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Review

Solid lipid based nanocarriers: An overview

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In the era of nanoparticulate controlled and site specific drug delivery systems, use of solid lipids to produce first generation lipid nanoparticles, solid lipid nanoparticles (SLN), became a revolutionary approach in the early nineties. The present review is designed to provide an insight into how SLN are finding a niche as promising nanovectors and forms a sound basis to troubleshoot the existing problems associated with traditional systems. Herein, authors had tried to highlight the frontline aspects prominent to SLN. An updated list of lipids, advanced forms of SLN, methods of preparation, characterization parameters, and various routes of administration of SLN are explored in-depth. Stability, toxicity, stealthing, targeting efficiency and other prospectives of SLN are also discussed in detail. The present discussion embodies the potential of SLN, now being looked up by various research groups around the world for their utility in the core areas of pharmaceutical sciences, thereby urging pharmaceutical industries to foster their scale-up.

Keywords: colloidal drug carrier, solid lipid nanoparticles (SLN), stability, targeting efficiency, cytotoxicity, stealthing of SLN

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INTRODUCTION

Multiparticulates, including micro- and nano-dimensional drug delivery vehicles have excellent and broad prospects in the pharmaceutical field. Scientists from various disciplines have been harnessed by the superior outcomes obtained from such carrier systems, *viz.* greater therapeutic efficacy with reduced dosing frequency (1). The *in vivo*

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biodistribution of any drug that longer depends on the properties of that drug but is mainly determined by a carrier system which enables a controlled release of bioactives to the localized site as per specific needs of the therapy (2).

When colloidal drug carriers (nanometric size range) are compared with microparticles (micrometer range) and implants (several millimeters), it has been found that microparticles and implants based on biodegradable polyesters permit controlled and localized drug release over a period of weeks to months after *s.c.* or *i.v.* injection/implantation, but are too large for drug targeting and intravenous administration. Therefore, colloidal drug carriers hold great promise for achieving the target of controlled and site specific drug release and have hence attracted wide attention of researchers and formulation scientists. Traditional colloidal drug carrier systems include nanoemulsions, nanosuspensions, nanoparticles, micelles, liposomes, polymer-drug conjugates (2, 4).

The existence of different colloidal drug carrier systems may raise the question to the readers' mind about which of these might be the most suitable carrier system for a desired purpose. To answer this question, the following aspects should be taken into consideration:

(i) drug loading capacity
(ii) sufficient drug targeting
(iii) *in vivo* fate of the carrier system (interaction with the surrounding biological fluid, degradation rate, accumulation in organs, *etc*.)
(iv) toxicity, acute as well as chronic
(v) storage stability, physical as well as chemical
(vi) large scale production
(vii) overall cost of formulation.

A new class of colloidal drug carriers, solid lipid nanoparticles (SLNs), emerged in the early nineties and is now being looked up by researchers around the world for exploitation of their potential applications in the core areas of pharmaceutical sciences, like drug delivery, clinical and therapeutic medicine as well as other sciences. Polymeric nanoparticles, instead of having the advantage of possible chemical modifications including synthesis of block- and co-polymers, have their own well-known limitations such as polymer cytotoxicity, polymer degradation, high cost due to lack of a suitable large scale production method and scarcity of their approval by the regulatory authorities that pose major hurdles for their utilization in clinical medicine (3). Thus, since the beginning of the nineties, attention of various research groups has been focused on an alternative to polymeric nanoparticles, the solid lipid nanoparticles (SLN).

Recent updates have shown that new drug development is not the prime concern for efficient drug therapy. Theoretical predictions and *in vitro* experimental data sometimes follow the disappointing *in vivo* results leading to therapy failure. The main reasons for therapy failure include:

(i) poor drug solubility excluding *i.v.* injection of aqueous drug solution
(ii) poor absorption, rapid metabolism and excretion (*e.g.* proteins, peptides, *etc*.)
(iii) drug distribution to non-targeted sites combined with high drug toxicity (*e.g.*, anticancer drugs)

(iv) high fluctuations in drug plasma levels due to unpredictable bioavailability after peroral administration, including influence of food on plasma levels (*e.g.*, cyclosporine).

To troubleshoot these formidable problems, a novel and promising nano-drug carrier system, solid lipid nanoparticles, was introduced. SLNs are one generation above the sub-micron sized lipid emulsions where the liquid lipid (oil) has been substituted by a solid lipid and are mainly composed of a physiological lipid dispersed in water or in aqueous surfactant solution. Replacement of liquid oil with solid lipid represents a milestone in achieving controlled drug release, because the mobility of a drug in solid lipid is usually lower compared to liquid oil, which made them attractive for their potential use in improving the performance of pharmaceuticals, nutraceuticals and other such materials (2–4).

In the present review, the authors have focused on the overview of SLNs, problems associated with them, broad prospects of SLNs as a drug delivery system in the pharmaceutical field and related trends in SLNs.

ADVANTAGES AND DISADVANTAGES OF SLNs

It has been proposed that SLNs combine the advantages of traditional colloidal drug carriers but avoid some of their major disadvantages (2, 5). The proposed advantages include:

(i) controlled and targeted drug release
(ii) possible high drug payload
(iii) feasibility of carrying both hydrophilic and lipophilic drug
(iv) water based formulation avoids organic solvents
(v) physiological lipids decrease the prevalence of acute or chronic toxicity; no reported biotoxicity of the carrier system
(vi) improved drug stability
(vii) most lipids being biodegradable, SLNs have excellent biocompatibility
*(viii)*less expensive than polymeric or surfactant based carriers
(ix) easy to scale up and sterilize
(x) easy to validate
(xi) easy to gain regulatory approval.

The potential disadvantages of SLNs include:

(i) poor drug loading capacity of drugs having limited solubility in lipid melt
(ii) relatively very high water content of dispersions (70–99.9 %)
(iii) drug expulsion after polymeric transition during storage.

The drug loading capacity of conventional SLNs is depends on the following factors:

(i) solubility of the drug in lipid melts

(ii) structure of the lipid matrix

(iii) polymorphic state of the lipid matrix.

If the lipid matrix consists of very similar molecules (*viz.* tristearin or tripalmitin), then a perfect crystal with few imperfections is formed. A highly ordered crystal lattice cannot accommodate large amounts of the drug, since the incorporated drugs are located between fatty acid chains, between lipid layers and also in imperfections. Therefore, the use of more complex lipids is more convenient for higher drug loading.

ADVANCED FORMS OF SLNs

Nanostructured lipid carriers (NLCs)

Nanostructured lipid carriers, introduced at the turn of the millennium, represent a new and improved generation of SLNs and are made of a solid lipid matrix entrapping liquid lipid nanocompartments, the blend being solid at body temperature (6). This new generation of lipid carriers (NLCs) was introduced to overcome the problems associated with SLNs, such as limited drug loading capacity, drug expulsion during storage and adjustment of drug release, long-term physical stability of the suspension, *etc.*

Production procedures are identical for both lipid particles, SLNs and NLCs. The solid lipid or solid-liquid lipid blend is melted, the pharmaceutical or cosmetic active is dissolved in melted lipid phase subsequently dispersed in a hot aqueous surfactant/stabilizer solution of equivalent temperature under high speed stirring. Obtained pre--emulsion is homogenized in a high pressure homogenizer yielding a hot o/w nanoemulsion. After cooling, emulsion droplets crystallize, forming lipid nanoparticles with solid particle matrices, depending on the starting material, either SLN or NLC (7).

Three models of NLCs were proposed. In the first model, also known as »imperfect type NLC«, particles are prepared from a lipid mixture of spatially different lipids (like glycerides) composed of different fatty acids. Use of spatially different lipids leads to larger distances between the fatty acid chains of glycerides and general imperfection of the crystal lattice. This would provide more space for accommodation of guest molecules in molecular form or as amorphous clusters. High drug loading could be achieved and drug expulsion from the lipid matrix during storage could be prevented with this model, due to distortion of the crystal lattice. This suggests that an increased number of imperfections leads to increased drug loading capacity and one could say that »the perfectness« of the NLC system lies in the »imperfectness« in its crystal lattice (Fig. 1) (7). Fang *et al.* (8) found enhanced permeation and controlled release of psoralens with NLC formulations compared to SLN formulations and concluded that NLCs can be potentially exploited as carriers for psoriasis therapies.

The second model is also known as »multiple type NLC«, where drugs showing higher solubility in oils than in solid lipids can be dissolved in oil and yet be protected from degradation by the surrounding solid lipids. Multiple type NLCs are analogous to w/o/w multiple emulsions since these are oil-in-solid lipid-in-water dispersions.

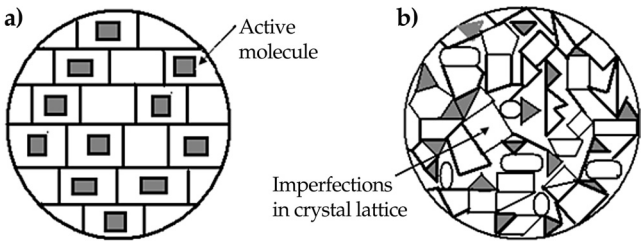

Fig. 1. Formation of: a) perfect crystal lattice in SLN using similar lipid molecules, and b) imperfections in NLC using spatially different lipids.

The third model, also known as »amorphous type NLC«, prevents the ongoing expulsion of the drug caused by crystallization or transformation of the solid lipid. Here, the particles are solid but crystallization upon cooling is avoided by using special lipids such as hydroxyl octacosanyl, hydroxyl stearate, isopropyl myristate, *etc.*

NLCs have mostly been extensively investigated for topical and dermatological preparations in the delivery of clotrimazole (9), celecoxib (10), ascorbyl palmitate (11), fluticasone (12) and so on.

Lipid drug conjugates (LDCs)

Lower drug loading capacity of hydrophilic actives was a major issue in SLNs due to partitioning effects during the production process. Highly potent-low dose drugs can be suitably incorporated only in the solid lipid matrix (13).

Polymer lipid hybrid nanoparticles (PLNs)

Polymer-lipid hybrid nanoparticles hold great promise as a drug delivery vehicle in the treatment of a myriad of diseases such as breast cancer (14, 15). A PLN comprises three distinct functional components:

(i) hydrophobic polymeric core to encapsulate poorly water-soluble drugs

(ii) hydrophilic polymeric shell to enhance PLN stability and circulation half-life

(iii) lipid monolayer at the core and shell interface to promote drug retention inside the polymeric core (16).

Interactions among these components play an important role for successful fabrication and performance of PLNs (17).

These hybrid NPs combine the merits of both liposomes and polymeric nanoparticles, two of the most popular drug delivery vehicles approved for clinical use, thereby serving as a robust drug delivery platform (16). It has been shown *in vitro* that hybrid NPs possess the ability of carrying poorly water-soluble drugs with high encapsulation and loading yields, tunable and sustained drug release profiles, excellent serum stability, and differential targeting of cells (18).

GENERAL INGREDIENTS USED IN THE PREPARATION OF SLNs

General ingredients used in the preparation of SLNs are given in Table I.

Table I. Ingredients used for the production of solid lipid nanoparticles

Ingredient	References
LIPIDS	
Triglycerides	
Tricaprin	[128]
Trilaurin	[57, 58, 129]
Trimyristin [Dynasan® 114]	[24, 57, 130]
Tripalmitin [Dynasan® 116]	[24, 57, 66, 131]
Tristearin [Dynasan® 118]	[57]
Hydrogenated coco-glycerides (Softisan® 142)	[132]
Hard fats	
Witepsol W® 35	[131]
Witepsol H® 35	[130]
Witepsol E® 85	[130]
Witepsol S® 51	[60]
Witepsol S® 55	[60]
Glyceryl monostearate [Imwitor® 900]	[104, 133]
Glyceryl behenate [Compritol 888® ATO]	[58, 63, 129, 134]
Glyceryl palmitostearate [Precirol®ATO 5]	[134]
Glyceryl caprate [Campul® MCM C10]	[122]
Cetyl palmitate	[134]
Stearic acid	[31, 32, 51, 133, 135, 136]
Palmitic acid	[137]
Decanoic acid	[137]
Behenic acid	[51]
Acidan N12	[51]
Beeswax	[138, 139]
Carnauba wax	[139]
Cacao butter	[140]
Emulsifiers	
Soybean lecithin	[23, 57, 58, 66, 130, 131]
Egg lecithin	[66]
Phosphatidylcholine	[23, 51, 133, 141]
Poloxamer 188	[23, 76, 131]
Poloxamer 182	[132]
Poloxamer 237	[142]
Poloxamer 238	[142]

Poloxamer 338	[142, 143]
Poloxamer 407	[142, 144]
Poloxamine 908	[142, 144]
Polysorbate 20	[145]
Polysorbate 21	[146]
Polysorbate 80	[34]
Tyloxapol	[21]
Sodium cholate	[132, 34]
Sodium glycocholate	[141]
Sodium taurocholate	[141]
Sodium taurodeoxycholate	[31, 32, 34, 51, 135, 136, 137]
Sodium tauroglycocholate	[147]
Butanol	[32, 135, 136]
Butyric acid	[31, 136]
PEG 660	[122]
Dioctyl sodium sulfosuccinate	[136]
Charge modifiers	
Stearylamine	[23]
Dicetyl phosphate	[23]
Dispersing agents	
Polyvinyl alcohol	[148]
Shell forming material	
Curdlan	[122]

Modified after ref. 2.

METHODS OF SLN PREPARATION

High shear homogenization

Since the fifties, high pressure homogenization technique has been used for the production of emulsions for parenteral nutrition on a commercial scale in the pharmaceutical sector, but it has emerged as the most reliable and powerful technique for preparation of solid lipid nanoparticles since the beginning of the nineties. Muller *et al.* (5) reported the influence of various process parameters, *viz.*, surfactant concentration, storage time, and crystallinity of the lipid matrix on degradation rate and crystallinity of nanoparticles after production of SLN formulations.

Two general approaches, hot homogenization and cold homogenization, can be used for the production of SLN by the high pressure homogenization technique. In both approaches, the primary step involves incorporation of the drug into bulk lipid by dissolving or dispersing the drug in lipid melt maintained at a temperature above the lipid melting point.

Schematic procedure for SLN production by hot and cold homogenization techniques is shown in Fig. 2.

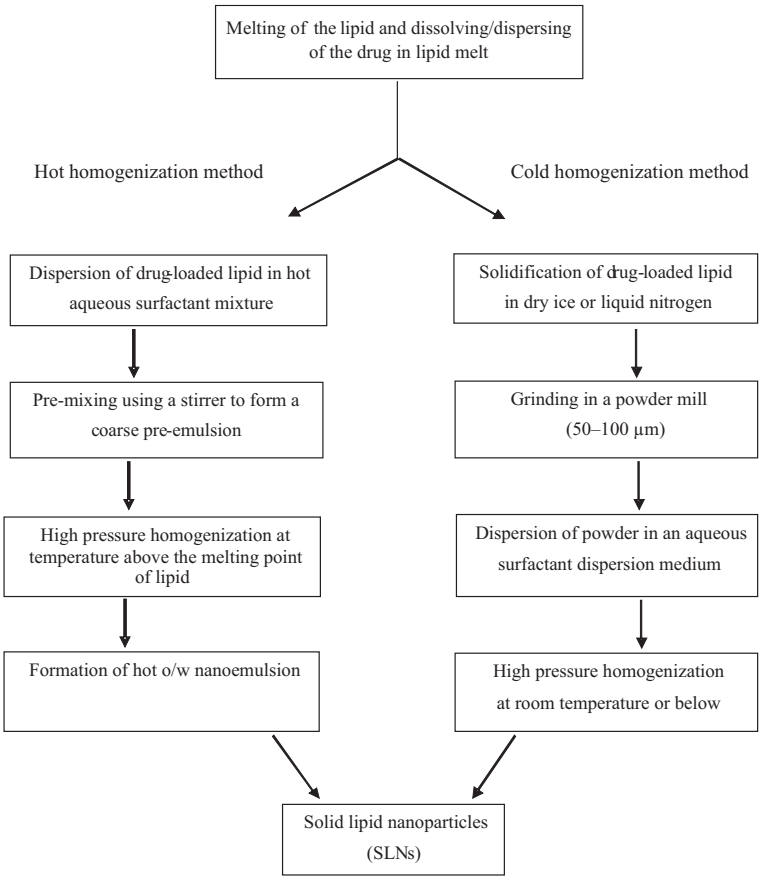

Fig. 2. Scheme of SLN production by hot and cold homogenization techniques.

Hot homogenization. – Hot homogenization is usually carried out at temperatures above the melting point of the lipid and can be thus regarded as homogenization of an emulsion. Drug loaded lipid melt and the preheated aqueous surfactant phase (the same temperature) are dispersed using a high shear stirring device such as silverson homogenizer to obtain a coarse o/w pre-emulsion. High pressure homogenization of pre-emulsion is carried out at temperatures above the melting point of the lipid. Pre-emulsion quality affects the quality of the final formulation to a great extent and is desirable for obtaining droplets in the size range of a few micrometers. Smaller particle size is usually obtained with higher processing temperature due to lowered viscosity of the lipid phase (19), although this might accelerate the drug and carrier degradation. Here, high pressure homogenization increases the sample temperature, approximately by 10 °C at 5×10^7 Pa. In most cases, 3–5 homogenization cycles at 5×10^7 to 5×10^9 Pa pressure are sufficient to have better products in hands (20). Increasing the homogenization pressure or number of homogenization cycles often leads to an increase in particle size. This hap-

pens because of the coalescence of particles as a result of their high kinetic energy (21). Hot homogenization primarily gives a nanoemulsion due to the presence of molten lipid and solid particles are expected to be produced by cooling of the obtained sample to room temperature or even below. Because of the nanometric particle size and the presence of emulsifiers or surfactants, lipid crystallization may be retarded and the sample may remain as a supercooled melt for several months (22).

There should be a difference between the lipid melting point and homogenization temperature big enough to prevent melting of the lipid in homogenizer. Here it is a point to be concentrated that the high pressure homogenization process itself increases the temperature of the product, approximately by 10 °C at 5×10^7 Pa per homogenization cycle. Hot homogenization technique can be also applied for temperature sensitive drugs because exposure to increased temperature is relatively short. In case of highly temperature-sensitive drugs, the cold homogenization technique may be preferred. The same technique can be used while formulating hydrophilic drugs because they would have a partition between the melted lipid and aqueous phase during the homogenization process (4). Venkateswarlu *et al.* (23) developed SLN delivery systems of clozapine by the hot homogenization technique followed by ultrasonication using a variety of lipids along with the charge modifier stearylamine and studied the effect of the charge modifier, various triglycerides, pH of the dialysis membrane and sterilization on the particle size, zeta potential, entrapment efficiency and drug release kinetics of clozapine from the solid lipid nanoparticle formulation.

Cold homogenization. – The prime objective of the cold homogenization technique development was to rectify the problems associated with the hot homogenization technique like temperature-mediated accelerated degradation of drug payload, partitioning and hence drug loss into aqueous phase during homogenization, uncertain polymorphic transitions of the lipids due to complexity of the nanoemulsion crystallization step leading to several modifications and/or supercooled melts (3).

The primary step is similar to the hot homogenization technique and it involves solubilization or dispersion of the drug into the lipid melt. The drug-loaded lipid melt is solidified rapidly by means of liquid nitrogen or dry ice to ensure homogeneous drug distribution into the lipid matrix. The drug containing solid lipid is then pulverized by ball or mortar milling to yield lipid microparticles of the typical size range of 50–100 microns. Low temperature enhances the lipid fragility, resulting in further particle communition. These solid lipid microparticles are then dispersed in cold surfactant solution yielding a pre-suspension, which is then homogenized at or below room temperature with appropriate temperature control and regulation, keeping in mind the usual rise in temperature during high pressure processing. At this stage, cavitational forces are playing their role and are strong enough to break lipid microparticles directly into the solid lipid nanoparticles (20). As cold homogenization is carried out with solid lipid and is thus regarded as »high pressure milling of a suspension«. This process of cold homogenization minimizes thermal exposure of the product but does not prevent it completely because of the melting of the lipid-drug mixture in the primary step. In general, compared to the hot homogenization technique, larger particle sizes and broader size distributions are observed with the cold homogenization technique. Cold homogenization is also preferred over hot homogenization, in fact it avoids or minimizes the melting of the lipid and thereby minimizes the loss of hydrophilic drugs to the aqueous phase. Further,

to minimize the loss of hydrophilic drugs to aqueous phase of SLN dispersion, it is advisable to replace water by liquids with low solubility for such drugs, like oils or PEG 6000. Production of SLN in oils or PEG 6000 is advantageous for oral drug delivery because this dispersion can be directly filled into soft gelatin capsules (4).

High shear homogenization and ultrasound

High shear homogenization and ultrasound are dispersing techniques that were initially used for the preparation of solid lipid nanodispersions. Both of these methods are easy to handle and hence widely used. However, the presence of microparticles and metal contaminants has a major influence on the nanodispersion quality and has to be compromised if ultrasound is used (1). Venkateswarlu (24) prepared lovastatin SLN using hot homogenization followed by ultrasonication.

Ultrasonication (high speed homogenization)

Vyas and Khar reviewed (25) the preparation of vesicular lipid particles (liposomes) of the nanometric size range using the ultrasonication technique. Two methods of sonication are commonly used based on the use of either a bath or probe tip ultrasonic disintegrator. Bath sonicator is employed for large volumes of diluted lipid dispersions whereas the probe tip sonicator is more suitable for dispersions, which require high energy in a small volume (*e.g.*, high concentration of lipids, or a viscous aqueous phase). This technique is most widely used since the equipments required are common in every lab.

Probe tip sonicators supply a high energy input to lipid dispersions but sometimes cause lipid degradation due to overheating of the lipid dispersion. Sonication tips also tend to release metal particles into the dispersion, which must be removed by centrifugation prior to use. For these reasons, bath sonicators are preferred over probe tip sonicators. The most formidable problem associated with this technique is broader particle size distribution of several micrometers. This, upon storage, may give signs of physical instability, like particle growth and particle aggregation. Efforts have been made by various research groups to prepare a stable formulation using combined high speed stirring and ultrasonication technique conducted at high temperature (3). Size and size distribution of the lipid dispersion are influenced by the composition and concentration of lipids, sonication time and power, and temperature (26).

Microemulsion based technique

Microemulsion has to be produced at a temperature above the melting point of the lipid so as to form a microemulsion with the lipid solid at room temperature (27). These microemulsions are clear or slightly bluish solutions composed of a lipophilic phase, surfactant, in most cases a co-surfactant, and water. Lipid (fatty acid and/or triglyceride) is melted first; a mixture of surfactant, co-surfactant and water is heated to the same temperature as the lipid phase and added to the lipid melt under mild stirring. When compounds are mixed in a correct ratio, a transparent and thermodynamically stable microemulsion system is formed. This microemulsion is then dispersed in a cold aque-

ous medium (2–3 °C) under mild mechanical mixing to precipitate the lipid phase forming fine particles (28, 29). Addition of a microemulsion to water leads to precipitation of the lipid phase forming fine particles. This effect is exploited in the SLN preparation method developed by Gasco (30).

Large-scale production of SLN by the microemulsion technique is also feasible and major process parameters that need to be considered during scaling-up include microemulsion and water temperature, temperature flows in water medium and thermodynamics of mixing, which should not change, or only a little, during scaling-up to maintain the same product characteristics. The first attempt was made by Morel *et al.* (31, 32), to encapsulate peptide drugs into SLN using a warm w/o/w microemulsion technique and incorporated [D-Trp-6] LHRH and thymopentin.

Double emulsion based technique

Hammady *et al.* (33) exploited biphasic polymeric nanospheres to co-encapsulate both hydrophilic and lipophilic drug molecules using the double emulsion technique, which is a two-step emulsification procedure. In the first step, a primary w/o emulsion is obtained by dispersing the aqueous solution of the hydrophilic drug molecule into organic phase (previously prepared by dissolving the polymer, lipophilic drug molecule and surfactant, with or without co-surfactant, into an organic solvent), and vortexing and simultaneous homogenization with a high speed stirrer. This primary w/o emulsion is then, in the second step, syringed into an aqueous solution to obtain w/o/w multiple emulsions by high pressure homogenization. Further stirring under reduced pressure results in extraction and evaporation of organic solvent with subsequent hardening of the nanospheres.

Solvent emulsification-diffusion technique

Trotta *et al.* (34) described a preparation method for nanoparticles based on emulsification of an organic solution of a solid lipid in an aqueous emulsifier solution, followed by dilution of the emulsion with water. The method is called »solvent emulsification-diffusion method«. In this proposed approach, the lipid was dissolved in a water-saturated organic solvent and this organic solution was first emulsified with a solvent-saturated aqueous solution containing emulsifier to form a pre-emulsion. Lipid nanoparticles were precipitated by quickly adding water into the initial pre-emulsion to extract the solvent into continuous phase and to give an SLN nanodispersion. This nanodispersion was then washed by ultrafiltration to remove residual solvent and lyophilized.

Battaglia *et al.* (35) reported the preparation of insulin-loaded SLN by the solvent-in-water emulsification-solvent diffusion technique using isovaleric acid (IVA) as organic phase, glyceryl monostearate (GMS) as lipid, soy lecithin and sodium taurodeoxycholate as emulsifiers. Their investigations demonstrated that SLN seem to have interesting possibilities as a delivery system for oral administration of insulin.

Yuan *et al.* (36) developed solid lipid nanoparticles in a nanoreactor system using the solvent diffusion method to improve the drug loading capacity and entrapment efficiency of SLN. Nanoemulsion is one of the nanoreactors generally referred to as miniemulsion or microemulsion (10–100 nm). The investigation demonstrated that the solvent

diffusion method with w/o miniemulsion as nanoreactor system favors the formation of SLN with higher drug loading capacity and smaller particle size, thereby enhancing the targeting potential and oral bioavailability of SLNs.

Emusification-solvent evaporation technique

This technique involves three steps of preparation:

(i) Preparation of organic phase: lipophilic material is first dissolved in an appropriate volume of organic solvent by magnetic stirring.

(ii) Pre-emulsification step: lipid containing organic phase is dispersed in an appropriate volume of aqueous solution using a high speed homogenizer in order to form a coarse pre-emulsion.

(iii) Nanoemulsification step: the resulting coarse pre-emulsion is immediately passed through a high-pressure homogenizer at an operating pressure to obtain nanodispersion. Obtained nanodispersion is then kept on the magnetic stirrer overnight, sometimes in a fume hood to drive off the organic solvent. Upon solvent evaporation, nanodispersion is formed by precipitation of lipid material in aqueous medium. Solidified nanodispersion is then filtered through a sintered glass filter to remove lipid and drug agglomerates.

Nanoparticles obtained by this method are small, monodisperse with high encapsulation efficiency. The process can be automated and scaled-up for the production of a large amount of nanoparticles (37, 38).

Spray-drying based technique

When particle dimensions are reduced to the nanometric size range, their physical and chemical properties deviate from bulk properties and such particles suffer from many problems related to their surface and thermal stability, shape preservation, handling and assembly into a suitable device (39). Prolonged long-term stability, especially for *i.v.* administered systems, can be achieved when stored as dry products. Thus a spray-drying technique was investigated for SLN as an alternative approach to lyophilization to convert liquid dispersions into a dry system. This less cost-intensive technique could produce fine, dust-free powder particles with a spherical shape, which are non-agglomerated and nearly monodisperse, with a controlled particle size (40). Spherical particles are of practical importance since, in general, they have better rheological properties than irregular particles (41). Freitas and Muller (42) recommend the use of lipids with the melting point > 70 °C for spray-drying. Fessi (2006) (43) reported the use of the spray--drying technique for conversion of the nanocapsule suspension into redispersible dried solid particles, as an alternative to the lyophilization technique.

Supercritical fluid based technique

This is a relatively novel technique for SLN production and has a major advantage of processing without organic solvent. SLN can be prepared by rapid expansion of supercritical carbon dioxide solutions (RESS method) (44). Carbon dioxide (99.99 %) was good choice of solvent for this method (3). Thote and Gupta (45) produced nanoparticles

of the hydrophilic drug, dexamethasone phosphate using supercritical carbon dioxide (CO_2), the technique being known as supercritical antisolvent technique with enhanced mass transfer (45).

Desolvation technique

Vandervoort and Ludwig (46) produced drug-loaded gelatin nanoparticles by using the desolvation technique. Here, the drug is dispersed into an aqueous gelatin solution by continuous stirring. Then, to induce desolvation, desolvating agent is added to the above solution until permanent faint turbidity is obtained. Finally, an aqueous solution of the cross-linking agent is added, with continuous stirring, to harden the nanoparticles. A desolvating agent (*e.g.*, alcohol, acetone or salt solution) is added to the aqueous gelatin solution to dehydrate the gelatin molecules, indicated by a rise in solution turbidity due to the change in conformation of gelatin molecules from stretched to coiled. In that study, the preparation and properties of gelatin nanoparticles loaded with both hydrophilic pilocarpine hydrochloride and lipophilic hydrocortisone were reported. If one tries to use a lipid matrix, instead of or along with the polymer, it would be possible to increase the loading and entrapment of lipophilic drugs.

EFFECT OF FORMULATION INGREDIENTS ON SLN QUALITY

Major formulation ingredients affecting SLN quality include lipid and emulsifier or surfactant and co-surfactant.

Influence of the lipid

Critical parameters for nanoparticle formation with different lipids include

(i) rate of lipid crystallization

(ii) lipid hydrophilicity (influence on self-emulsifying properties)

(iii) shape of the lipid crystal (and therefore the surface area).

It was also a common finding that most of the lipids used were composed of a mixture of several chemical compounds. A small difference in lipid composition (*e.g.*, presence of impurities) might have a great impact on the quality of SLN dispersions (by changing the zeta potential, retarding the crystallization process) (2).

Paliwal *et al.* (47) reported the effect of lipid core material on the particle size of methotrexate-loaded SLN for enhancing the oral bioavailability of methotrexate *via* lymphatic delivery, produced by the solvent-diffusion method in an aqueous system and stabilized by 1 % (*m*/*m*) soya lecithin. Particle size of Compritol® 888 ATO (glyceryl behenate) SLNs was found to be smaller (120.1 ± 0.8 nm) than the size of stearic acid (130.0 ± 3.2 nm), tristearin (140.4 ± 6.4 nm) and glycerol monosterate (166.2 ± 8.1 nm) SLNs. It was concluded that SLNs produced by a highly lipophilic lipid (Compritol® 888 ATO) showed more promising results, both *in vitro* and *in vivo*, as compared to other lipid core materials.

Influence of the emulsifier

Choice of emulsifier and its concentration is of great importance in determining the quality of SLN dispersion (2). Investigating the influence of four different emulsifiers (*i.e.*, cholic acid sodium salt, Lipoid E80, Poloxamer 407 and Tween 80) of the same concentration (0.5 %) on the degradation rate of SLN prepared by the hot homogenization technique using three different lipids (*i.e.*, cetylpalmitate, Dynasan 116 and Dynasan 118). Olbrich and Muller (48) concluded that the longer the ethyleneoxide chains in emulsifier molecule, the more hindered is the anchoring of the lipase/colipase complex and consequently the SLN degradation. Also, the longer are the fatty acid chains in glycerides, the slower the degradation rate of SLN. These results could be used to adjust SLN degradation and consequently drug release in a controlled way.

Chen *et al.* (49) studied the influence of lipophilic emulsifiers on oral absorption of lovastatin from NLC formulations and found NLCs with Myverol to be more stable in the gastric environment compared to those with soybean phosphatidylcholin (SPC). Xie *et al.* (50) worked out the effect of the polymeric emulsifier poly(lactic-co-glycolic acid) (PLGA) at concentration levels of 4 and 16 % on the entrapment efficiency and loading capacity of hydrophilic protein-loaded SLN prepared by the w/o/w double emulsion--solvent evaporation technique and both were found to be enhanced significantly as PLGA concentration was increased from 4 to 16 %, in all formulations.

STERILIZATION AND LYOPHILIZATION OF SLNs

Sterilization

SLN formulations intended for parenteral and ocular administration must be sterile. Sterilization by autoclaving at 121 °C cannot be possible for lipid-based formulations prepared using poloxamers, sterically stabilized polymers, since this temperature seems to be too close to the critical flocculation temperature (CFT) of polymers. This is known to collapse partially the polymer adsorption layer and due to insufficient stabilization of the system, particle aggregation occurs. This problem could be avoided by reducing the autoclaving temperature from 121 to 110 °C and prolonging the autoclaving time (51).

However, autoclaving is possible in lecithin-stabilized SLN formulations. Formulation melts at autoclaving temperature lead to the formation of a hot o/w microemulsion in the autoclave and recrystallize during the cooling cycle producing SLN particles larger than the initial ones due to coalescence of some nanodroplets during recrystallization. But, these nanodroplets may not be sufficiently stabilized because SLNs are washed off before and after sterilization and amounts of surfactant and co-surfactant are smaller in a hot emulsion system (52).

Lyophilization

Lyophilization or freeze drying has been considered an excellent technique of drying and improving the long-term stability of various pharmaceutical products, including

vaccines, viruses, proteins, peptides and colloidal carriers like nanoparticles, nanoemulsions, and liposomes.

Stresses that destabilize the colloidal nanoparticulate suspension may be generated during freeze drying, particularly stresses of freezing and dehydration. Aggregation and sometimes irreversible fusion of nanoparticles may destabilize the colloidal nanoparticulate system. To get rid of this problem, special excipients must be added before freezing to protect the nanoparticulate suspension. The added excipients that protect the system from freezing stress are called cryoprotectants and those protecting from the drying stress are called lyoprotectants.

Among the long list of cryoprotectants cited in literature, the most frequently used cryoprotectants include sugars like trehalose, glucose, sucrose, and mannitol. These sugars get vitrified at a specific temperature, denoted as *T*g (glass transition temperature). These cryoprotectants immobilize the nanoparticles within their glassy matrix and protect them against destabilization by aggregation. In general, freezing must be carried out below *T*g of an amorphous sample or below *T*eu (eutectic crystallization temperature) of crystalline samples of cryoprotectants (53).

Particle isolation hypothesis is one of the mechanisms for nanoparticle stabilization by cryoprotectants during the freezing step. Sugars isolate individual particles in unfrozen fraction and thereby prevent particle aggregation during freezing above *T*g. Sugar vitrification is not required for this effect (54).

Water replacement hypothesis is a major mechanism for nanoparticle stabilization by lyoprotectants during the drying step. These lyoprotectants are known to form hydrogen bonds with the surface polar groups of nanoparticles and serve as water substitutes to preserve the native structure of nanoparticles, thereby stabilizing the system (55).

Freeze dried nanoparticles should have the following desirable characteristics:

(i) preservation of primary physical and chemical characteristics of the product

(ii) long-term stability

(iii) acceptable relative humidity.

DRUG INCORPORATION INTO SLNs

A vast number of drugs with a great variety of lipophilicity and structure have been studied to investigating their incorporation into SLNs. Drug incorporation implies localization of the drug in the solid lipid matrix (2). Drug loading capacity is one of important parameters to judge the suitability of a drug carrier system. Loading capacity is expressed in percent related to the lipid phase (matrix lipid + drug) (4).

Factors determining drug loading capacity in lipid include:

(i) drug solubility in melted lipid

(ii) miscibility of drug melt and lipid melt

(iii) chemical and physical structure of solid lipid matrix

(iv) polymorphic state of lipid material.

Sufficiently high solubility of the drug in the lipid melt is a prime prerequisite to obtain sufficient loading capacity. Typically, solubility should be higher than required because it decreases while the lipid melt is being cooled down and might even be lower in solid lipid. Addition of solubilizers and use of mono- and diglycerides in the lipid, used as matrix material, serves to promote drug solubilization in the lipid melt.

Chemical nature of the lipid also needs to be considered because lipids which form highly crystalline particles with a perfect crystal lattice lead to drug expulsion. More complex lipids being mixtures of mono-, di- and triglycerides along with fatty acids of different chain length, form less perfect crystals with many imperfections offering more space to accommodate drugs. Chemically polydisperse lipids, which are more likely to be used in cosmetics and dermal preparations, showed very efficient drug incorporation capacities.

Polymorphic state of the lipid is also a parameter of great importance in determining drug incorporation. Crystallization of the lipid in nanoparticles is different compared to that in bulk material; lipid nanoparticles recrystallize at least partially in α-form, whereas bulk lipids tend to recrystallize preferentially in β′-modification transforming rapidly into the β-form (56). Increasing formation of more stable modifications leads to a more perfect lattice and the number of imperfections decreases, which means formation of β′/β-modifications promoting drug expulsion. In general, this transformation is slower for long-chain than for short-chain triglycerides. An optimal SLN carrier can be produced in a controlled way when a certain fraction of α-form can be created and preserved during storage time. By doing this, a normal SLN carrier transforms to an intelligent drug delivery system by having a built-in trigger mechanism to initiate transformation from α- to β-forms (57). Triggering factors for the transformations are, *e.g.*, temperature and water loss of SLN dispersion, *e.g.*, after topical application. Thus, it was concluded that drug incorporation decreases in the following order:

$$\text{supercooled melt} < \alpha\text{-form} < \beta'\text{-form} < \beta\text{-form}.$$

Muller (4) reported three drug incorporation models (as shown in Fig. 3): solid solution model, core-shell model (drug-enriched shell) and core-shell model (drug-enriched core).

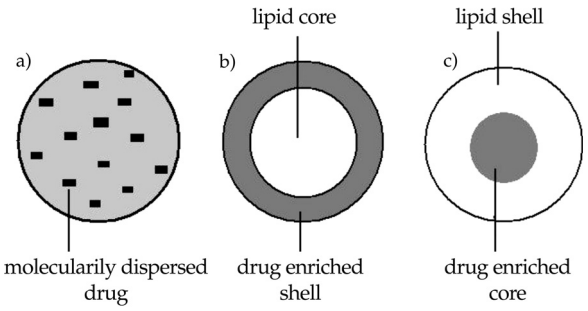

Fig. 3. Drug incorporation models: a) solid solution model, b) core--shell model with drug-enriched shell, c) core-shell model with drug-enriched core (4).

SLN matrix is a solid solution (*i.e.*, drug molecularly dispersed in lipid matrix) when particles are produced by the cold homogenization technique and using no surfactant or no drug-solubilizing surfactant.

The core-shell model with a drug-enriched shell could be obtained when there is re-partitioning of the drug to the lipid phase during cooling of the obtained nanoemulsion, in the hot homogenization technique.

The core-shell model with the drug-enriched core could be obtained when the drug precipitates first, before recrystallization of the lipid. It should be obtained when dissolving a drug in the lipid melt at or close to its saturation solubility. The proposed mechanism behind this fact is that nanoemulsion cooling will lead to supersaturation of the drug in the melted lipid and ultimately to drug crystallization prior to lipid crystallization. Further cooling will finally lead to recrystallization of the lipid surrounding the drug core as a membrane. This lipid membrane will only contain the drug in a concentration corresponding to saturation solubility of the drug at recrystallization temperature of the lipid. Finally, it will result in a drug-enriched core surrounded by a lipid shell.

DRUG RELEASE FROM SLNs

Investigations have shown that several studies concerning optimization of production parameters, long-term stability, recrystallization behavior, morphological characterization and *in vivo* toxicity have been undertaken to date. Additionally, investigations of drug incorporation and release served as an important tool in the design, development, and evaluation of potential drug carrier systems. However, there are many studies concerning drug incorporation into SLN but data on release mechanisms are still scarce. A major problem in early work with lipid nanoparticles was the generally observed burst release of drugs (58). The amount of drug in the outer shell and on particle surface is generally released in the form of a burst, while the drug incorporated in particle core releases in a prolonged manner. Therefore, the extent of burst release can be controlled by controlling drug solubility in the aqueous phase during production, which, in turn, can be controlled *via* the temperature employed and the surfactant concentration used. Higher temperature and higher surfactant concentration increase the burst whereas production at room temperature avoids partitioning of the drug into water phase and subsequent re-partitioning into lipid phase, thereby showing no burst release at all; the partitioning behavior is depicted in Fig. 4. To avoid or minimize the burst release, SLN can be produced surfactant-free or with surfactants unable to solubilize the drug (4). Olbrich and Muller (48) showed that the lipid and the emulsifier are responsible for enzymatic degradation of the lipid matrix by lipase. Since lipases require a lipid interface for enzyme activation, appropriate balance between steric stabilizers and other surfactants should be optimized to modify drug release and particle degradation. Therefore, hydrophilic coating over lipid nanoparticles is not easily recognized by these enzymes (3).

Venkateswarlu and Manjunath (23) observed an inverse relationship between the percent drug release and the partition coefficient of clozapine from clozapine-loaded solid lipid nanoparticles prepared by the cold homogenization technique.

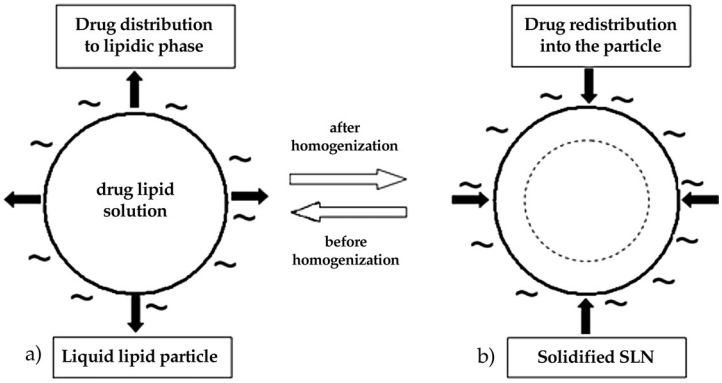

Fig. 4. Partitioning behavior of the drug during SLN production by hot homogenization technique: a) drug partitioning from the lipid phase to aqueous phase at increased temperature, b) re--partitioning of the drug to the lipid phase during cooling of the produced o/w nanoemulsion.

Factors that contribute to rapid release of the drug include large surface area, high diffusion coefficient due to small molecular size, low viscosity in the matrix and short diffusion distance for the drug, *i.e.*, release from the outer nanoparticulate surface.

STABILITY CONSIDERATIONS IN SLNs

I. Physicochemical mechanisms of destabilization of SLNs

Various physicochemical mechanisms implied in the destabilization process of solid lipid nanoparticles are highlighted below.

Chemical stability (stability of lipid excipients)

Lipids provide endless opportunities, as drug carriers, in the area of drug delivery due to their ability to enhance bioavailability and thereby the therapeutic efficacy of lipophilic drugs with poor aqueous solubility (59).

Results have been published on the chemical stability of pharmaceutical drugs and cosmetic actives loaded into SLN formulations, but nobody considered at the stability of lipid excipients used therein. The first report regarding chemical stability of lipids in SLN was given by Radomska-Soukharev (60). SLN formulations made from triglycerides (Dynasan 112 to 118) were compared with those made from lipids with different ratios of mono-, di- and triglycerides (Softisan and Imwitor) and lipids having different hydroxyl number (Witepsol S51 and S55). Chemical stability was revealed by the gas chromatographic method. Obtained results proved that the SLN formulations obtained with triglycerides were more stable than those with mono- and diglycerides. The observed excipient stability is a prerequisite for introducing SLN as a formulation into the pharmaceutical market.

Triglycerides undergo a hydrolytic reaction, leading to the formation of mono- and di-glycerides with free fatty acids, but due to their internal location in SLNs, they are less susceptible to hydrolysis than phospholipids located externally in liposomes (61).

Physical stability

Modifications in the used lipids and colloidal lipid dispersions mostly affect the physical stability of SLNs.

Lipid modifications

Crystallization-polymorphic transitions. – Sustained drug release of lipoidal nanoparticulate systems essentially relies on the solid state of the lipid matrix. Solidification of particles after the production process must, therefore, be ensured. However some lipid materials may not crystallize in the colloidally dispersed state and dispersions should be regarded as emulsions of supercooled melts rather than dispersions of solid particles. Crystallization of bulk triglycerides from the melt usually occurs in the metastable α-form after rapid cooling (57).

In case of supercooled melts, the lipid crystallization may not occur although the dispersion is stored at a temperature below the melting point of the lipid (2).

Transformation of lipid particles, after crystallization, from less stable to more stable is referred to as polymorphic transition. Crystallization of bulk triglycerides from the melt after rapid cooling usually occurs in the metastable α-form, which transforms *via* β′-form into the stable β-form upon heating or storage. The kinetics of these polymorphic transitions are faster in colloidally dispersed mixed triglycerides than in bulk and also in shorter-chain triglycerides than in those with longer chains (57).

Thermoanalysis and X-ray diffraction pattern of SLN dispersions helps reveal the crystallization behavior, time course of polymorphic transitions, enthalpy, fusion temperature and the degree of crystallinity of melt-homogenized glyceride nanoparticle dispersions (62).

Gelling phenomena. – Very rapid and unpredictable process of transformation of a low-viscosity SLN dispersion into a viscous gel is referred to as a gelling phenomenon. In most cases, the process of gelation is irreversible (2). High temperature, light, and shear stress are some of the promoters of gelation (63). Gel formation may also occur due to high lipid concentration, high ionic strength (64), rapid crystallization of the lipid (57) and intense contact of the SLN dispersion with other surfaces, like the surface of the packing material (65). All gelation promoters increase the kinetic energy of the particles and favor their collision.

Addition of co-emulsifying surfactants with high mobility (*e.g.*, glycocholate) was found to retard or prevent gel formation (66). Storage in dark at 8 °C prevented particle growth. Also, fat samples stored under a nitrogen atmosphere were more stable than the samples filled under regular air (63). Zeta potential is a good predictor of gelation phenomena. An increased or decreased zeta potential indicates the beginning of gelation (67).

Dispersion modification

Ostwald ripening. – Ostwald ripening in SLN dispersions corresponds to an increase in particle size due to the dissolution of smaller crystals and deposition of dissolved material on larger surfaces, leading to the growth of large particles at the expense of smaller ones (61).

Coalescence. – Rigid solid particles are expected to be stable against coalescence but SLN dispersions tend to form a cream or gel after particle aggregation.

II. Storage stability of SLNs

Solid lipid nanoparticles based on solid lipids and stabilized by surfactant, even in aqueous dispersions, possess long-term physical stability of at least two years. However, depending on the composition and storage conditions, lipoidal systems are critical in terms of their stability (68). Gelation phenomena, increase in particle size, particle aggregation and drug expulsion from the lipid carrier systems are the most formidable problems of storage stability of SLN (2). Storage conditions like exposure to light, temperature and packing material are also some major stability determining factors.

Freitas and Muller (63) studied the effect of light, temperature and packing material on zeta potential, particle size and gelation of SLN using an SLN dispersion consisting of Compritol 888 ATO stabilized with Poloxamer 188.

Influence of light

SLN dispersions were exposed to various light conditions such as dark, daylight and artificial light during storage in white and brown glass vials. Storage in white glass vials under artificial light induced rapid gelation with immediate particle growth, while the gelation process was slower under daylight. Dark exposure results in slow particle growth and gelation after one month of storage. Under all conditions, zeta potential dropped compared to that immediately after production, with slight variations under different conditions. The SLN dispersion system changed after exposure to different light conditions, leading to reduced zeta potential due to modification of the crystallization form of lipids. Results proved that light radiation had a destabilizing effect and further increase in the intensity of light radiation accelerated particle growth and gelling process significantly. In general, introduction of energy to the system leads to particle growth and subsequent gelation and high energetic radiations like UV lead to increased destabilization of a SLN system (63).

Physical stability for more than 3 years of lipid suspensions stored at room temperature in white glass bottles under daylight has been reported (4).

Influence of temperature

Temperature, unlike light exposure, corresponds to energy input to the system and can lead to changes in the crystalline structure of the lipids and high temperatures generally cause destabilization of the SLN system due to reduction in zeta potential (61). Freitas and co-workers (63) studied the effect of different temperature conditions such as

8, 20 and 50 °C on the particle size and zeta potential of Compritol SLN and observed that storage at higher temperatures led to rapid particle growth with reduction of zeta potential of the SLN system fastest at 50 °C.

Influence of packing material

The influence of packing material on SLN physical stability was less pronounced than temperature. But aggregation and gelling can be promoted by the inner surface. Stability improvement has been observed with some SLN systems with a gelling tendency when they were packed in plastic containers instead of glass vials (62).

Freitas and Muller (63) packed Compritol SLN in siliconized and in untreated glass vials and stored under optimum conditions (at 8 °C, in dark) to assess whether siliconization of the glass surface had any influence on the stability. They observed that the particle diameters of SLN systems in untreated glass vials were slightly larger compared to those in siliconized ones while the zeta potential of SLN systems in siliconized vials was slightly higher than those in untreated vials. It was found that minimization of SLN adherence to the surface of the vial by siliconization minimizes aggregation and consequently improves physical stability. Hence, it was concluded that siliconization of the glass surface has a stabilizing effect.

ROUTES OF ADMINISTRATION AND BIODISTRIBUTION OF SLNs

The *in vivo* biodistribution of solid lipid nanoparticles will mainly depend on the route of administration and interactions of SLN with biological surroundings which, in general, include two types of processes: distribution processes (adsorption of biological materials on the particle surface and desorption of SLN components into the biological surrounding) and enzymatic processes (lipid degradation by lipases and esterases).

Physiological or physiologically related lipids or waxes generally constitute the SLNs. Therefore, the *in vivo* fate of the carrier, to a large extent, occurs through the pathways of transportation and metabolism present in the body. Lipases, the enzymes present in various organs and tissues of the body, are most responsible for SLN degradation. Lipases split the ester linkage and form partial glycerides or glycerol and free fatty acids. Activation by an oil/water interface, which opens the catalytic centre, is a prerequisite for lipases to act (69, 70). Solid lipid nanoparticles show different degradation rates, *in vitro*, by the lipolytic enzyme pancreatin lipase as a function of their composition (lipid matrix, stabilizing surfactant (48).

Peroral administration

Aqueous dispersions or SLN-loaded traditional dosage forms (tablets, capsules, pellets or powders in sachets) may serve as peroral administration forms of SLN. Particle aggregation of SLN dispersion occurs in the microclimate of the stomach due to acidity and high ionic strength. It can be expected that food will have a great impact on SLN performance; however, to our knowledge no experimental data have been published on this issue till date. The question concerning the effect of the stomach and pancreatic li-

pases on SLN degradation *in vivo* still remains open. Unfortunately, only a few *in vivo* studies have been performed so far.

Pandey *et al.* (71) formulated and evaluated the chemotherapeutic potential of solid lipid nanoparticles incorporating antitubercular drugs following oral administration to mice and suggested that oral SLN based antitubercular drug therapy forms a sound basis for reducing dosing frequency and improving patient compliance for better management of tuberculosis. Zhang *et al.* (72) administered orally insulin-loaded SLN and WGA-modified SLN to rats and demonstrated that both of these formulations promoted the intestinal absorption of insulin after oral administration.

Parenteral administration

Parenteral drug delivery took a major leap after successful development of the submicronic parenteral fat emulsion in the 1960s. Quick commercialization of submicron emulsion based products, such as Diazemuls (diazepam) and Diprivan (propofol), indicated the interest of pharmaceutical industries in colloidal carriers. Since then, there have been continuous efforts to develop novel colloidal nanocarriers for improved parenteral delivery (73). Wissing *et al.* (74) reviewed, in detail, the bioactivity of SLN after parenteral administration, *i.e.*, tolerability, toxicology, cellular uptake, albumin adsorption, pharmacokinetics, tissue distribution and drug targeting.

Gasco and coworkers (75) studied the pharmacokinetics and tissue distribution of stealth and non stealth SLN loaded with doxorubicin after *i.v.* administration to rats and found prolonged circulation time of SLN compared to the commercial doxorubicin solution.

Yang *et al.* (76) studied the pharmacokinetics, body distribution and specific drug targeting of camptothecin after *i.v.* injection in mice. In comparison with commercial camptothecin solution, SLN was found to give much higher *AUC*/dose and mean residence times (*MRT*), especially in brain, heart and reticuloendothelial cells containing organs. The highest *AUC* ratio of SLN to drug solution among the tested organs was found in the brain.

Reddy *et al.* (77) studied the influence of the route of administration on tumor uptake and biodistribution of etoposide loaded solid lipid nanoparticles in mice bearing Dalton's lymphoma after subcutaneous, intravenous and intraperitoneal injections. It was observed that subcutaneous injection reduced the biodistribution of SLN to all the tissues studied, whereas intravenous injection resulted in lower levels of etoposide-loaded SLN in RES rich organs compared to free etoposide. SLN experienced significantly higher brain distribution after intraperitoneal injection, indicating its potential application in targeting etoposide to brain tumors.

Transdermal administration

Since the epidermal lipids are found in high amounts in the penetration barrier, lipid carriers (liposomes, SLN, NLC, *etc.*) attaching themselves to the skin surface and allowing lipid exchange between the outermost layers of the stratum corneum and the carrier appear promising (78).

Incorporation of SLN dispersion in an ointment or gel, by reduction of the lipid content of the SLN dispersion, is necessary to achieve a formulation that can be easily administered to the skin (3). Cosmetic field offers interesting applications of SLN due to their UV reflecting properties. UV reflectance is related to the solid state of lipids and was not evident in nanoemulsions of comparable composition. These observations open a new application area for the development of SLN-based UV protective systems (2).

The short time-to-market and big potential area of SLNs are topical products based on SLN technology, which means pharmaceutical and cosmetic formulations. A major step forward was the development of »intelligent SLNs« (ISLN), which release incorporated drug in a controlled fashion after it receives a triggering impulse. Such triggering impulses are an increase in temperature or loss of water from the SLN dispersion or SLN-containing cream (2). This effect was exploited for controlled release of vitamin A loaded in a glyceryl behenate SLN dispersion (79, 80) and flurbiprofen-loaded SLN-gel formulation (81).

Pulmonary administration

Growing attention has been given to the potential of pulmonary route as an alternative to the non-invasive local and systemic delivery of therapeutic agents using lipid particles, since it provides a large absorptive mucosal area. The lung offers a large surface area for drug absorption and the alveolar epithelium allows rapid drug absorption. What makes pulmonary administration of many drugs very promising, particularly for proteins and peptides, is the presence of various metabolic activities and pathways, which may be different from those present in the GI tract. The superior physicochemical characteristics of SLNs make them more suitable as an appropriate delivery system due to correlation between the diameter within the nanometric range, biocompatible composition and deep-lung deposition ability. Prolonged drug serum concentration and lung retention are both achievable by means of the particulate colloidal drug carrier system including SLNs (82).

Liu *et al.* (83) developed novel nebulizer-compatible solid lipid nanoparticles for pulmonary delivery of insulin by the reverse micelle-double emulsion technique and their finding suggested that SLN could be used as a potential carrier for pulmonary delivery of insulin improving its stability as well as bioavailability.

Ocular administration

Eyes are among the most readily accessible organs in terms of their location in the body, yet drug delivery to eye tissues is particularly problematic. Delivery of drugs *via* nanotechnology-based products fulfils three main objectives: enhanced drug permeation, controlled drug release and higher targeting potential. Attama *et al.* (84) prepared sodium diclofenac-loaded lipid nanoparticles combining the homolipid from goat (goat fat) and a phospholipid, with high encapsulation efficiency applying hot high-pressure homogenization technique. Administration of this formulation to bioengineered human cornea demonstrated sustained release of the analgesic drug. Furthermore, permeation of sodium diclofenac through the corneal construct was improved by surface tailoring of nanoparticles with phospholipid, which showed better performance for ocular administration.

TARGETING EFFICIENCY OF SLNs

One of the most challenging aspects in pharmaceutical research is targeted delivery of drug molecules to a specific organ, tissue or specific cellular sites. By developing colloidal delivery systems such as liposomes, micelles and nanoparticles, a new frontier was opened for improving drug delivery.

Brain targeting

Nanosystems employed for the development of drug delivery systems intended for CNS targeting include polymeric nanoparticles, nanospheres, nanosuspensions, nanoemulsions, nanogels, nano-micelles, nano-liposomes, nanofibres, nanorobots, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs) and lipid drug conjugates (LDCs) (85). The most formidable obstacles that often impede drug delivery to the brain are characterized by the presence of relatively impermeable endothelial cells with tight junctions, enzymatic activity and the presence of active efflux transporter mechanisms like P-glycoprotein efflux. However, the bioacceptable and biocompatible nature of SLNs makes them less toxic compared to polymeric nanoparticles and they are taken up by the brain because of their lipidic nature. SLNs below 200 nm have increased blood circulation and hence an increase in the time during which the drug remains in contact with BBB and is taken up by the brain (86).

Mistry *et al.* (87) suggested that the existence of a direct nose-to-brain delivery route for nanoparticles administered to the nasal cavity and transported *via* the olfactory epithelium and/or the trigeminal nerves directly to the CNS is relevant in the field of drug delivery as well as new developments in nanotechnology. Proteins and peptides (P/P) have been pointed out as holding great promise for the treatment of various neurodegenerative diseases. A major challenge in this regard, however, is the delivery of P/P drugs over the blood-brain-barrier (BBB). Technology based approaches (comprising functionalized nanocarriers and liposomes) and pharmacological strategies (such as the use of carriers and chimeric peptide technology) appear to be the most promising ones to facilitate enhanced P/P drug delivery over BBB (88).

Microphage targeting

Microphages play a central role in mediating inflammatory responses and also serve as a reservoir for microorganisms involved in life-threatening infectious diseases. The use of drug-loaded nanoparticles represents a good alternative to avoid or at least decrease side effects and increase efficacy, compared to potent drugs which often induce unwanted side effects when applied as free forms for treating the microphage-mediated diseases (89).

Epidermal targeting

Chen *et al.* (90) formulated solid lipid nanoparticles loaded with podophyllotoxin (POD) stabilized with Poloxamer 188 (P-SLN) and Polysorbate 80 (T-SLN) and evaluated as topical carriers for epidermal targeting of POD. Penetration of POD from P-SLN,

by fluorescence microscopy, seemed to follow two pathways along the stratum corneum and hair follicle route. Imaging revealed that P-SLN had a strong localization of POD within the epidermis. To sum up, P-SLN provides a good epidermal targeting effect and may be a promising carrier for topical delivery of drugs like POD.

CELLULAR UPTAKE AND CYTOTOXICITY/NEUROTOXICITY ASPECTS OF SLNs

Solid lipid nanoparticles are well tolerated in a biological system because they are composed of physiological lipids. *In vitro* cytotoxicity and viability determinations were performed on suspensions of human granulocytes using the dimethylthiazolyl-diphenyltetrazolium (MTT) test to assess their tolerability at cellular levels. The results of cytotoxicity studies (MTT assay) indicated that SLNs are less toxic than polymeric nanoparticles (91, 92).

Yuan *et al.* (93) investigated the cellular uptake and cytotoxicity of paclitaxel-loaded solid lipid nanoparticles composed of different lipid materials. The order of cellular uptake ability was: glycerol tristearate SLN > monostearin SLN > stearic acid SLN > Compritol 888 ATO SLN. Incorporation of polyethylene glycol monostearate (PEG-SA) into SLN could enhance cellular uptake but it did not increase cytotoxicity whereas the introduction of folic acid-stearic acid (FA-SA) into SLN could enhance cellular uptake and cytotoxicity, which indicated a potential application of the later for targeting tumor therapy.

Recent observations suggested that several NPs such as Polysorbate-80 coated polybutyl cyanoacrylate (PBCA) NPs are able to cross the BBB through intravenous administration followed by accumulation in the brain resulting in severe neurotoxicity (94). There are already a few reports that observed the neurotoxicity of nanoparticles both *in vitro* (95) and *in vivo* (96, 97).

CHARACTERIZATION OF SLN QUALITY AND STRUCTURE

Adequate and appropriate characterization of SLNs is essential for quality control of formulations. However, characterization of SLN is not an easy task due to the colloidal size range and complex nature of delivery systems. The key parameters to be evaluated and the techniques used for SLNs characterization are the following:

Particle size/size distribution

Particle size/size distribution of SLN may be studied using photon correlation spectroscopy (PCS), laser diffraction (LD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM), scanning tunneling microscopy (STM) or freeze fracture electron microscopy (FFEM). Among these, PCS and LD are the most commonly employed techniques for routine measurement of particle size. PCS measures the fluctuation of the intensity of scattered light caused by particle movement. PCS covers a range from a few nanometers to about 3 μm. However, PCS is not able to detect larger microparticles. They can be visualized by LD measurements.

The method of laser diffraction (LD) is based on the dependence of diffraction angle on particle radius (Fraunhofer spectra). Smaller particles cause more intense scattering at high angles compared to larger ones. LD covers a broader size range from nanometers to a lower millimeter range. Development of the polarization intensity differential scattering (PIDS) technique greatly enhanced the sensitivity of LD to smaller particles. However, both of these techniques are always recommended to be used simultaneously. It should be noted that both of these techniques do not »measure« particle size, but they detect light scattering effects which are further used to calculate particle size. Difficulties may arise both in PCS and LD measurements in case of samples which are non-uniform in size and, therefore, use of additional techniques like light microscopy, although not sensitive enough to the nanometric size range, is recommended or electron microscopy which gives direct information on the particle shape (3, 98).

Zeta potential

One could draw predictions regarding the storage stability of colloidal dispersions from zeta potential measurements. This is an important formulation characteristic of SLN since, in general, high zeta potential values are likely to cause particle deaggregation due to electric repulsion. However, systems containing steric stabilizers or hydrophilic surface appendages do not follow strictly this rule, because adsorption of steric stabilizers will decrease the zeta potential due to a shift in the particle shear plane (3, 4).

Dynamic light scattering (DLS)

Dynamic light scattering (DLS), also known as Photon Correlation Spectroscopy (PCS) or quasi-elastic light scattering (QELS), records the variation in intensity of scattered light on the microsecond time scale. This variation results from the interference of light scattered by individual particles under the influence of Brownian motion and is quantified by compilation of an autocorrelation function. This function is fitted to an exponential, or some combination or modification thereof, with the corresponding decay constant(s) being related to the diffusion coefficient(s). Particle size can be calculated from this coefficient using standard assumptions of spherical size, low concentration, and known viscosity of the suspending medium. Rapid analysis, lack of required calibration, and sensitivity to submicron sized particles are some of the merits of this method. Spectral decomposition capability of dynamic light scattering enables one to estimate the molecular weight of each nanoparticle (3, 99). Thus, DLS is a nonintrusive, sensitive and powerful analytical tool used to characterize macromolecules and colloids in solution (100).

Static light scattering/Fraunhofer diffraction (SLS)

Static light scattering (SLS) is an ensemble method in which the pattern of light scattered from a solution of particles is collected and fitted to fundamental electromagnetic equations in which particle size is the primary variable. This method is rapid and rugged, but it requires more cleanliness than DLS and advanced knowledge of optical properties of the particles (3).

Field flow fractionation (FFF)

Field flow fractionation (FFF) was used to separate particles due to their Stoke's radius. In this technique, particles are separated in a flow through a thin channel under the influence of a field force perpendicular to the flow. The flow in the channel has a parabolic pattern, with highest speed in the centre, such that smaller particles are transported faster and eluted earlier. All eluted fractions are analyzed by multi-angle light scattering (MALS) where a photometer records scattering signals of the particles and calculates the size-weighted radius. MALS is able to measure the particles ranging from 10 nm to approximately 1 μm. The two subtechniques of FFF, thermal FFF and cross--flow FFF, were successfully employed to separate and analyze the particles according to their size and diffusion coefficients. Two variations of flow FFF, symmetric flow and asymmetric flow, were reported by Dulog and Schauer (101).

Acoustic method

Acoustic spectroscopy deals with determination of the size of nanoparticles by measuring the attenuation of sound waves through the fitting of physically relevant equations. In addition, the oscillating electric field generated by the movement of charged particles under the influence of acoustic energy can be detected to provide information on surface charge (3, 102).

Nuclear magnetic resonance (NMR)

NMR can be used to determine both the size and the qualitative nature of nanoparticles. Selectivity afforded by the chemical shift compliments the sensitivity to molecular mobility to provide information on the physicochemical status of the components within nanoparticles (103).

Electron microscopy

SEM (scanning electron microscopy) and TEM (transmission electron microscopy) are useful for direct observation of nanoparticles and the former method is preferentially used for physical characterization of nanoparticles, particularly their morphological examination. However, vacuum drying used in sample preparation of SEM causes shrinkage of the SLN size (104). TEM has a smaller size limit of detection, provides good validation for other methods, but one must be cognizant of the statistically small sample size (3).

Force microscopy/scanning force microscopy

Atomic force microscopy (AFM). – In AFM, a probe tip with atomic scale sharpness is rastered across the sample to produce a topological map based on the forces at play between the tip and the surface. The probe can be dragged across the sample (contact mode), or is allowed to hover just above (noncontact mode) (3). AFM is ideally suited for characterizing the nanoparticles because it offers the capability of 3D visualization, both qualitative and quantitative information of the sample topology, including morphology,

surface texture and roughness and, more important in this case, the size of the nanoparticles (100). Also, ultrahigh resolution can be obtained with this technique. However, the deposition method used for AFM causes the SLN to form small clusters (104).

Magnetic force microscopy (MFM). – Albrecht *et al.* (105) combined the AFM and MFM techniques to study bacterial magnetosomes, comprised of membrane-bound magnetite particles. AFM imaging confirmed the existence of single isolated magnetosomes with a size of about 40 nm while MFM addressed the magnetic properties of single magnetosomes. Their results demonstrated that AFM and MFM could be used as powerful tools for the development of magnetosomes for medical applications.

Freeze fracture electron microscopy (FFEM). – Freeze fracture electron microscopy is used for morphological examination of lipidic particles. Škalko *et al.* (106) used FFEM for detection of the appearance of protein bound to the surface of intact and microfluidized liposomes and its influence on their surface morphology. Surface bound protein was observed by FFEM and was confirmed by immunogold cryo microscopy.

Scanning tunneling microscopy (STM). – The technique of scanning tunneling microscopy (STM) was developed to examine the surface characteristics of desiccated and hydrated phospholipid bilayers in liposomes. It is expected to be useful in assessing the molecular organization of liposomes with distinct surface characteristics, molecular processes occurring during membrane dehydration, and the organization of phospholipids in the viscinity of membrane-bound proteins, lectins or polymers (107).

X-ray diffraction (XRD)

The degree of crystallinity of a solid can be assessed from the geometric scattering of radiation from the crystal planes of that solid using the X-ray powder diffraction technique. Robach *et al.* (108) investigated the structure of ~100 nm thick films of FePt nanoparticles by the same technique. Investigations of the physical state, melting behavior and recrystallization of lipids in the colloidal carrier systems can be performed using both X-ray diffraction and synchrotron radiation X-ray diffraction (small and wide angle) techniques (56).

Thermal analysis

Differential scanning calorimetry (DSC) can be used to determine the nature and degree of crystallinity of lipids within the nanoparticles by measuring the glass and melting temperatures, and their associated enthalpies (2). Schubert *et al.* (109) evaluated by thermoanalysis the influence of lecithin, an amphiphilic lipid, within the lipid matrix on the crystallization behavior of lipids.

APPLICATIONS OF SLNs

Long-term stability and ease of large scale production impart immense importance to the solid lipid nanoparticles, which forms the basis of biodegradable and biocompa-

tible colloidal drug delivery systems. SLNs find potential applications in the following areas of interest:

Cosmeceuticals

SLN have been employed for dermal application of cosmeceuticals like molecular sunscreens (110) and as carriers for UV blockers (111). Cosmetic benefits of lipid nanoparticles include enhancement of the chemical stability of actives, film formation on skin, controlled occlusion, skin hydration, drug targeting, enhanced skin bioavailability and physical stability of lipid nanoparticles as topical formulations (8, 112). An *in vivo* study showed increased skin hydration, by 31 %, after 4 weeks after addition of 4 % SLN to a conventional cream formulation (113).

Topical delivery

Among lipid nanoparticles, both SLN and NLC have been used in topical application of various pharmaceutical actives such as podophyllotoxin (90), tretinoin (114), isotretinoin (115), flurbiprofen (81), psoralen (8), vitamin A (79, 80). Lipid-based nanoformulation fulfils three main objectives: controlled drug release, enhanced drug permeation and site specific drug delivery (84). Dermal penetration barriers contain a high concentration of epidermal lipids and lipid based carriers appear to be promising by attaching themselves to the skin surface, allowing lipid exchange between the outermost layers of stratum corneum (116).

Gene vector carrier delivery

Rapid elimination of naked plasmid DNA from the circulation after intravenous administration calls for the development of an optimized DNA delivery system for effective and successful clinical use. Cationic solid lipid nanoparticles can bind to the DNA directly *via* electrostatic interactions, thereby mediating gene transfection *in vitro* as well as *in vivo* (117) and acting as a non-viral gene delivery vector carrier for DNA, plasmid DNA and nucleic acids (118, 119).

Chemotherapy

Khuller and co-workers exploited the chemotherapeutic potential of oral solid lipid nanoparticles for the delivery of antitubercular drugs such as rifampicin, isoniazid and pyrazinamide. The system was found to reduce the dosing frequency and enhance patient compliance (71).

SLN-based anticancer drug delivery systems have been shown to be superior over the conventional ones and promising for improved cancer chemotherapy (120). Doxorubicin, an anticancer drug encapsulated into PLN, an advanced form of SLN, was found to be very effective in treating multidrug resistant breast cancer (121). Inspiring efficacy and tumor targeting have been reported to be enhanced with SLNs loaded with various anticancer drugs like doxorubicin (122) and mitoxantron (123).

Vaccine adjuvant

Adjuvants are used in vaccination to enhance the immune response (2). The lipid--based nanocarriers for antigen delivery mainly include liposomes, immunostimulating complexes (ISCOMs), and NLC, again an advanced form of SLN. Major characteristics that make nanocarriers unique vaccine adjuvants are firstly their effective increase of the amount of antigens reaching the immune system, secondly that they control the release of antigens over prolonged periods of time and finally that they can add their immunostimulating or immunomodulating actions synergistically to their primary antigen delivery function (124).

STEALTHING OF SLNs

Major obstacles to the systemic use of nanoparticulate colloidal drug carriers designed to deliver the active pharmaceutical ingredients to their destination sites are posed by the reticuloendothelial system (RES) and opsonization process which recognize them as foreign products and quickly clear them from systemic circulation. The rate of RES uptake of colloidal nanoparticulates is so rapid that it significantly reduces the circulation half-life of these carriers. Opsonization refers to the clearance of nanoparticulates from the blood stream through their interaction with blood plasma proteins (opsonins), resulting in attachment of particles to the membrane of macrophages.

A great deal of work has been recently devoted for the surface modification of nanoparticulate carriers to deter them from the opsonization process and RES uptake through coating, binding or grafting of certain materials to the surface of nanoparticles, which could impart stealth behavior (25, 125).

Hydrophilic and flexible polymer coating on the surface of nanoparticles is thought to mask the surface from marking the particles with serum components (opsonins) for RES uptake. These surface modified nanoparticles are called sterically stabilized nanoparticles or stealth nanoparticles. The stealthing phenomenon has been successfully employed and explored in SLNs (126).

FUTURE PROSPECTS AND CHALLENGES

Research must continue in such a direction to provide improved efficacy, drug loading, targeting and lowering of the drug dose, thereby overcoming the toxicity challenges of this carrier system (127).

In addition, structure and dynamics of SLNs on the molecular level, both *in vitro* and *in vivo*, stability, targeting, toxicity and aspects related to interactions of SLNs with their biological surrounding pose a challenge that should be explored in the near future by various research groups around the globe.

CONCLUSIONS

A drug delivery system is said to be successful only if, along with the academic research groups, pharmaceutical industries undertake its development. Since SLNs have already been proven as good formulations in cosmeceuticals and other allied fields, they must occupy a considerable place in the pharmaceutical market. To exploit the broad applications of lipid based nanoparticulate formulations, it is essential that the pharmaceutical industries specialized in the development of new drug delivery systems should engage in novel formulation technology to promote their scale up and bring them onto the pharmacist's shelves.

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S A Ž E T A K

Nanonosači na bazi čvrstih lipida: Pregled

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Pojava nanočestica za kontroliranu i ciljanu isporuku lijekova izrađenih iz čvrstih lipida (SLN) imala je ranih devedesetih godina revolucionarno značenje. U ovom preglednom radu opisani su SLN sustavi kao korisni nanovektori za isporuku lijekova. Autori ističu prednosti SLN sustava, daju pregled lipida za njihovu izradu, opisuju metode priprave, karakterizacijske parametre i različite načine primjene SLN-a. Osim toga, detaljno se raspravlja o njihovoj stabilnosti, toksičnosti te mogućnosti ciljane isporuke. Istaknute su mogućnosti koje pružaju SLNi u području farmaceutskih znanosti i njihova moguća primjena u farmaceutskoj industriji.

Ključne riječi: koloidni nosači lijekova, nanočestice na bazi čvrstih lipida (SLN), stabilnost, mogućnost ciljane isporuke, citotoksičnost, pritajenost

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