.1)

F = 100 [(A-B)/A] …….....Equation1.1

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 Wissinget al.[64], 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 skin

SLN can be admixed to an already commercially available and established topical formulation, e.g. a cosmetic day cream. Admixing of SLN leads to an increase in occlusivity 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.

NLC 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 whom 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%[65]. Little or no increase in elasticity was observed.

Advantages of NLC as a Topical Drug Delivery system

Increase of skin occlusion[66,67]. Increase of skin hydration and elasticity[68,69]. Enhancement of skin permeation and drug targeting[70,71]. Improve benefit/risk ratio[72,73]. Enhanced chemical stability of chemically liable compounds[74,75].

Cosmetic Applications of NLC

Lipid nanoparticles—SLN and NLC—can be used to formulate active compounds in cosmetics, e.g. prolonged release of perfumes. Incorporation of cosmetic compounds and modulation of release is even more flexible when using NLC. In addition, the release of insect repellents has been described[76,77].

A feature of general interest is the stabilisation of chemically labile compounds. The solid matrix of the lipid nanoparticle protects them against chemical degradation, e.g. Retinol[78]and

coenzyme Q10. A recently discovered feature is the sunscreen blocking effect of lipid nanoparticles. Similar to particles such as titanium dioxide the crystalline lipid particles scatter UV light, thus protecting against UV irradiation[79]. In addition, it was found that incorporation of sunscreens leads to a synergistic UV blocking effect of the particulate blocker lipid nanoparticle and the molecular blocker. In vitro, crystalline lipid nanoparticles with the same sunscreen concentration exhibited twice the UV protection effect compared with an O/W emulsion loaded with the sunscreen[80].

REFERENCES

1. Müller RH, Mader K, Gohla S, Solid lipid nanoparticles (SLN) for controlled drug delivery - A review of the state of the art, Eur. J. Pharm Biopharm, 2000, 50, 161-177.
2. Radomska-Soukharev A, Stability of lipid excipients in solid lipid nanoparticles Adv Drug Deliv Rev. 59: 411-418 DOI:10.1016/j.addr., 2007, 04, 004.
3. Lucks, JS, Müller, R. H., Medication vehicles made of solid lipid particles (solid lipid nanospheres SLN), in EP0000605497, 1996, Germany.
4. Westesen K, Siekmann B, Investigation of the gel formation of phospholipid-stabilized solid lipid nanoparticles, Int. J. Pharm, 1997, 151, 35-45.
5. Westesen K, Bunjes H, Koch MH, Physiocochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential J. Control Release, 1997, 48, 223-236.
6. Zauner W, Farrow NA, Haines AM, *In vitro* uptake of polystyrene microspheres: effect of particle size cell line and cell density. J. Control Release, 2001, 71, 39-51.
7. Müller RH, Mäder K, Lippacher A, Jenning V, Solid-liquid (semi-solid) liquid particles and method of producing highly concentrated lipid particle dispersions, in PCT/EP00/04565, 2000.
8. Souto EB, Müller RH, Investigation of the factors influencing the incorporation of clotrimazole in SLN and NLC prepared by hot high pressure homogenization J. Microencapsul, 2006, 23, 377-388.
9. Müller RH, Radtke M, Wissing SA, Nanostructured lipid matrices for improved microencapsulation of drugs Int. J. Pharm. 2002, 242, 121- 128
10. Muller RH, R¨uhl D, Runge S, Schulze-Forster K, Mehnert W, Cytotoxicity of solid lipid nanoparticles as a function of the lipid matrix and the surfactant Pharm. Res, 1997, 14, 458–462.
11. Wissing SA, Muller RH, A novel sunscreen system based on tocopherol acetate incorporated into solid lipid nanoparticles. Int. J. Cos. Sci., 2001, 23, 233–243.
12. Wissing SA, Muller RH, a. Solid lipid nanoparticles as carrier for sunscreens: *in vitro* release and in vivo skin penetration. J. Controlled Release, 2002, 81, 225–233.
13. Wissing SA, Muller RH, b. The influence of the crystallinity of lipid nanoparticles on their occlusive properties Int. J. Pharm., 2002, 242, 377– 379.
14. Uner M, Wissing SA, Yener G, Muller RH, Skin moisturizing effect and skin penetration of ascorbylpalmitate entrapped in solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) incorporated into hydrogel. Pharmazie, 2005, 60, 751–755.
15. Souto EB, Wissing SA, Barbosa CM, Muller RH, Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. Int. J. Pharm., 2004, 278, 71–77.
16. Souto EB, Muller RH, SLN and NLC for topical delivery of ketoconazole, J. Microencapsul, 2005, 5, 501–510.
17. Souto EB, Muller RH, The use of SLN® and NLC® as topical participate carriers for imidazole antifungal agents. Pharmazie, 2006, 61, 431–437.

18. Müller RH, Radtke M, Wissing SA, Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations, Adv Drug Deliv Rev, 2002, 54(1), 131-55.

19. Wissing S, Lippacher A, Muller R, Investigations on the occlusive properties of solid lipid nanoparticles (SLN), J CosmetSci, 2001, 52(5), 313-24.

20. Wissing SA, Müller RH, Cosmetic applications for solid lipid nanoparticles (SLN), Int J Pharm, 2003, 254(1), 65-68.

21. Wissing S, Müller R, The influence of the crystallinity of lipid nanoparticles on their occlusive properties, Int J Pharm, 2002, 242(1-2), 377-379.

22. Souto EB, Wissing SA, Barbosa CM, Müller RH, Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery, Int J Pharm, 2004, 278, 71–77.

23. Teeranachaideekul V, Boonme P, Souto EB, Müller RH, Junyaprasert VB, Influence of oil content on physicochemical properties and skin distribution of Nile red-loaded NLC, J Control Release, 2008, 128(2), 134-141.

24. Müller RH, Hommoss A, Pardeike J, Schmidt C, Lipid nanoparticles (NLC) as novel carrier for cosmetics - Special features & state of commercialisation, SÖFW, 2007, (9), 40-46.

25. Zhai H, Maibach HI, Effects of skin occlusion on percutaneous absorption: an overview, Skin PharmacolAppl Skin Physiol, 2001, 14(1), 1-10.

26. Schäfer-Korting M, Mehnert W, Korting HC, Lipid nanoparticles for improved topical application of drugs for skin diseases, Adv Drug Deliv Rev, 2007, 59(6), 427-443.

27. Pardeike J, Müller RH, Coenzyme Q10 loaded NLCs: preparation, occlusive properties and penetration enhancement Pharm TechnolEur, 2007, July.

28. Jenning V, Schäfer-Korting M, Gohla S, Vitamin A-loaded solid lipid nanoparticles for topical use: drug release properties, J Control Release, 2000, 66(2-3), 115-126.

29. Stecova J, et al., Cyproterone acetate loading to lipid nanoparticles for topical acne treatment: particle characterisation and skin uptake, Pharm Res, 2007, 24(5), 991-1000.

30. Mei Z, Chen H, Weng T, Yang Y, Yang X, Solid lipid nanoparticle and microemulsion for topical delivery of triptolide, Eur J Pharm Biopharm, 2003, 56(2), 189-196.

31. Chen H, Chang X, Du D, Liu W, Liu J, Weng T, Yang Y, Xu H, Yang X, Podophyllotoxin-loaded solid lipid nanoparticles for epidermal targeting, J Control Release, 2006, 110(2), 296-306.

32. Liu J, Hu W, Chen H, Ni Q, Xu H, Yang X, Isotretinoin-loaded solid lipid nanoparticles with skin targeting for topical delivery, Int J Pharm, 2007, 328(2), 191-5.

33. Ricci M, Puglia C, Bonna F, Di Giovanni C, Giovagnoli S, Rossi C, Evaluation of indomethacin percutaneous absorption from nanostructured lipid carriers (NLC): in vitro and in vivo studies, J Pharm Sci, 2005, 94(5), 1149-59.

34. Joshi M, Patravale V, Nanostructured lipid carrier (NLC) based gel of celecoxib, Int J Pharm, 2008, 346(1-2), 124-32.

35. Maia CS, Mehnert W, Schäfer-Korting M, Solid lipid nanoparticles as drug carriers for topical glucocorticoids, Int J Pharm, 2000, 196(2), 165-167.

36. Schäfer-Korting M, Mehnert W, Korting HC, Lipid nanoparticles for improved topical application of drugs for skin diseases, Adv Drug Deliv Rev, 2007, 59(6), 427-43.

37. Joshi M, Patravale V, Formulation and evaluation of Nanostructured Lipid Carrier (NLC)-based gel of Valdecoxib, Drug DevInd Pharm, 2006, 32(8), 911-918.

38. Shah KA, Date AA, Joshi MD, Patravale VB, Solid lipid nanoparticles (SLN) of tretinoin: Potential in topical delivery, Int J Pharm, 2007, 345(1-2), 163-71.

39. Müller RH, Radtke M, Wissing SA, Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations, Adv Drug Deliv Rev, 2002, 54(1), 131-55.

40. Wissing SA, Müller RH, Cosmetic applications for solid lipid nanoparticles (SLN), Int J Pharm, 2003, 254(1), 65-68.

41. Wissing SA, Müller RH, Solid lipid nanoparticles (SLN)--a novel carrier for UV blockers, Pharmazie., 2001, 56(10), 783-786.

42. Wissing SA, Müller RH, Solid lipid nanoparticles as carrier for sunscreens: in vitro release and in vivo skin penetration, J Control Release, 2002, 81(3), 225-233.

43. Song C, Liu S, A new healthy sunscreen system for human: solid lipid nanoparticles as carrier for 3,4,5-trimethoxybenzoylchitin and the improvement by adding Vitamin E, Int J BiolMacromol, 2005, 36(1-2), 116-119.

44. Villalobos-Hernandez JR, Müller-Goymann CC, Novel nanoparticulate carrier system based on carnauba wax and decyloleate for the dispersion of inorganic sunscreens in aqueous media, Eur J Pharm Biopharm, 2005, 60(1), 113-122.

45. Teeranachaideekul V, Müller RH, Junyaprasert VB, Encapsulation of ascorbylpalmitate in nanostructured lipid carriers (NLC)--effects of formulation parameters on physicochemical stability, Int J Pharm, 2007, 340(1-2), 198-206.

46. Jenning V, Gohla SH, Encapsulation of retinoids in solid lipid nanoparticles (SLN), J Microencapsul, 2001, 18(2), 149-158.

47. Jenning V, Solid lipid Nanoparticles (SLN) as a carrier system for the dermal application of retinol. PhD thesis. 1999, FreieUniversität Berlin: Berlin.

48. Domb AJ, Lipospheres for controlled delivery of substances, United States Patent, 1993, USS 188837.

49. Muller RH, Mehnert W, Lucks JS, Schwarz C, ZurMuhlen A, Weyhers H, Freitas C, Ruhl D, Solid lipid nanoparticles (SLN) — An alternative colloidal carrier system for controlled drug delivery, Eur. J. Pharm. Biopharm., 1995, 41, 62–69.

50. ZurMuhlen A, Mehnert W, Drug release and release mechanism of prednisolone loaded solid lipid nanoparticles, Pharmazie, 1998, 53, 552–555.

51. ZurMuhlen A, Schwarz C, Mehnert W, Solid lipid nanoparticles (SLN) for controlled drug delivery — Drug release and release mechanism, Eur. J. Pharm. Biopharm., 1998, 45, 149–155.

52. Gasco MR, Method for producing solid lipid microspheres having a narrow size distribution. United States Patent, 1993, USS 188837

53. Moulik SP, Paul BK, Structure, dynamics and transport properties of microemulsions, Adv. Coll. Interf. Sci., 1998, 78, 99–195.

54. Hu FQ, Yuan H, Zhang HH, Fang M, Preparation of solid lipid nanoparticles with clobetasol propionate by a novel solvent diffusion method in aqueous system and physicochemical characterization, Int. J. Pharm., 2002, 239, 121–128

55. Yuan H, Wang LL, Du YZ, You J, Hu FQ, Zeng S, Preparation and characteristics of nanostructured lipid carriers for control-releasing progesterone by meltemulsification, Colloids and Surfaces B: Biointerfaces, 2007, 60, 174–179.

56. Hu FQ, Zhang Y, Du YZ, Yuan H, Nimodipine loaded lipid nanoparticles prepared by solvent diffusion method in a drug saturated aqueous system, International Journal of Pharmaceutics, 2008, 348, 146–152.

57. ZurMuhlen A, Mehnert W, Drug release and release mechanism of prednisolone loaded solid lipid nanoparticles, Pharmazie, 53, 1998, 552.

58. ZurMuhlen A, Schwarz C, Mehnert W, Solid lipid nanoparticles (SLN) for controlled drug

delivery: drug release and release mechanism, Eur. J. Pharm. Biopharm, 1998, 45, 149–155.

59. T. de Vringer, Topical preparation containing a suspension of solid lipid particles, European Patent Application EP 0 506 197 A1, 1992.

60. Wissing SA, Lippacher A, Muller RH, Investigations on the occlusive properties of solid lipid nanoparticles (SLN), J. Cosmet. Sci, 2001, 52, 313–323.

61. Muller RH, Dingler A, The next generation after the liposomes: solid lipid nanoparticles (SLN, Lipopearls) as dermal carrier in cosmetics, Eurocosmetics, 1998, (7–8), 19–26.

62. Wissing SA, Muller RH, The influence of solid lipid nanoparticles (SLN) on skin hydration and viscoelasticity: in vivo study, Eur. J. Pharm. Biopharm., 2002. [submitted for publication.]

63. Sjostrom B, Bergenstahl B, Preparation of submicron drug particles in lecithin-stabilized o/w emulsions I. Model studies of the precipitation of cholesteryl acetate, Int. J. Pharm., 1992, 88, 53–62.

64. Muller RH, Schwarz C, ZurMuhlen A, Mehnert W, Incorporation of lipophilic drugs and drug release profiles of solid lipid nanoparticles (SLN), Int. Symp. Control. Release Bioact. Mater, 1994, 21,146–147.

65. Siekmann B, Westesen K, Investigations on solid lipid nanoparticles prepared by precipitation in o/w emulsions, Eur. J. Pharm. Biopharm, 1996, 43, 104–109.

66. Westesen K, Bunjes H, Koch MH Physiocochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential, J. Control Release, 1997, 48: 223-236.

67. Zauner W, Farrow NA, Haines AM, In vitro uptake of polystyrene microspheres: effect of particle size cell line and cell density. J. Control Release, 2001, 71, 39-51.

68. Müller RH, Mäder K, Lippacher A, Solid-liquid (semi-solid) liquid particles and method of producing highly concentrated lipid particle dispersions, in PCT/EP00/04565, 2000.

69. Souto EB, Müller RH, Investigation of the factors influencing the incorporation of clotrimazole in SLN and NLC prepared by hot highpressure homogenization, J Microencapsul, 2006, 23, 377-388.

70. Müller RH, Radtke M, Wissing SA, Nanostructured lipid matrices for improved microencapsulation of drugs, Int. J. Pharm., 2002, 242, 121- 128.

71. Muller RH, Ruhl D, Runge S, Schulze-Forster K, Mehnert W, Cytotoxicity of solid lipid nanoparticles as a function of the lipid matrix and the surfactant, Pharm. Res., 1997, 14, 458–462.

72. Wissing SA, Muller RH, A novel sunscreen system based on tocopherol acetate incorporated into solid lipid nanoparticles. Int. J. Cos. Sci, 2001, 23, 233–243.

73. Wissing SA, Muller RH, a. Solid lipid nanoparticles as carrier for sunscreens: in vitro release and in vivo skin penetration. J. Controlled Release, 2002, 81, 225–233.

74. Wissing SA, Muller RH, b. The influence of the crystallinity of lipid nanoparticles on their occlusive properties, Int. J. Pharm, 2002, 242, 377– 379.

75. Wissing SA, Mader K, Muller RH, b. Prolonged efficacy of the insect repellent lemon oil by incorporation into solid lipid nanoparticles (SLN), Third World Meeting APGI/APV, Berlin, 2000, 439–440.

76. Wissing SA, Mader K, Muller RH, 2000b. Prolonged efficacy of the insect repellent lemon oil by incorporation into solid lipid nanoparticles (SLN™), Third World Meeting APGI/APV, Berlin, 439–440.

77. Yaziksiz-Iscan Y, Wissing SA, Muller RH, Hekimoglu S, 2002. Different production methods for solid lipid nanoparticles (SLN) containing the insect repellent DEET. Fourth

World Meeting APGI/APV, Florenz, submitted for publication.

78. Jenning V, Gohla S, Encapsulation of retinoids in solid lipid nanoparticles (SLN) J. Microencaps, 2001, 18, 149–158.

79. Wissing SA, Muller RH, The development of an improved carrier system for sunscreen formulations based on crystalline lipid nanoparticles, Abstract book 13th Internatioal Symposium on Microencapsulation, Angers, 2001, P- 055.