Solid Lipid Nanoparticles and Nanostructured Lipid Carriers – Innovative Generations of Solid Lipid Carriers

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Abstract: The first generation of solid lipid carrier systems in nanometer range, Solid Lipid Nanoparticles (SLN), was introduced as an alternative to liposomes. SLN are aqueous colloidal dispersions, the matrix of which comprises of solid biodegradable lipids. SLN are manufactured by techniques like high pressure homogenization, solvent diffusion method etc. They exhibit major advantages such as modulated release, improved bioavailability, protection of chemically labile molecules like retinol, peptides from degradation, cost effective excipients, improved drug incorporation and wide application spectrum. However there are certain limitations associated with SLN, like limited drug loading capacity and drug expulsion during storage, which can be minimized by the next generation of solid lipids, Nanostructured lipid carriers (NLC). NLC are lipid particles with a controlled nanostructure that improves drug loading and firmly incorporates the drug during storage. Owing to their properties and advantages, SLN and NLC may find extensive application in topical drug delivery, oral and parenteral administration of cosmetic and pharmaceutical actives. Cosmeceuticals is emerging as the biggest application target of these carriers.

Carrier systems like SLN and NLC were developed with a perspective to meet industrial needs like scale up, qualification and validation, simple technology, low cost etc. This paper reviews present status of SLN and NLC as carrier systems with special emphasis on their application in Cosmeceuticals; it also gives an overview about various manufacturing techniques of SLN and NLC.

Keywords: Solid Lipid Nanoparticles (SLN), Nanostructured lipid carriers (NLC), colloidal drug carriers, drug delivery systems, particulate drug delivery.

INTRODUCTION

Particulate drug carriers investigated for many years include oil-in-water (O/W) emulsions, liposomes, microparticles and nanoparticles based on synthetic polymers or natural macromolecules [1, 2]. Despite the excellent tolerability of these carrier systems the number of products on the market is relatively low due to problems such as limited physical stability; technological problems like, lack of a suitable low cost, qualified, large scale production method yielding a product of a quality accepted by the regulatory authorities, presence of solvent residues left over from production or the cytotoxicity of polymers [3-8].

In order to overcome the drawbacks associated with the traditional colloidal dispersions, Solid Lipid Nanoparticles (SLN) were developed at the beginning of the nineties as an alternative carrier system [9]. Drug loaded SLN combines the best features of polymeric nanoparticle formulations and liposomal formulations by maximizing drug targeting, creating uniform particle size distribution, thus offering low toxicity and good tissue compatibility without the disadvantages associated with such systems [10].

Colloidal particles are generally taken up and cleared by the cells of the Reticuloendothelial System(RES) upon intravenous injection. Through modification of the nanoparticles surface properties by using steric stabilizers, it is possible to escape clearance by the RES resulting in long circulating carriers thus, improving the bio-distribution. Moreover sustained action from long circulating carriers as an intravenous depot formulation may be desired. SLN may therefore represent an alternative parenteral delivery to influence pharmacokinetics and drug distribution in the body. Podio *et al.* [11] have investigated that polyethylene glycol 2000 conjugated radiolabeled stealth stearic acid nanoparticles escape recognition and uptake by the RES more efficiently than nonstealth particles. Zara *et al.* [11] reported that the blood levels of doxorubicin incorporated as ion pair in stearic acid nanoparticles were much higher than that with the solution. Doxorubicin nanoparticles showed a more than 17 fold increase in plasma AUC (0-180min), a lower rate of clearance and a smaller volume of distribution than the commercial drug solution. The drug concentration was also higher in the lungs, spleen and brain with lipid nanoparticle-incorporated doxorubicin, whereas drug levels were low in liver, heart and kidney.

SLN are aqueous colloidal dispersions composed of well tolerated and regulatory acceptable excipients and they possess a matrix which comprises of solid biodegradable lipids [9, 12]. Although they exhibit major advantages such as modulated release, improved bioavailability, protection of chemically labile molecules from degradation, wide application spectrum, there are certain limitations associated with SLN, like limited drug loading capacity and drug expulsion during storage [3, 5, 13]. Nanostructured lipid carriers (NLC) are lipid particles with a controlled nanostructure that improves drug loading and firmly incorporates the drug during storage. SLN and NLC find extensive application in topical, oral and parenteral administration of pharmaceutical actives as well as cosmetics [8, 9, 13]. Cosmeceuticals is emerging as the biggest application target of these carriers.

This paper reviews present status of SLN and NLC as carrier systems with special emphasis on their applications; it also gives an overview about various manufacturing techniques of SLN and NLC along with their properties and advantages and limitations.

SOLID LIPID NANOPARTICLES (SLN)

SLN represent innovative drug carrier systems composed of solid lipids and drug (hydrophilic and lipophilic), with mean size ranging from 50 to 1000 nm. The matrix of SLN comprises of solid lipids like triglycerides (trimyristin, tripalmitin, tristearin), glyceryl behenate (Compritol® 888 ATO), glyceryl monostearate (Imwitor® 900), glyceryl palmitostearate (Precirol® ATO 5), or the wax cetyl palmitate, biodegradable lipids are also used [5, 12]. Lipid concentration ranges between 5 and 40% [5]. Depending on the type and concentration of the lipid, 0.5 to 5% emulsifier (surfactant) has to be added for physical stabilization. In particular poloxamer 188, polysorbate 80, lecithin, tyloxapol, polyglycerol methylglucose distearate (TegoCare® 450), sodium cocoamphoacetate

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(Miranol® Ultra C32) or saccharose fatty acid ester are very often employed [5].

SLN Structure and Drug Loading Mechanism

Based on the drug incorporation technique the types of SLN are as follows [14]**:**

a) The SLN Type I or homogeneous matrix model is derived from solid solutions of lipid and active ingredient. A solid solution can be obtained when SLNs are produced by cold homogenization method. A lipid blend can be produced containing the active in a molecularly dispersed form. After solidification of this blend, it is ground in its solid state thus avoiding or minimizing of the enrichment of active molecules in different parts of the lipid nanoparticle.

b) The SLN Type II or drug enriched shell model are produced by the hot homogenization technique. Initially the drug partitions from the lipid phase to the water phase at increased temperature. Then as the O/W nanoemulsion cools, the drug re-partitions to the lipid phase to form the drug enriched shell.

c) The SLN Type III or drug enriched core model -these particles are formed when the drug precipitates first before the lipid re-crystallizes. This is seen when the drug is dissolved in the lipid phase at or close to its saturation stability. Cooling of such a nanoemulsion will lead to the super-saturation of the drug in the lipid melt and subsequently to drug crystallization prior to lipid crystallization.

SLN structure depends on the chemical nature of active ingredients and excipients. The expected advantages of solid lipid particles, e.g., modified release properties, essentially rely on the solid state of the particles. After processing in the melted state (e.g., in melt-homogenization), some matrix materials do, however, not crystallize readily in colloidal dispersions. Retarded or suppressed crystallization has been observed for shorter chain monoacid triglycerides like tricaprin, trilaurin or trimyristin as well as for more complex glyceride mixtures, e.g., some hard fats. Without special thermal treatment, dispersions of such matrix lipids may remain in the emulsion state for months or even years instead of forming the desired solid particles. The admixture of longer chain triglycerides or the use of emulsifiers with long, saturated alkyl chains may reduce this effect. On the other hand, the presence of liquid drugs or oil can further decrease the crystallization tendency. Monitoring of the crystalline status is thus a very important point in the characterization of solid lipid nanoparticle dispersions, particularly when novel compositions or preparation procedures are introduced [15-17].

After crystallization, the particles may undergo polymeric transitions, a phenomenon typical for lipidic materials. This process and other crystal aging phenomena may proceed over several days or weeks after solidification. The matrix material determines the type of polymorphs that can be formed in the suspensions. For fatty acid nanoparticles investigated in the dried state mainly polymorph C has been found along with the presence of polymorph B in some cases. For, glycerides, the presence of main polymorphs α, β'and β as well as an intermediate form β_i has been confirmed in nanoparticles prepared by melt-homogenization. In some cases, polymorphic forms not observable in the corresponding raw materials were detected in triglyceride nanosuspensions. The rate of polymeric transitions was found to be accelerated in the nanoparticles compared to the bulk material. It depends on the type of matrix lipid, the stabilizer composition and also on the particle size; e.g., shorter chain saturated monoacid triglyceride transforms more quickly from the least stable α into the stable β-modification than triglycerides with longer chains and smaller particles have a higher transformation rate than larger ones. During polymorphic transitions, the particle undergoes a rearrangement of the matrix molecules into more dense packing and may also change their shape [18, 19].

The highly ordered and tightly packed crystalline particle matrix should be expected to present rather unfavorable localization for the incorporation of at least larger amounts of the drugs because the drug will disturb the order of the crystal lattice. The incorporation capacity will depend on the physicochemical properties of the drug, but also on the type of matrix material (e.g., pure triglycerides are assumed to have a lower incorporation capacity than complex lipid) and the matrix state (in particular the degree of crystallinity and polymorphic form). Drug molecules that cannot be accommodated within the crystalline matrix may adsorb to the nanoparticle surface or separate from the particles. This will lead to the formation of drug crystals or a droplet or to distribution into the aqueous phase or additional colloidal structure present in the dispersion (e.g., micelles or phospholipids vesicles) [15, 20, 21]. Investigations of the drug distribution between nanoparticles and aqueous phase have confirmed that the drugs are not always completely distributed towards the nanoparticles and may sometime even be present the aqueous phase in considerable amount [16, 22, 23].

SLN are obtained by methods like high- pressure homogenization, ultrasound, solvent evaporation technique and microemulsion technique [5, 24]. SLN were primarily designed for i.v. administration, further they were exploited for oral and per oral drug delivery, recently the attention has been focused on the use of SLN in topical formulations with not only pharmaceutical, but also cosmetic applications [5, 9].

Advantages of SLN [3, 5, 13, 25, 26, 27]

- Use of biodegradable physiological lipids.
- Avoidance of organic solvents related to the production method or methods.
- Wide application spectrum (oral, i.v., dermal).
- Improved bioavailability of poorly water- soluble molecules.
- Site specific delivery of drugs via i.v. injection route.
- Enhanced drug penetration into the skin, localization in certain skin layers, via dermal application.
- Possibility of scaling up to industrial production level, by high-pressure homogenization, at low cost and in a relatively simple way.
- Protection of chemically labile agents from degradation in the gut and sensitive molecules from outer environment.

Disadvantages of SLN [25, 28, 29]

- Limited drug loading capacity due to crystalline structure of solid lipid.
- Adjustment of drug release profile.
- Drug expulsion during storage due to the formation of a perfect crystal.
- Particle growing.
- Unpredictable gelation tendency.
- Unexpected dynamics of polymorphic transitions.
- High water content of SLN dispersions.

NANOSTRUCTURED LIPID CARRIERS (NLC)

The limitations posed by SLN are overcome or minimized by the development of NLC. For NLC three models have been proposed:

a) NLC Type I is defined as the imperfect crystal model, because once in its matrix there are many imperfections which are able to accommodate the active molecules. This model is obtained when mixing solid lipids with small amounts of liquid lipids (oils). Owing to the different chain lengths of the fatty ac-

ids and the mixtures of mono-, di-, triacylglycerols, the matrix of NLCs is not able to form a highly ordered structure [30].

b) NLC Type II is called the amorphous model because it is created when mixing special liquids which do not recrystallize anymore after homogenization and cooling, such as hydroxyoctacosanylhydroxystearate and isopropyl myristate. These liquids are able to create solid particles of amorphous lipid structure, which can avoid the occurance of crystallization, minimizing drug expulsion, because the matrix is maintained in polymorphic α form.

c) NLC Type III is described as the multiple model. This model has been developed to improve the loading capacity of several drugs, such as the ones whose solubility in liquid lipids is higher than in solid lipids [31, 32]. This type is derived from w/o/w emulsions, which consist of an oil-in-fat-in-water dispersion. Very small oil nanocomparments are created inside the solid lipid matrix of the nanoparticles generated by a phase separation process. This model is obtained when mixing solid lipids with liquid lipids (oils) in such a ratio that the solubility of oil molecules in the solid lipid is exceeded. The melted lipids and the hot oil are blended; thus, the two lipids must show a miscibility gap at the used concentrations, at approximatel 40°C. A hot o/w nanoemulsion is produced at a higher temperature (approx. 80°C), then the lipid droplets are cooled. When reaching the miscibility gap, the oil precipitates forming tiny oil droplets in the melted solid lipid. Subsequent solidification of the solid lipid as solid nanoparticles matrix leads to fixation of the oiling nanocompartments.

The new generation of lipid carriers consists of a lipid matrix with a special nanostructure [8]. In the SLN the drug is mainly dispersed in molecular form, whereas NLC are composed of oily droplets embedded in a solid lipid matrix. In contrast to SLN being produced from solid lipids, the NLC are produced by controlled mixing of solid lipids with spatially incompatible liquid lipids. In the NLC different lipids are blended to form the matrix, like solid lipids and liquid lipids (e.g. hydroxyoctacosanylhydroxystearate, isopropylmyristate) .Since liquid lipids solubilize lipophilic molecules to a much higher extent than solid lipids, the NLC particles would provide a high incorporation capacity and better control of release [5]. Also due to their differences in structure they cannot fit together very well to form a perfect crystal. The matrix contains lots of imperfections to accommodate drug in molecular form, thus avoiding drug expulsion [13].

These NLCs can be produced by high-pressure homogenization and the process can be modified to yield lipid particle dispersions with solid contents from 30–80% [8]. The different types of NLCs are [8, 28]:

1. The imperfect structured type.

2. The structureless type/ non-crystalline amorphous NLC.

3. The multiple types (O/F/W) – these particles contain liquid oil nanocompartments within the lipid particle matrix. These particles are achieved by lipid-lipid precipitation technique. The solid lipids are melted and mixed with a large amount of hot liquid oil; the droplets were formed by homogenization at elevated temperature. During the cooling process a phase separation of the two lipids occurs. At a certain temperature they have a miscibility gap leading to the precipitation of liquid oil in small droplets (similar to precipitation of a drug from a solvent).

ADVANTAGES OF NLC [8, 13, 27, 28, 33]

- Improves drug loading.
- Firmly incorporates the drug during storage.
- Flexible modulation of release profile.
- Improved performance in producing final dosage forms such as creams, tablets, capsules and injectables.
- Suspensions of higher solid content can be produced (e.g. 30-50% solid).

MANUFACTURING TECHNOLOGIES:

SLNs and NLCs are produced by various techniques like, high-pressure homogenization [3, 34], microemulsion technique [3], precipitated lipid particles [3], using membrane contractor [35], solvent diffusion method [36], ultrasound method [13, 37] etc. The two techniques explained are:

1) Preparation of SLN Using Membrane Contractor

A new process for the preparation of SLN using a membrane contractor is being investigated. The lipid phase is pressed, at a temperature above the melting point of the lipid, through the membrane pores allowing the formation of small droplets. The aqueous phase circulates inside the membrane module, and sweeps away the droplets forming at the pore outlets. SLN are formed by cooling of the preparation to room temperature. Process parameters like aqueous phase and lipid phase temperatures, aqueous phase cross-flow velocity, lipid phase pressure and membrane pore- size influence the particle size of SLN. The advantages of this new process are its facility of use, the control of the SLN size by appropriate process parameters, and its scaling-up abilities [35].

2) Preparation by High-Pressure Homogenization

Melt emulsification by high-pressure homogenization was introduced as preparation method for SLN. The solid matrix lipids are melted, and after free dispersion (usually by Ultra Turrax vortexing or ultrasonication) the melt is dispersed in an aqueous phase by high-pressure homogenization in the heat with the aid of emulsifying agent [15, 20]. Subsequently, the droplets of the resulting hot colloidal emulsion have to be crystallized. This may occur by simply cooling the dispersion to room temperature but, depending on the composition, can also require specific thermal treatment such as cooling to refrigerator or even to subzero temperatures. The resulting super cooled lipid particles may have higher drug incorporation capacity and may be easier to stabilize than SLN but do not have the potential advantages expected for nanoparticles in the solid state [20]. The mean particle size of high pressure melt emulsified lipid suspension is typically in the range of 50-400nm. The effects of composition and homogenization parameters are basically similar to those found for o/w emulsions, and thus reflect the emulsion like state of the dispersed molten lipids during emulsification [38, 39].

SLN can also be prepared by cold homogenization by passing the predispersed matrix lipid through a high pressure homogenizer at a temperature below its melting point. This allows processing also of lipid matrix material with melting temperature distinctly above 100°C, e.g., cholesterol. Active agents are incorporated into matrix by dissolving or dispersing them in the melted lipid which is subsequently solidified and ground into fine powder at low temperature (e.g., under liquid nitrogen or dry ice cooling). This powder is processed into a submicron suspension by high-pressure homogenization in an aqueous surfactant solution (e.g., sodium cholate, Tween 80). Because the dispersion of solid liquids requires a higher energy input than that of liquid melt, harsher homogenization conditions with respect to homogenization pressure and number of homogenization cycles are often applied. The dispersions obtained are still typically of larger mean particle size and of broader size distribution than that resulting from processing of melted lipids, often with particles sizes in the upper nm or even in the μm range [40-42].

APPLICATIONS

SLN and NLC find extensive application in topical, oral, parenteral, ocular and pulmonary administration of cosmetic and pharmaceutical actives.

1) Topical Applications

Dermally applied lipid nanoparticles exhibit following features:

1. Increased Hydration

On application of the lipid nanodispersions to the skin surface, the evaporation of water induces the ultrafine lipid particles to form an adhesive layer applying occlusion to the surface. Consequently, an increase in the hydration of the stratum corneum occurs [3, 5, 43]. Occlusion prevents transepidermal water loss and improves penetration of drugs through stratum corneum [44]. The occlusive effects depend on the particle size, lipid concentration, applied sample volume (lipid mass) and crystallinity of the matrix [5, 45]. Skin hydration was measured as a function of time using Corneometer 825 [29]. To detect adhesive properties of SLN on human skin Tesa strip test have been performed [29, 43].

2. Wrinkle Smoothing

A reduction in wrinkle depth is observed following SLN application [5], also a cream containing retinol-loaded SLN showed improved skin smoothness when compared against SLN –free cream [3].

3. Drug Release Modification, Modulation of Penetration

The SLN releases the incorporated drug in a controlled way after it receives a triggering impulse like, increase in temperature or the loss of water from SLN dispersion or an SLN-containing cream [3]. The penetration of active compounds in the skin was studied using the Tesa stripping test. 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. The chemical composition of the formulation and factors like film forming properties, skin hydration, and interaction of SLN lipids and surfactants with skin lipids affect the degree of penetration [29].

4. Protection of Chemically Labile Compounds

The solid matrix of SLN can improve chemical stability of drugs, like retinol, coenzyme Q10, vitamin E, tocopherol, as compared to aqueous dispersions, by protecting molecules from hydrolysis and oxidation [3, 5, 43]. Cosmetic products containing retinol are manufactured under special, cost-intensive safety conditions and packed in less aesthetic packing in order to retain its stability. Incorporation of retinol in SLN would offer a cost- effective manufacturing technique and a more appealing consumer-oriented packaging.

5. Increased Efficiency of Molecular UV-Blockers and Reduced Side Effects

A completely new, recently discovered area of application is the use of SLN in unprotective creams; they were initially introduced as carriers for molecular and particular sunscreens (UV-blockers). Side effects of molecular sunscreens are penetration into the skin; causing photo allergies, phototoxic reactions and skin irritations [46]. Particulate sunscreens like titanium dioxide were also found to penetrate into the skin and interact with the immune system [3, 29]. This can be avoided or minimized by entrapping molecular and particulate sunscreens into the SLN matrix. Interestingly, it was found that the SLN themselves also have a sun protective effect; due to their particulate character they scatter UV-light (similar to titanium dioxide) [3, 29]. Incorporation of molecular sunscreens into SLN thus shows a synergistic effect on the protective characteristics [3, 28, 29, 47]. Therefore the concentration of molecular sunscreens in formulations decreases; while retaining the protection level; at the same time side effects are reduced. This opens the perspective to a new class of sun protective creams [3, 48]. Cetyl palmitate-nanodispersions act both as particulate UV blockers themselves and as carriers or UV absorbing agents (e.g. 2- hydroxyl-4-methoxy benzophenone; Eusolex® 4360) [5, 34]. The Transpore test serves as an *in vitro* method to investigate the UV-blocking ability of the sunscreen formulations [48].

6. Pigment Effect

The SLN possess a pigment effect covering undesired colours leading to improved appearance of the product [43].

SLN loaded with Clobetasol propionate (Cp) were incorporated in suitable cream base. Invitro studies and clinical evaluation against an equivalent marketed formulation was carried out to assess drug release, drug permeation. Skin uptake studies from Cp creams were carried out in a validated Franz static diffusion cell across human cadaver skin (HCS). Both formulations were found to be responsive to manifestations of chronic eczema, while Cp-SLN cream prepared in this investigation registered significant improvement in therapeutic response (1.9 fold; inflammation, 1.2 fold; itching) in terms of per cent reduction in degree of inflammation and itching against marketed cream [49].

Prednicarbate (PC) was incorporated into solid lipid nanoparticles of various compositions. Local tolerability as well as drug penetration and metabolism were studied in excised human skin and reconstructed epidermis. Conventional PC cream of similar strength and ointment served for reference. PC incorporation into nanoparticles appeared to induce a localizing effect in the epidermal layer which was pronounced at 6 h and declined later. Lipid nanoparticle-induced epidermal targeting may increase the benefit/risk ratio of topical therapy and this may contribute to a reduction of skin atrophy by the inhibition of fibroblasts induced by long term topical glucocorticoid treatment [50].

Clotrimazole-loaded SLN and NLC were prepared, the physical stability of these particles, as well as the entrapment efficiency of this lipophilic drug and its *in vitro* release profile was assessed, and the particle size analyzed by photon correlation spectroscopy (PCS) and laser diffractometry (LD) showed that the particles remained in their colloidal state during 3 months of storage at 4ºC, 20ºC and 40ºC. For all tested formulations the entrapment efficiency was higher than 50%. The obtained results also demonstrate the use of these lipid nanoparticles as modified release formulations for lipophilic drugs over a period of 10h [51].

Methotrexate- (MTx) loaded SLN were prepared, incorporated in suitable gel base and evaluated, *in-vitro* and clinically to justify the role of the developed gel in treatment of psoriasis. *In vitro* skin deposition studies were carried out over dermatomed and prepared human cadaver skin (HCS). Findings of the studies suggest that there is significant improvement in therapeutic index in treatment of psoriasis by MTx-SLN incorporated gel base. More benefits of the gel were observed in terms of reduction in adverse effects of therapy, fostering better patient compliance [52].

Glyceryl behenate SLN loaded with chemically labile vitamin A, (retinol and retinyl palmitate) and incorporated in a hydrogel and o/w-cream, were tested with respect to their influence on drug penetration into porcine skin. Franz diffusion cells were used to assess the release kinetics over a period of 24 h. Vitamin A concentrations in the skin tissue suggested a certain drug localizing effect. Due to the polymorphic transition of the lipid carrier, drug expulsion follows the application onto the skin; the drug localizing action appears to be limited for 6-24 h [53, 54]. Invitro penetration studies were carried on NLC containing retinol. Initially the concentrations were low (due to prolonged release from particle), after a period of 24hrs high retinol levels were found in the residual skin [29].The stabilization of retinol incorporated into SLN offers more cost-effective ways of production and the use of more appealing, consumer-orientated packaging [3].

Chemical sunscreen vitamin E (tocopherol acetate) was incorporated into the SLN to protect it from chemical degradation and improve the UV blocking capacity. The SLN dispersion was incorporated into gels. Characterization, film formation, occlusivity, stability testing, thermoanalytical testing along with the UV-blocking capacity, was investigated. The occlusion promotes the penetration of vitamin E into the skin, as shown by the stripping

test. SLN dispersions penetrated twice as effectively as compared to the conventional emulsions, used as reference. Placebo SLN showed greater UV blocking efficacy as compared to emulsions containing tocopherol acetate as molecular sunscreen. In addition to chemical stabilization of active ingredients, occlusive effects, UV blocking effect and enhanced penetration of compounds, the SLN possess a pigment effect covering undesired colours leading to an increased aesthetic acceptance by the customer [43, 55].

Molecular sunscreen Oxybenzone was incorporated into two different carrier systems, SLN and conventional o/w emulsions. The influence of the carrier on the rate of release was studied *in vitro* with a membrane-free model. Invitro penetration studies were performed using static Franz diffusion cells. Tape stripping method was used to investigate *in vivo* penetration of oxybenzone into stratum corneum. The rate of release is strongly dependent upon the formulation and total concentration of oxybenzone; and could be decreased by 30–60% in SLN formulations [47, 48].

Ascorbyl palmitate (AP) entrapped in SLN, NLC and for comparison, Nanoemulsion (NE), were incorporated into a hydrogel and assessed for its moisturizing effect, the occlusive effect of the carriers on skin hydration was compared to placebo SLN, NLC and NE incorporated into hydrogel. Penetration of AP from these carriers through human skin was studied invitro. Chemical stability was also conducted at **room temperature**, 4ºC and 40ºC. According to the data of 3 months AP was found to be most stable at 4ºC for each formulation [56].

Ketoconazole was loaded in SLN and NLC, the lipid particles targeted the drug into topical route thus minimizing adverse side effects and providing a controlled release. Compritol®888 ATO (glyceryl behenate) was selected as the solid lipid and the lipidsoluble antioxidant _-tocopherol was chosen as liquid lipid for preparation of NLC. Stability of Ketoconazole in aqueous SLN and NLC dispersions, along with the physicochemical stability of these lipid nanoparticles, was assessed. Ketoconazole loading capacity was identical for both SLN and NLC systems (5% of particle mass). SLN were physically stable as suspensions during 3 months of storage, but the SLN matrix was not able to protect the chemically labile ketoconazole against degradation under light exposure, this problem is resolved by adequate packaging. In contrast, the NLC were able to stabilize the drug, but the aqueous NLC dispersion showed size increase during storage which was resolved by forming a gel network [57].

SLN containing a novel potential sunscreen *n*-dodecyl-ferulate (ester of ferulic acid) were developed. Parameters like particle size, surface electrical charge (zeta potential) and matrix crystallinity were investigated. The chemical stability of *n*-dodecyl-ferulate at high temperatures was also assessed by thermal gravimetric analysis. *N*-dodecyl-ferulate loaded SLN prepared with cetyl palmitate among other lipids showed the lowest mean particle size and polydispersity index, as well as the highest physical stability during storage time of 21 days at 4ºC, 20ºC and 40 ºC [58].

Indomethacin (IND)-loaded SLN and NLC were prepared by ultrasonication. The organization and distribution of the different ingredients of the nanoparticle system were studied by differential scanning calorimetry (DSC) technique. Further mean particle size and percentage of drug encapsulation were also determined. The results obtained showed that NLC lipid organization guaranteed an increased Indomethacin encapsulation in comparison with SLN. DSC static and dynamic measurements showed that oil nanocompartments incorporated into NLC solid matrix drastically influenced drug distribution inside the nanoparticle system. Controlled release from NLC system could be explained considering both drug partition between oil nanocompartments and solid lipid and a successive partition between solid lipid and water [59].

Topical therapy is employed as first-line treatment in mild acne but side effects and the undesirable physicochemical characteristics of certain important agents like tretinoin and benzoyl peroxide affect their utility and patient compliance. Novel drug delivery strategies like SLN play a pivotal role in optimizing and improving the topical delivery of antiacne agents by enhancing their dermal localization with a concomitant reduction in their side effects [60].

2) Oral Applications

Oral administration of SLN and NLC is possible as aqueous dispersion or after transformation into dosage forms, i.e. tablets, pellets, capsules or powders in sachets [3, 8]. For the production of tablets the aqueous SLN dispersion can be used instead of a granulation fluid in the granulation process. Alternatively SLN can be transferred to a powder (e.g. by spray-drying) and added to the tableting powder mixture. For the production of pellets the SLN dispersion can be used as wetting agent in the extrusion process [61]. SLN powders can be used for the filling of hard gelatin capsules; or the SLN can be produced directly in liquid PEG 600 and filled into soft gelatin capsules. Sachets are also possible using spray dried or lyophilized powders. In both cases it is beneficial to have a higher solid content to avoid the necessity of having to remove too much water. For cost reasons spray drying might be the preferred method for transferring SLN dispersions into powders. Primary drugs of interest are compounds undergoing chemical degradation in the gastrointestinal tract [3].

Camptothecin (CA)-SLN were produced from stearic acid and stabilized with Poloxamer 188. Encapsulation efficiency of 99.6% and invitro-release for one week was achieved. The plasma levels and body distribution were also determined after administration of CASLN suspension vs. CA solution. It was found that incorporation of CA into SLN prevented it from hydrolysis. Thus proving that, SLN are a promising sustained release system for CA and other lipophilic drugs after oral administration [62].

Piribedil (PD), whose aqueous solubility is low and elimination half-life short, was incorporated into a lipid matrix to give prolonged release and higher oral bioavailability. Thereby preventing known side effects and high frequency of oral administration [63].

Cyclosporine, the peptide, was incorporated in SLN and an oral formulation was developed as a competitive product to Sandimmun Neoral/Optoral by Pharmtec (Milan/Italy) [64]. An *in vivo* study was performed on three pigs comparing the cyclosporine SLN dispersion with the commercial product Sandimmun Neoral/Optoral. The animal study shows that the cyclosporine SLN combines the advantages of the old Sandimmun and the new Sandimmun microemulsion: avoidance of nephrotoxic high plasma peak and low variability in plasma profile [3, 24, 64].

Highly lipophillic Mifepristone was incorporated in SLN, by modified high shear homogenization and ultrasound techniques. The mean particle size measured by laser diffractometry (LD) was found to be 106nm with a narrow particle distribution of polydispersity index, 0.278. Differential scanning calorimetry and X-ray diffraction measurements suggested that the majority of the SLNs were less ordered arrangement of crystals, and this was favorable for increasing the drug loading capacity. The drug entrapment efficiency (EE%) of SLNs was more than 87 percent and showed relatively long-term physical stability, as the leakage was very small after being stored for one month [24].

3) Parenteral Applications

SLN and NLC can be injected either intravenously, intramuscularly, subcutaneously or to the target organ, because of their small size. The particles are cleared from the circulation by the liver and spleen. SLN formulations can be used for systemic body distribution with a minimized risk of blood clotting and aggregation leading to embolism. Also SLN and NLC provide a sustained release depot of the drug when administered subcutaneously or intramuscularly [8, 65]. The drug can be incorporated between fatty acid chains, lipid layers or imperfections; in the lipid nanoparticles. De-

pending on the drug/lipid ratio and solubility, the drug is located mainly in the core of the particles, in the shell or molecularly dispersed throughout the matrix [65]. Incorporated drug is gradually released on erosion (e.g. degradation by enzymes) or by diffusion from the particles. The rate of release may be controlled by the nature of the lipid material, particle size, and choice of surfactant and also by inner structure of the lipid particles [66, 67, 68, 69]. To facilitate drug targeting, for example in tumor tissue, reticuloendothelial system avoidance (stealth) facility may be incorporated. This may be achieved using block polyoxyethylene polypropylene copolymers like Pluronic F188 in which the hydrophobic portion of the molecule forms the nanoparticle matrix while the water soluble polyoxyethylene block forms a hydrophilic coating on the particle. Stealth SLN increases the tumor accumulation [70], antibacterial activity of antiparasitic and antifungal drugs [71] and allows brain delivery of anticancer drugs that are not capable of crossing the blood brain barrier (BBB) [72].

Stealth and non-stealth solid lipid nanoparticles were produced and studied in cultures of macrophages [73-75]. They were then loaded with Paclitaxel and assessed *in vivo*. The i.v. administered SLN led to higher and prolonged plasma levels of Paclitaxel. A low uptake by the liver and the spleen macrophages, and an increased uptake in the brain were observed [3]. As an i.v. system, the NLC can be loaded with paclitaxel thereby avoiding critical solubilizers like Cremophor EL [8, 28].

Camptothecin (CA)-loaded SLN were produced by high pressure homogenization. The SLN employed were composed of stearic acid, soybean lecithin and Poloxamer 188. The distribution of CA in the body was studied. The i.v. administration showed increased uptake in some organs especially in the brain [3, 76].

Three types of solid lipid nanoparticles (SLN) containing three different percentages of tobramycin were prepared (Tobra-SLN). *In vitro* tobramycin diffusion through a hydrophilic/lipophilic membrane was determined, tobramycin diffusion varied with the percentage of drug incorporated in SLN: the higher the percentage of tobramycin incorporated, the greater the amount of the drug diffused. *In vivo* uptake and transport were determined after administering a fixed dose of tobramycin in each of the three types of SLN intraduodenally to rats. Blood and lymph sampled at fixed intervals were examined by transmission electron microscopy (TEM) analysis to detect the presence, size, and shape of SLN. The pharmacokinetic variations observed may be related to the differences among the three types of SLN; namely, the number of SLN administered and the mean diameter, the total surface area, and the drug content in each nanoparticle. TEM analysis showed that Tobra-SLNs were targeted to the lymph. Tobra-SLN may act as a reservoir of the drug in the lymphatic system, thereby favoring its sustained release [77].

Etoposide-SLN was administered intraperitoneally in Dalton's lymphoma ascites bearing mice. The cell cycle perturbation, cytogenetic damage, cell death (apoptosis), tumor regression, and animal survival were investigated as parameters of response with time to determine the tumoricidal effects of etoposide after a single-dose administration. Of the 3 different formulations, apoptosis induction and survival time of mice was higher when treated with etoposide-loaded tripalmitin (ETP) nanoparticles, followed by etoposide-loaded glycerol monostearate (EGMS) and etoposide-loaded glycerol distearate (EGDS) compared with free etoposide. This indicates that the ETP-SLN formulation may be a promising delivery system for the effective treatment of ETPsensitive peritoneal carcinomas and peritoneal metastasis [78].

Significant nanoparticle-mediated drug transport of various drugs into the brain using polysorbate-coated polybutylcyanoacrylate (PBCA) SLN was observed. After i.v. administration the polysorbate coated nanoparticles immediately interact with the plasma proteins. Other surfactants such as poloxamer 407, poloxamine 908 or Cremophorw RH 40 led to no effects. An enrichment of apolipoprotein E (apoE) was observed on the surface of polysorbate-coated solid lipid nanoparticles after incubation in human citrate plasma, whereas no apoE adsorption resulted after incubation with the other mentioned surfactants. ApoE plays an important role in the transport of lipoproteins into the brain via the low density lipoprotein (LDL) receptor on the BBB. Thus, it was supposed that apoE is the responsible protein mediating the uptake into the brain. Intravenous administration of Dalargin, Tubocurarine, Camptothecin, Doxorubicin and 30, 50- dioctanoyl-5-fluoro-20- deoxyuridine showed in principle the potential of SLN to deliver drugs to the brain. However, the PBCA nanoparticles show some disadvantages such as limited drug loading capacity, too slow *in vivo* biodegradation and release of toxicologically problematic formaldehyde residues [76].

A polymeric microparticulate delivery system for corticoids to treat arthritis of joints was developed in 1980. The microparticles loaded with a corticoid were to be injected into the joint; the macrophages would then phagocytose the particles. The drug released inside the macrophages would reduce their hyperactivity which was causing the inflammation. But the polymer particles proved to be cytotoxic in the concentrations necessary for the treatment. As an alternative the lipophilic corticoids could be incorporated into SLN and developed further [3].

4) Potential as a Vaccine Adjuvant

Adjuvants are used in vaccines to enhance the immune response. The adjuvant frequently used are aluminium hydroxide particles, emulsion systems like SAF 1 and MF 59, Freund's complete adjuvant (FCA) and Freund's incomplete adjuvant (FIA); however they exhibit side effects or rapidly degrade in the body. The lipid components of SLN will degrade more slowly due to their solid state thus providing a longer lasting exposure to the immune system. Degradation of SLN can be slowed down even more when using sterically stabilizing surfactants that hinder the anchoring of enzyme complexes. Advantages compared to traditional adjuvants are the biodegradation of SLN and their good tolerability by the body [3].

SLN have been tested as adjuvant in comparison to FIA in sheep. The two unoptimized SLN formulations exhibited 43 and 73% of the immune response (antibody titer) of FIA investigated as standard [3].

SLN have also been tested as a vaccine adjuvant in hens. Hens were vaccinated under the addition of SLN and the egg yolk concentrations of IgY were determined. The adjuvant effect was compared to FCA/FIA and to the vaccine without any adjuvant. The SLN induced characteristic changes of the chronological titer development. This is an indication of an adjuvant effect. The tissue tolerability was very good. Antibody titers were enhanced only slightly [79].

5) Potential as Transfection Agent

Cationic solid lipid nanoparticles (SLN) for gene transfer are formulated using the same cationic lipids as for liposomal transfection agents. The differences and similarities in structure and performance between SLN and liposomes were investigated. A SLN preparation, its counterpart formulation without matrix lipid, a commercially available liposomal preparation — all based on the cationic lipid DOTAP (1, 2- Dioleyl-snglycero- 3-trimethylammoniumpropane)—and a liposomal formulation that additionally contained the helper lipid dioleoylphosphatidylethanolamine (DOPE) (EscortTM) were compared. Photon correlation spectroscopy (PCS) showed that the SLN were smaller in diameter than the corresponding liposomes while atomic force microscopy (AFM) supported the expected structural differences. DNA binding differed only marginally. Cationic lipid composition governs the *in vitro* transfection performance than the colloidal structure it is arranged in. Hence, cationic SLN extends the range of highly potent nonviral transfec-

tion agents by one with favorable and distinct technological properties [80]. Combination of cationic SLN with the nuclear localization signal TAT2 increased transfection efficiency hundredfold [79].

6) Ocular Delivery

Prolonged retention of the drug in the eye using nanoparticles is mentioned in many papers however there are no products in the market. This is due to toxicity problem of non-accepted polymer polyalcylcyanoacrylate. SLN showed an increased retention time in the eye (Gasco personal communication); it would be even more beneficial to use NLC due to improved drug accommodation properties [28].

7) Controlled Release of Perfumes and Insect Repellents

SLN and NLC are used to incorporate perfumes [28]. The perfume Allure was incorporated in SLN and the release studied was compared to a nanoemulsion of identical lipid content and surfactant composition. The initial release was similar; this could be due to the presence of perfume in the outer shell of the SLN. The release of the perfume from SLN was delayed further to 8h. This opens the prospect of developing long lasting perfume formulations based on the prolonged release of the perfume from lipid matrix [81].

Prolonged release is also desired for insect repellents while simultaneously the releasing carrier systems should firmly adhere to the skin. SLN as carrier systems are therefore preferred for incorporating insect repellents. The insect repellent DEET (N, N- diethyl toluamide) was incorporated in different SLN formulations. A loading of 10% could be achieved stearic acid SLN stabilized with Tween 80 as surfactant. The particles were observed to be physically stable for a long- term after incorporation in a ready- to- use gel [29].

7) Pulmonary Applications

The potential of SLN in pulmonary drug delivery has not been sufficiently explored. Aqueous SLN dispersions were nebulized with a Pari-Boy, the aerosol droplets were collected and the size of SLN analyzed. It was observed that the particle size distributions of SLN before and after nebulization were almost identical, only very little aggregation could be detected which was of no significance for pulmonary administration. SLN powders might be used in dry powder inhalers. SLN could be spray-dried using, lactose. Basic advantages of drug release from SLN in the lung are control of the release profile, achievement of a prolonged release and having a faster degradation compared to particles made from some polymeric materials. High tolerability of SLN can be exploited for drug targeting to lung macrophages [3].

CONCLUSION

The lipid nanoparticles, SLN and NLC, are carrier systems that possess the potential to develop as the new generation of the carrier systems after the liposomes. They have good perspectives to be developed and marketed very successfully. The reason for this is that they were developed considering industrial needs. The promising results of SLN and NLC prove their potential as versatile carrier systems for application in cosmetic and pharmaceutical formulations. The novel generations of lipid carriers also offer much more flexibility in drug loading, modulation of release and improved performance in producing final dosage forms.

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