ISSN: 0975-766X
CODEN: IJPTFI
Available through Online
www.ijptonline.com
Review Article

SOLID LIPID NANOPARTICLES- A NOVEL SOLID LIPID BASED TECHNOLOGY FOR POORLY WATER SOLUBLE DRUGS: A REVIEW

P.Shanmukhi*
Assistant Professor, Department of Pharmaceutics, DCRM College, Inkollu, Prakasam dist.
Email: shanmukhi.pharmacy@gmail.com

Received on 12-06-2013 *Accepted on 05-07-2013*

Abstract

Solid lipid nanoparticles (SLNs) are the effective lipid based colloidal carriers which were introduced in 1991, as an alternative to the conventional carriers such as microemulsions, liposomes, microparticles and nanoparticles based on synthetic polymers or natural macromolecules. The bioacceptable and biodegradable nature of SLNs makes them less toxic as compared to polymeric nanoparticles. The small size SLN which prolongs the circulation time in blood, feasible scale up for large scale production and absence of burst effect makes them interesting candidates for study. This paper presents an overview about the choice of the drug candidates, advantages, methods of preparations, appropriate analytical techniques for characterization, applications, routes of administration, etc are elaborated in detail.

Keywords: Solid lipid nanoparticles, Colloidal carriers, Bioavailability enhancement, Polymeric nanoparticles, Gastrointestinal solubilization.

Introduction

Solid lipid nanoparticles (SLNs) are introduced as a carrier system for poorly water soluble drug and cosmetic active drug. Colloidal particles ranging in size between 10 and 1000 nm are known as nanoparticles. They are synthesized from synthetic/natural polymers and suited to optimize drug delivery and reduce toxicity. They have emerged as a variable substitute to liposomes as drug carriers. The successful implementation of nanoparticles for drug delivery depends on their ability to penetrate through several anatomical barriers, sustained release of their contents and their stability in the nanometer size. They have some limitations due to their high cost and scarcity of safe polymers with

regulatory approval[1]. To overcome this limitation polymeric nanoparticle lipid is used as an alternative carrier. These nanoparticles are known as solid lipid nanoparticles (SLNs)[2].

SLNs are developed as an alternative system for polymeric nanoparticles, liposome and emulsion. SLNs have unique property like small size, large surface area, high drug loading and interaction of phase at the interphase[3]. SLNs are attracting major attention in novel colloidal carrier for intravenous application. SLNs are a new generation of submicron-sized lipid emulsions where the liquid lipid (oil) has been substituted by a solid lipid. SLNs are submicron colloidal carrier composed of physiological lipid, dispersed in water or in an aqueous surfactant solution[4]. The proposed structure of SLN is as shown in the figure 1.

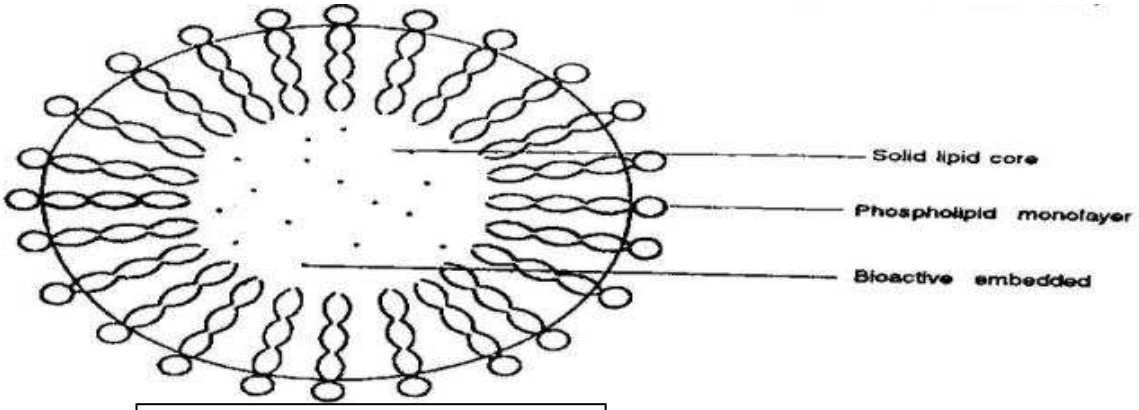

Fig.1: Proposed structure of SLN.

Mechanisms for enhancing the oral bioavailability of drug molecules by SLNs[5]

- Enhance dissolution / solubilization
- Stimulation of lymphatic transport
- Increased gastric residence time
- Enhace intestinal permeability
- Reduce metabolism and efflux activity
- Prevent first pass metabolism

Advantages

1. The solid matrix provides highest flexibility in controlling the release profile. The slower degradation velocity *in vivo* (e.g. compared to liposomes) allows drug release for prolonged periods.[6,7,8]

2. Surface modification can easily be accomplished further by coating with or attaching ligands to SLNs there is a increased scope of drug targetting[9,10].

3. High drug payload[11].

4. The solid matrix can (but need not) protect incorporated active ingredients/ drugs against chemical degradation[12].

5. In SLNs the lipid matrix is made from physiological lipid which is biodegradable hence decreases the danger of acute and chronic toxicity hence safe[3].

6. No toxic metabolites are produced[13].

7. Incorporation of drug can reduce distinct side effects of drug[14].

e.g. Thrombophlebitis that is associated with i.v. injection of diazepam

8. The nanoparticles and the SLNs particularly those in the range of 120–200 nm are not taken up readily by the cells of the RES (Reticulo Endothelial System) and thus bypass liver and spleen filtration[6].

9. SLN formulations stable for even three years have been developed. This is of paramount importance with respect to the other colloidal carrier systems[15,16].

10. Excellent reproducibility with a cost effective high pressure homogenization method as the preparation procedure[17].

11. The feasibility of incorporating both hydrophilic and hydrophobic drugs[18,19].

12. Avoidance of organic solvents [11].

13. Feasible large scale production and sterilization[6].

14. SLNs can be enhancing the bioavailability of entrapped bioactive[20].

15. SLNs can be lyophilized as well as spray dried[13,14].

SLN Versus Other colloidal carriers [21, 22]

SLN have been proven to be a better alternative carrier system than conventional 0/w emulsion in the following aspects.

- If protection of drug against chemical degradation is required. Incorporation of drug in the solid lipid matrix surely offer a better protection than can be achieved in the oily internal phase of emulsion and liposomes.
- Prolonged release of drug from emulsion is not feasible which can be achieved to a certain extent from SLN .

SLNs is found to be a better carrier than polymeric nanoparticles in the following aspects

- Lower cytotoxicity due to the absence of solvents
- Low cost of excipients
- Large scale production is possible by the simple process of high-pressure homogenization

SLNs versus liposomes:

In comparison with liposomes SLNs offer better protection to drug against chemical degradation there is no or little access of water to the inner core of lipid particles. Depending upon the nature of the drug higher payload might be achieved.

Disadvantages

1. Poor drug loading capacity [23]

2. Drug expulsion after polymeric transition during storage

3. Relatively high water content of the dispersions (70-99.9%)[24]

4. The low capacity to load hydrophilic drugs due to partitioning effects during the production process [25]

Types of solid nanoparticles [26]: The types of SLNs depend on the chemical nature of the active ingredient and lipid, the solubility of actives in the melted lipid, nature and concentration of surfactants, type of production and the production temperature. Therefore incorporation models have been proposed for study.

SLN, Type I or homogenous matrix model

SLN, Type II or drug enriched shell model

SLN, Type III or drug enriched core model

General Ingredients and the Emulsifiers [27]: The matrixes of SLN are the natural or the synthetic lipids which can be degradated, including triglyceride (tri-stearic acid, tri-palmitic acid, tri-lauric acid *etc*. long-chain fatty acid), steroid (e.g., cholesterin), waxes (e. g. , microcrystal paraffin wax, whale esterwax).

- The choice of the emulsifiers depends on the administration of the drug, to the parenteral system, there are limits to choose the emulsifiers including the phospholipids [e.g., soybean phospholipids (LS 75, LS loo), yolk phospholipids (LE80) , lecithin , nonionic wetting agent (e.g., poloxamer 188, 182, 407, 908), chleolate (e. g. , sodium cholate,

sodium glycocholate sodium taurocholate , deoxy-sodium taurocholate , short-chain spirits (e. g. , butanol, butanoic acid).

- Amphipathicity materials (e. g. , ionic and nonionic type) can stabilize the dispersion of SLN, on the surface of SLN, hydrophobic parts stretch to the core, hydrophilic parts stretch to the disperse medium, so drug with low water-solubility can be entrapped in the SLN to form the colloidal drug system.

Toxicity and Status of Excipients[28,29,30,31]

Toxicity and the status of excipients are a major issue for the use of a delivery system. The status of excipients for SLN has to be discussed as a function of the administration routes. Topical and oral administration of SLN are absolutely non-problematic regarding the excipients. For topical SLN, all excipients can be used which are currently employed for the formulation of pharmaceutical and cosmetic ointments and creams. For oral SLN, all the lipids and surfactants used in traditional dosage forms such as tablets, pellets and capsules can be exploited. In addition all compounds of GRAS status or accepted GRAS status can be employed. There is also the option to use lipids and surfactants from the food industry. Of course, use in the food industry does not allow directly its use in pharmaceutical products. However, the toxicity material available for the food area can be used for submission to the pharmaceutical regulatory authorities, that means it is a relatively easy case. The situation is slightly different for parenteral administration. Up to now there are no products on the market containing solid lipid particles for parenteral injection. Therefore, a toxicity study would be necessary. However, one can use glycerides composed of fatty acids which are contained in oils of parenteral fat emulsions. Therefore, no toxic effects are expected from the SLN degradation products. In addition, one has to consider that a toxicity study with the parenteral new product has to be made anyway, that means the lipid itself might contribute very little to the total costs of the study required. To formulate parenteral SLN, surfactants accepted for parenteral administration can be used, that means, e.g. lecithin, Tween 80, Poloxamer 188, PVP, sodium glycocholate, Span 85 etc. For the intravenous route it is recommended to focus on the i.v. accepted surfactants (e.g. lecithin, Tween 80, Poloxamer 188, sodium glycocholate).

PREPARATION METHODS OF SLNs

High pressure homogenization (HPH)[8,34,36]**:** It is a reliable and powerful technique, which is used for the production of SLNs. High pressure homogenizers push a liquid with high pressure (100–2000 bar) through a narrow gap (in the

range of a few microns). The fluid accelerates on a very short distance to very high velocity (over 1000 km/h). Very high shear stress and cavitation forces disrupt the particles down to the submicron range. Generally 5-10% lipid content is used but up to 40% lipid content has also been investigated (32, 7). Two general approaches of HPH are hot homogenization and cold homogenization; work on the same concept of mixing the drug in bulk of lipid melt.

Hot homogenization[67]: For the hot homogenization technique the drug-containing melt is dispersed under stirring in a hot aqueous surfactant solution of identical temperature. Then the obtained pre-emulsion is homogenized using a piston-gap homogenizer (e.g. Micron LAB40), the produced hot O/W nanoemulsion is cooled down to room temperature, the lipid recrystallizes and leads to solid lipid nanoparticles (Fig. 2). Of course, care needs to be taken that recrystallization of the lipid occurs. For glycerides being composed of short chain fatty acids (e.g. Dynasan 112) and glycerides with a low melting point (too close to room temperature) it might be necessary to cool the nanoemulsions to even lower temperatures to initiate recrystallization. Recrystallization can also be initiated, e.g. by lyophilization.

In general, higher temperatures result in lower particle sizes due to the decreased viscosity of the inner phase . However, high temperatures increase the degradation rate of the drug and the carrier. Further, one should remember that the high pressure homogenization increases the temperature of the sample (approximately 108°C for 500 bar) . Increasing the homogenization pressure or the number of cycles often results in an increase of the particle size due to high kinetic energy of the particles .

Advantages

- The hot homogenization technique is also suitable for drugs showing some temperature sensitivity because the exposure to an increased temperature is relatively short.

Demerits[26]

- It cannot be employed to incorporate hydrophilic active ingredients/ drugs because due to dispersing the lipid melt in the aqueous surfactant solution would lead to the partitioning of the drug to the water phase, which means that with hydrophilic drugs more than 90% would be lost to the water phase.

Cold homogenization: For the cold homogenization technique the drug containing lipid melt is cooled, the solid lipid ground to lipid microparticles (approximately 50±100 mm) and these lipid microparticles are dispersed in a cold

surfactant solution yielding a pre-suspension. Then this pre-suspension is homogenized at or below room temperature, the cavitation forces are strong enough to break the lipid microparticles directly to solid lipid nanoparticles. This process avoids, or minimizes, the melting of the lipid and therefore minimizing loss of hydrophilic drugs to the water phase. Of course, the difference between the melting point of the lipid and the homogenization temperature needs to be large enough to avoid melting of the lipid in the homogenizer. The homogenization process itself increases the product temperature (e.g. 10±208C per homogenization cycle). There are also temperature peaks in the homogenizer. To further minimize the loss of hydrophilic compounds to the aqueous phase of the SLN dispersion, water can be replaced by liquids with low solubility for the drug, e.g. oils or PEG 600. Production of SLN in oil or PEG 600 is advantageous for oral drug delivery because this dispersion could be directly filled into soft gelatin capsules.

Advantages [35]

Cold homogenization has been developed to over come the following three problems of the hot homogenization technique:

- Temperature induced drug degradation for highly temperature-sensitive compounds.
- It is also necessary to use this technique when formulating hydrophilic drugs because they would partition between the melted lipid and the water phase during the hot homogenization process.
- Complexity of the crystallization step.

The schematic procedures for hot and cold homogenization techniques for SLN production is shown in the figure 2.

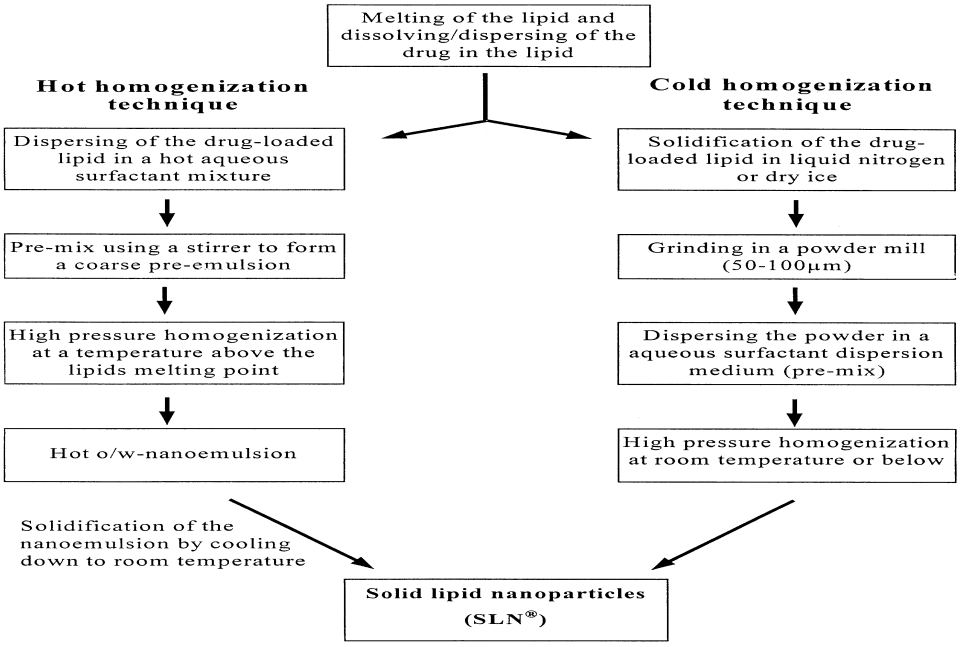

Fig 2: Schematic procedure of hot and cold homogenization techniques for SLN production

Ultrasonication or high speed homogenization [4,36]

SLN were also developed by high speed stirring or sonication. A most advantages is that, equipment whatever use here are very common in every lab. The problem of this method is broader particle size distribution ranging into micrometer range. This lead physical instabilities likes particle growth upon storage. Potential metal contamination due to ultrasonication is also a big problem in this method. So for making a stable formulation, studies have been performed by various research groups that high speed stirring and ultrasonication are used combined and performed at high temperature.

Spray drying method [37]

It's an alternative procedure to lyophilization in order to transform an aqueous SLN dispersion into a drug product. It's a cheaper method than lyophilization. This method cause particle aggregation due to high temperature, shear forces and partial melting of the particle. Freitas and Mullera recommends the use of lipid with melting point $>70^0$ for spray drying.

SLN preparation by using supercritical fluid [38,39]

This is a relatively new technique for SLN production and has the advantage of solvent less processing. There are several variations in this platform technology for powder and nanoparticle preparation. SLN can be prepared by the rapid expansion of supercritical carbon dioxide solutions (RESS) method. Carbon dioxide (99.99%) was the good choice as a solvent for this method.

Double emulsion method [40]

For the preparation of hydrophilic loaded SLN, a novel method based on solvent emulsification-evaporation has been used. Here the drug is encapsulated with a stabilizer to prevent drug partitioning to external water phase during solvent evaporation in the external water phase of w/o/w double emulsion.

Micro emulsion based SLN preparations[4, 38, 41,42,43]

Gasco and coworkers develop a new technique for production of SLNs based on the dilution of micro emulsions . As microemulsions are two-phase systems composed of an inner and outer phase (e.g. o/w microemulsions). They are made by stirring an optically transparent mixture at 65-70^0 which is typically composed of a low melting fatty acid (stearic acid), an emulsifier(polysorbate 20, polysorbate 60, soy phosphatidylcholine, and sodium taurodeoxycholate),

co-emulsifiers (sodium monooctylphosphate) and water. The hot micro emulsion is dispersed in cold water (2-3^0) under stirring. Typical volume ratios of the hot micro emulsion to cold water are in the range of 1:25 to 1:50.The dilution process is critically determined by the composition of the micro emulsion. According to the literature, the droplet structure is already contained in the micro emulsion and therefore, no energy is required to achieve submicron particle sizes.

SLN prepared by solvent emulsification/evaporation[36,44]:

This technique is used for of nanoparticle dispersions by precipitation in o/w emulsions. The lipophilic material is dissolved in water immiscible organic solvent (cyclohexane) that is emulsified in an aqueous phase. Upon evaporation of the solvent nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium. The mean diameter of the obtained particles was 25 nm with cholesterol acetate as model drug and lecithin/sodium glycocholate blend as emulsifier. The reproducibility of the result was confirmed by Siekmann and Westesen, who produced the cholesterol acetate nanoparticles of mean size 29 nm.

Characterization of Sln Quality and Structure[45]

Adequate and proper characterization of the SLNs is necessary for its quality control. The important parameters which need to be evaluated for the SLNs are, particle size, size distribution kinetics (zeta potential), degree of crystallinity and lipid modification (polymorphism), coexistence of additional colloidal Structures (micelles, liposome, super cooled, melts, drug nanoparticles), time scale of distribution processes, drug content, *in vitro* drug release and surface morphology. The particle size/size-distribution may be studied using photon correlation spectroscopy (PCS), transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM), scanning tunneling microscopy (STM), or freeze fracture electron microscopy (FFEM).

Measurement of particle size and zeta potential[46]

Photon correlation spectroscopy (PCS) and laser diffraction (LD) are the most powerful techniques for routine measurements of particle size. The Coulter method is rarely used to measure SLN particle size because of difficulties in the assessment of small nanoparticle and the need of electrolytes which may destabilize colloidal dispersions. PCS (also known dynamic light scattering) measures the fluctuation of the intensity of the scattered light which is caused by the particle movement. This method covers a size range from a few nanometers to about 3 microns. This means that

PCS is a good tool to characterize nanoparticles, but it is not able to detect larger microparticles. They can be visualized by means of LD measurements. This method is based on the dependence of the diffraction angle on the particle radius (Fraunhofer spectra). Smaller particles cause more intense scattering at high angles compared to the larger ones. A clear advantage of LD is the coverage of a broad size range from the nanometer to the lower millimeter range. The of polarization intensity differential scattering (PIDS) technology greatly enhanced the sensitivity of LD to smaller particles. However, despite this progress, it is highly recommended to use PCS and LD simultaneously. It should be kept in mind that both methods do not 'measure' particle size. Rather, they detect light scattering effects which are used to calculate particle size. Further, difficulties may arise both in PCS and LD measurements for samples which contain several populations of different size. Therefore, additional techniques might be useful. For example, light microscopy is recommended, although it is not sensitive to the nanometer size range. It gives a fast indication of the presence and character of microparticles . Electron microscopy provides, in contrast to PCS and LD, direct information on the particle shape. However, the investigator should pay special attention to possible artifacts which may be caused by the sample preparation. Zeta potential is an important product characteristic of SLNs since its high value is expected to lead to deaggregation of particles in the absence of other complicating factors such as steric stabilizers or hydrophilic surface appendages. It is usually measured by zetameter.

Dynamic light scattering (DLS)[47]

DLS, also known as PCS or quasi-elastic light scattering (QELS) records the variation in the intensity of scattered light on the microsecond time scale. This variation results from 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 fit to an exponential, or some combination or modification thereof, with the corresponding decay constant(s) being related to the diffusion coefficient(s). Using standard assumptions of spherical size, low concentration, and known viscosity of the suspending medium, particle size is calculated from this coefficient. The advantages of the method are the speed of analysis, lack of required calibration, and sensitivity to submicrometer particles.

Static light scattering/Fraunhofer diffraction[20]: Static light scattering (SLS) is an ensemble method in which the pattern of light scattered from a solution of particles is collected and fit to fundamental electromagnetic equations in

which size is the primary variable. The method is fast and rugged, but requires more cleanliness than DLS, and advance knowledge of the particles' optical qualities.

Acoustic methods

Another ensemble approach, acoustic spectroscopy, measures the attenuation of sound waves as a means of determining size 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.

Nuclear magnetic resonance (NMR)

NMR can be used to determine both the size and the qualitative nature of nanoparticles. The selectivity afforded by chemical shift complements the sensitivity to molecular mobility to provide information on the physicochemical status of components within the nanoparticle.

Electron microscopy

SEM and TEM provide a way to directly observe nanoparticles, physical characterization of nanoparticles. TEM has a smaller size limit of detection, is a good validation for other methods, and affords structural required, and one must be cognizant of the statistically small sample size and the effect that vacuum can have on the particles.

Atomic Force Microscopy (AFM)[47]

In this technique, a probe tip with atomic scale sharpness is rastered across a 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 allowed to hover just above (noncontact mode), with the exact nature of the particular force employed serving to distinguish among the subtechniques. That ultrahigh resolution is obtainable with this approach, which along with the ability to map a sample according to properties in addition to size, e.g., colloidal attraction or resistance to deformation, makes AFM a valuable tool.

X-ray diffraction (powder X-ray diffraction) and differential scanning calorimetry (DSC)[30,48]

The geometric scattering of radiation from crystal planes within a solid allow the presence or absence of the former to be determined thus permitting the degree of crystallinity to be assessed. Another method that is a little different from

its implementation with bulk materials, DSC can be used to determine the nature and speciation of crystallinity within nanoparticles through the measurement of glass and melting point temperatures and their associated enthalpies.

Drug incorporation and loading capacity[49]

Many different drugs have been incorporated in SLN, examples are given in Table 1. A very important point to judge the suitability of a drug carrier system is its loading capacity. The loading capacity is generally expressed in percent related to the lipid phase (matrix lipid + drug). Westesen et al. studied the incorporation of drugs using loading capacities of typically 1±5%, for Ubidecarenone loading capacities of up to 50% were reported . For Tetracaine and etomidate capacities of 10±20% are reported , for retinol up to 5% [55±57], for coenzyme Q10 20% and for cyclosporin 20±25% .

Factors determining the loading capacity of drug in the lipid are, for example:

1. Solubility of drug in melted lipid
2. Miscibility of drug melt and lipid melt
3. Chemical and physical structure of solid lipid matrix
4. Polymorphic state of lipid material.

The examples of drugs incorporated in SLN, all SLN were prepared by high pressure homogenization apart from the SLN by Gasco (microemulsion technique) is shown in the table 1.

Drug	Corresponding author/research group	Reference
Timolol	Gasco	75,76
Deoxycorticosterone	Gasco	77
Doxorubicin	Gasco	78
Idarubicin	Gasco	78
Pilocarpine	Gasco	79
Thymopentin	Gasco	80
Diazepam	Gasco	81

Progesterone	Gasco	82
Hydrocortisone	Gasco	82
Paclitaxel	Gasco	83
Retinol	Gohla	84,85
Coenzyme Q10	Gohla	10
Aciclovir	Lukowski	86,87
Cyclosporin	Muller	59
Azido thymidine palmitate	Phillips	88
Betamethasone valerate	Westesen	89
Camptothecin	Yang	90,91
Piribedil	Yazan	92

Drug release from SLN[49]

As can be seen from Table 1 there are many studies dealing with drug incorporation, however, there are distinctly less data available about drug release, especially information about the release mechanisms. Most of the data about in vitro drug release mechanisms were generated by Mehnert et al. studying the model drugs tetracaine, etomidate and prednisolone. A major problem during the work with lipid nanopellets was the burst release observed with these systems. A similar burst release was obtained when incorporating tetracaine and etomidate into SLN independent on the production method (hot vs. cold homogenization). A prolonged drug release was first obtained when studying the incorporation of prednisolone. This demonstrated the principle suitability of the SLN system for prolonged drug release. Even more important it was possible to modify the release profiles as a function of lipid matrix, surfactant concentration and production parameters (e.g. temperature).

In vitro drug release could be achieved for up to 5±7 weeks. The profiles could be modulated showing prolonged release without any burst at all, but also generating systems with different percentages of burst followed by prolonged release. The burst can be exploited to deliver an initial dose when desired. It is highly important that it could be shown that the release profiles are not or only slightly affected by the particle size, dominant factors for the shape of the profiles are the production parameters (surfactant concentration, temperature) and also the nature of the lipid matrix. The profiles obtained could be explained by partitioning effects of the drug between the melted lipid phase and the aqueous surfactant phase during particle production. During particle production by the hot homogenization technique, drug partitions from the liquid oil phase to the aqueous water phase. The amount of drug partitioning to the water phase will increase with the solubility of the drug in the water phase, that means with increasing temperature of the aqueous phase and increasing surfactant concentration. The higher the temperature and surfactant concentration, the greater is the saturation solubility of the drug in the water phase. During the cooling of the produced O/W nanoemulsion the solubility of the drug in the water phase decreases continuously with decreasing temperature of the water phase, that means a re-partitioning of the drug into the lipid phase occurs. When reaching the recrystallization temperature of the lipid, a solid lipid core starts forming including the drug which is present at this temperature in this lipid phase. Reducing the temperature of the dispersion further increases the pressure on the drug because of its reduced solubility in water to further re-partition into the lipid phase. The already crystallized core is not accessible anymore for the drug, consequently the drug concentrates in the still liquid outer shell of the SLN and/or on the surface of the particles. The amount of drug in the outer shell and on the particle surface is released in the form of a burst, the drug incorporated into the particle core is released in a prolonged way. Therefore, the extent of burst release can be controlled via the solubility of the drug in the water phase during production, that means via the temperature employed and the surfactant concentration used. Higher temperatures and higher surfactant concentrations increase the burst production at room temperature avoids partitioning of drug into the water phase and subsequent re-partitioning to the oil phase, thus showing no burst at all. To avoid or minimize the burst, SLN can be produced surfactant free or using surfactants which are not able to solubilize the drug. No burst occurs at room temperature and 0% surfactant, the burst increases with increasing temperature and also with increasing surfactant concentration at a given temperature.

Based on the data published by the group of Mehnert, three drug incorporation models resulted:

1. Solid solution model

2. core-shell model, drug-enriched shell

3. core-shell model, drug-enriched core.

In-vitro and ex-vivo methods for the assessment of drug release from SLNs

In-vitro drug release

Dialysis tubing [50,51]

In-vitro drug release could be achieved using dialysis tubing. The solid lipid nanoparticle dispersion is placed in a prewashed dialysis tubing which can be hermetically sealed. The dialysis sac is then dialyzed against a suitable dissolution medium at room temperature, the samples are withdrawn from the dissolution medium at suitable intervals, centrifuged and analyzed for drug content using a suitable analytical method (U.V. spectroscopy, HPLC etc). The maintenance of sink conditions is essential. This method however suffers from the limitation of a lack of direct dilution of the SLNs by the dissolution medium. The drug release of camptothecin SLN using a dynamic dialysis method in phosphate buffered saline has been reported .

Reverse dialysis[50]

In this technique a number of small dialysis sacs containing 1 ml of dissolution medium are placed in SLN dispersion. The SLNs are then displaced into the dissolution medium. The direct dilution of the SLNs is possible with this method; however the rapid release cannot be quantified using this method .

Franz diffusion cell[50]

The solid lipid nanoparticle dispersion is placed in the donor chamber of a Franz diffusion cell fitted with a cellophane membrane. The dispersion is then dialyzed against a suitable dissolution medium (simulated gastric medium/simulated intestinal medium/simulated plasma) at room temperature, the samples are withdrawn from the dissolution medium at suitable intervals and analyzed for drug content using a suitable instrumental method (U.V. spectroscopy, HPLC). The maintenance of sink condition is essential and the method suffers from the limitation of lack of direct dilution of the SLNs by the dissolution medium.

Ex-vivo model for determining permeability across the gut[52]

Ahlin et al. demonstrated passage of Enalaprilat SLNs across rat jejunum. In short, the rat jejunum was excised from the rats after sacrificing the animal. The jejunum 20–30 cm distal from the pyloric sphincter was used. The jejunum was rinsed to remove the luminal contents after washing with ice cold standard Ringer solution. The tissue was then cut into segments, opened up along the mesenteric border and placed between two Easy Mount side-by-side diffusion chambers with an exposed tissue area of 1 cm^2. The mucosal side was bathed with ringer buffer containing 10mM mannitol and the serosal side with ringer buffer containing 10mM glucose. The enalaprilat loaded nanoparticles were placed on the mucosal side, dispersed in ringer containing the paracellular transporter sodium fluorescein confirming for tissue integrity. Similar type of study will be carried out here also.

Animals and administration of drug formulations

Male Wistar rats and Swiss albino mice are used for pharmacokinetic and tissue distribution studies respectively.

Intravenous administration[53]

Rats are anaesthetized and the selected samples are given. Time taken for administration is 30 sec. Blood samples are drawn by retro-orbital venous plexus puncture at 15, 30, 45, 60, 90, 120, 240 and 480 min post i.v. dose. The samples are centrifuged (5000×g, 15 min) and serum are collected and stored at -20^{0}C until analysis.

Intraduodenal administration

Rats are anaesthetized by an intraperitoneal injection of 60 mg/kg of thiopentone sodium (short acting anaesthetic agent). Small incision are made at abdomen, duodenum is located and similar formulations are administered directly into the duodenum with syringe. Blood samples are collected and processed as described in intravenous route.

Biodistribution studies

Tissue distribution studies are carried out in Swiss albino mice after a 7-day acclimatization period. At predetermined time points (like 15, 30, 45, 60, 90, 120, 180, 360 and 720 min) three animals at each time point from each group is given anaesthesia and blood is collected via cardiac puncture. Tissues of interest (brain, liver, spleen, kidney, and heart) are collected immediately after cervical dislocation at different time points and they were blotted dry with tissue paper. Serum and tissue samples are frozen at -20^0 C until analysis.

Serum and tissue sample analysis

Serum and tissue samples are evaluated. The method involves extraction of drug. The data is recorded and calculated using Winchrome software .

Pharmacokinetic analysis[53]

Serum concentration versus time data for drug in individual rats are analyzed by non-compartmental estimations using WinNonlin software (version 1.1). Relative bioavailability (F) of SLNs are obtained. Maximum serum concentration (Cmax) and the time to reach Cmax (Tmax) are taken directly from the observed concentration versus time profiles. The area under the concentration – time curve (AUC) and the area under the first moment curve (AUMC) is calculated using the linear trapezoidal rule. Mean residence time (MRT) is determined by dividing AUMC by AUC. The relative bioavailability (Fr) is defined as ratio of AUC of drug loaded SLN to the AUC of other drug formulation when same doses are administered and calculated.

Assay and entrapment efficiency[54]

The amount of drug entrapped within SLN is measured by UV spectrophotometry or by HPLC.

Entrapment efficiency

The entrapment efficiency of the drug is determined by measuring chamber through filter membrane. The amount of the drug in the aqueous phase is determined by HPLC.

Statistical analysis

Size and entrapment efficiency of SLNs are compared using the Student's t-test. Statistical analyses are also performed.

Stability studies

Drug loaded SLNs are stored at 25°C for 6 months and average size and entrapment efficiency are determined.

Storage stability[35]**:** SLN and nanoemulsions have remarkable similarities with respect to their composition and production methods. However, SLN cannot simply be regarded as colloidal lipid dispersions with solidified droplets. The problems connected with the presence of additional colloidal structures (micelles, mixed micelles, liposomes) exist for both carrier systems. However, SLN have additional features (supercooled melts, different modifications, non-spherical shapes) which are contributing to or determining the stability of the colloidal lipid suspension. Gelation

phenomena, increase in particle sizes and drug expulsion from the lipid carrier are the major problems of storage stability. As described above, there is a close relation between the modification of the lipid, gelation, particle aggregation and drug expulsion. A supercooled melt, which is the first product formed after hot homogenization, represents a nanoemulsion. It is characterized by spherical lipid droplets and a high incorporation rate for guest molecules (e.g. The transformation of the lipid melt to lipid crystals results in an increase of particle surface,a of the loading capacity of the lipid and it leads to increased stability problems. Stability of the lipid dispersions decreases as stability of the lipid modification increases.

Sterlization of Slns [20]: Intravenous and ocular administration SLN must be sterile. The high temperature reach during sterilization by autoclaving causes a hot o/w micro emulsion to form in the autoclave, modifies the size of the hot nanodroplets. On subsequent slow cooling, the SLN reformed, but some nanodroplets may coalesce, producing larger SLN than the initial ones. Amounts of surfactant and co surfactant present in the hot system are smaller. There for the nanoparticles are not stabilized.

Effect of Sterilization [54]

To see the effect of sterilization on particle size, zeta potential and entrapment efficiency, blank and drug dispersions are autoclaved at 121°C for 20 min. Coexistence of additional colloidal structures (micelles , liposomes , supercooled melts, drug nanoparticles) and time scale of distribution procases Nuclear Magnetic Resonance (NMR) and Electron Spin Resonance (ESR) are powerful tools for investigating dynamic phenomena and the characteristics of nanocompartments in colloidal lipid dispersions. This method is based on the different proton relaxation times in the liquid and semisolid/ solid state. Protons in the liquid state give sharp signals with high signal amplitudes, while semisolid/ solid protons give weak and broad NMR signals under these circumstances. It also allows for the the concentration of free drug in the dispersion medium. Ultracentrifugation was carried out using Centrisart, which consist of filter membrane (molecular weight cutoff 20,000 Da) at the base of the sample recovery chamber. The solid lipid nanoparticles along with encapsulated drug remained in the outer chamber and aqueous phase moved into the sample recovery characterization of liquid nanocompartments (NLC) in recently developed lipid particles, which are made from blends of solid and liquid lipids. The corresponding ESR spectra give information about the microviscosity and micropolarity.

CLINICAL BATCH PRODUCTION AND LARGE SCALE PRODUCTION OF SLN [49]

An important step towards a pharmaceutical product are first human trials, a prerequisite for this is the availability of a GMP production unit to provide first clinical batches. A GMP production unit was developed to produce clinical batches between 2 kg up to a maximum of 10 kg SLN dispersion. Such a unit exists at the company Pharmatec (Milan, Italy) and will be very soon available also at SkyePharma (Muttenz/Basel, Switzerland). For topical products, i.e. creams containing SLN, a batch size of approximately 50 kg to a few hundred kg is required. For this batch size a production line was developed having a capacity of 50 kg SLN dispersion/hour. It consists of two homogenizers being placed in series, that means instead of running a dispersion twice through one homogenizer (two homogenization cycles), the product is run continuously through two homogenizers placed in series (APV LAB60, Gaulin 5.5). Such solutions are possible because it is low cost equipment from the shelf. The size of the batch is given by the size of the feeding vessel and product container, respectively. Meanwhile a production line has been designed running on a continuous basis and having a capacity of 150 kg/h. The melted lipid and the hot aqueous surfactant solution are mixed by static blenders instead of mixing them batch wise in a large feeding container. A basic advantage of the homogenizers employed is their ability to be cleaned in place (CIP) and sterilized in place (SIP). The homogenizers can be sterilized by streaming steam, the product containers (e.g. employed for cosmetic batches) can be autoclaved.

Administration Routes

Transdermal application [27]

SLN used to this domain because its clear advantages can avoid the chemicals to degrade. At the same time, SLN forms a film, when the film moisture evaporates, SLN deformates, the drug is wapped out, in order to increase the absorption of SLN. As the transdermal carrier, SLN can significantly increase drug absorption of the skin.

Local application

The particle size of SLN can not only form a film on the skin, but also protect the active compounds not to be chemical decomposition, at the same time; SLN also controls the release of the drug. The latest research indicates that SLN has UV reflecting properties, when it entraps the physical and chemical sunscreen agents, it has also been found to modulate drug release into the skin and to improve drug delivery to particular skin layers. Besides it avoids the

stimulus which the sunscreen agents permeate the skin layers. Therefore, the cosmetic field offers interesting applications about SLN.

Oral administration[45,55,56,57,58,59]

Oral administration is the most convenient mode to the patients. Oral administration of SLN is possible as aqueous dispersion or alternatively after transform into a traditional dosage form, i.e. tablets, pellets, capsules or powders in sachets. 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 tabletting powder mixture. For the production of pellets the SLN dispersion can be used as wetting agent in the extrusion process. SLN powders can be used for the filling of hard gelatin capsules, alternatively, 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.An example for orally administered SLN are Camptothecin (CA)-loaded particles.

Pulmonary administration

Until now the SLN system has not yet been fully exploited for pulmonary drug delivery, very little has been published in this area. To demonstrate the suitability in principle of SLN for pulmonary delivery, aqueous SLN dispersions were nebulized with a PariBoy, the aerosol droplets were collected and the size of SLN analyzed. It could be shown that the particle size distributions of SLN before nebulization and after nebulization were almost identical, only very little aggregation could be detected which is of no significance for pulmonary administration. Alternatively SLN powders might be used in dry powder inhalers. SLN could be spray-dried using, e.g. lactose as excipient in the spray-drying process.

Parenteral administration[49]

Basically SLN can be used for all parenteral applications suitable for polymeric nanoparticles. This ranges from intra articular to intravenous administration. Studies using intravenously administered SLN have been performed by various groups. Gasco et al. produced stealth and non-stealth solid lipid nanoparticles and studied them in cultures of macrophages and also after loading them with Paclitaxel in vivo. The i.v. administered SLN lead to higher and

prolonged plasma levels of Paclitaxel. Interestingly both non-stealth and stealth SLN showed a similar low uptake by the liver and the spleen macrophages, a very interesting point was the increased uptake observed in the brain. This study demonstrates nicely the potential of SLN to achieve prolonged drug plasma levels. The observed similar low uptake by the liver and spleen macrophages might be explained by a similar low surface hydrophobicity of both types of particles avoiding the adsorption of any blood proteins mediating the uptake by liver and spleen macrophages. The uptake of the SLN by the brain might be explained by adsorption of a blood protein mediating the adherence to the endothelial cells of the blood brain barrier, an effect described previously by Kreuter.

Applications[30,60]

SLNs as gene vector carrier[61,62]

SLN can be used in the gene vector formulation. In one work, the gene transfer was optimized by incorporation of a diametric HIV-1 HAT peptide (TAT 2) into SLN gene vector. There are several recent reports of SLN carrying genetic/peptide materials such as DNA, plasmid DNA and other nucleic acid. The lipid nuclic acid nanoparticles were prepared from a liquid nanophase containing water and a water miscible organic solvent where both lipid and DNA are separately dissolved by removing the organic solvent, stable and homogeneously sized lipid-nuclic acid nanoparticle (70-100 nm) were formed. It's called genospheres. It is targeted specific by insertion of an antibody lipo polymer conjugated in the particle.

Topical use[63-66]

SLNs and NLCs have been used for topical application for various drugs such as tropolide, imidazole antifungals, anticancers, vitamin A, isotretinoin, ketoconazole, DNA, flurbiprofen and glucocorticoids. The penetration of podophyllotoxin SLN into stratum corneum along with skin surface lead to the epidermal targeting. By using glyceryl behenate, vitamine A-loaded nanoparticles can be prepared. The methods are useful for the improvement of penetration with sustained release.

Cosmeceuticals[68]

The SLNs have been applied in the preparation of sunscreens and as an active carrier agent for molecular sunscreens and UV blockers. The *in vivo* study showed that skin hydration will be increased by 31% after 4 weeks by addition of 4% SLN to a conventional cream. SLN and NLCs have proved to be controlled release innovative occlusive topicals.

Better localization has been achieved for vitamin A in upper layers of skin with glyceryl behenate SLNs compared to conventional formulations.

Potential agriculture application

Essential oil extracted from *Artemisia arboreseens* L when incorporated in SLN, were able to reduce the rapid evaporation compared with emulsions and the systems have been used in agriculture as a suitable carrier of ecologically safe pesticides. The SLN were prepared here by using compritol 888 ATO as lipid and poloxamer 188 or Miranol Ultra C32 as surfactant.

Targeted carrier for anticancer drug to solid tumors[69,70]

SLNs have been reported to be useful as drug carriers to treat neoplasms. Tamoxifen, an anticancer drug incorporated in SLN to prolong release of drug after i.v. administration in breast cancer and to enhance the permeability and retention effect. Tumour targeting has been achieved with SLNs loaded with drugs like methotrexate and camptothecin.

Breast cancer and lymph node metastases[71]

Mitoxantrone loaded SLN local injections were formulated to reduce the toxicity and improve the safety and bioavailability of drug. Efficacy of doxorubicin (Dox) has been reported to be enhanced by incorporation in SLNs. In the methodology the Dox was complexed with soybean-oil-based anionic polymer and dispersed together with a lipid in water to form Dox-loaded solid lipid nanoparticles. The system is enhanced its efficacy and reduced breast cancer cells.

Antitubercular chemotherapy[46]: Antitubercular drugs such as rifampicin, isonizide, pyrazinamide-loaded SLN systems, were able to decrease the dosing frequency and improve patient compliance. By using the emulsion solvent diffusion technique this anti tubercular drug loaded solid lipid nanoparticles are prepared. The nebulization in animal by incorporating the above drug in SLN also reported for improving the bioavailability of the drug.

Stealth nanoparticles[73,74]

These provide a novel and unique drug delivery system they evade quick clearance by the immune system. Theoretically, such nanoparticles can target specific cells. Studies with antibody labelled stealth lipobodies have

shown increased delivery to the target tissue in accessible sites. Stealth SLNs have been successfully tested in animal models with marker molecules and drugs.

Potential new adjuvant for vaccines [49]

Adjuvants are used in vaccination to enhance the immune response. The more safer new subunit vaccines are less effective in immunization and therefore effective adjuvants are required. Increase the amount of antigen delivered is not a solution because this also increases the costs. Especially with regard to the third world such a solution prohibits the desired broad vaccinations in these countries.

Conclusion

SLNs delivery can be an innovative way to administer molecules into the target site in a controlled manner by possibly overcoming or alleviating the solubility, permeability and toxicity problems associated with the respective drug molecules. High physical stability of these systems is another advantage. On the other hand the use of solid lipids as matrix material for drug delivery is well known from lipid pellets for oral drug delivery (Runge S et al., 1996). So SLNs is a new era technology which has been taken over by the pharmaceutical industry. The possibility of incorporating both the lipophillic and hydrophilic molecules and the possibility of the several administration make the SLNs delivery system all the more promising. SLNs will open a new channel for an effective delivery of a vast variety of drug molecules including analgesics, antitubercular, anticancerous, antiaging, antianxiety, antibiotics, and antiviral agents to the target site.

References

1. Scheffel, U. Rhodes, BA. Natajaran, TK. Wagner, HN. 'Albumin microspheres for study of the reticulo endothelial system', J Nucl Med, vol.13, 1970 pp.498-503.
2. Jumaa, M. Muller, BW. 'Lipid emulsions as a novel system to reduce the hemolytic activity of lytic agents: Mechanism of protective effect', Eur J Pharm Sci, vol.9, 2000 pp.285-90.
3. Cavalli, R. Caputo, O. Gasco, MR. 'Solid lipospheres of doxorubicin and idarubicin', Int J Pharm, vol.89, 1993 pp.9-12.
4. Gasco, MR. 'Method for producing solid lipid microspheres having a narrow size distribution', United states patent, US 188837; 1993.

5. Sunil Kamboj. Suman Bala and Anroop Nair, B. 'Solid Lipid Nanoparticles: An Effective Lipid Based Technology For Poorly Water Soluble Drugs', vol.5, no.2, 2010.

6. Muller, RH. Mäder, K. Gohla, S. 'Solid lipid nanoparticles (SLN) for controlled drug delivery—a review of the state of the art', Eur J Pharm Biopharm, vol.50, 2000 pp.161–177.

7. Muller, RH. Mehnert, W. Lucks, JS. Schwarz, C. Muhlen, AZ. Weyhers, H. Freitas, C. Ruhl, D. 'Solid lipid nanoparticles (SLN) — an alternative colloidal carrier systems for controlled drug delivery', Eur J Pharm Biopharm, vol.41, 1995; pp.62–69.

8. Muhlen, AZ. Schwarz, C. Mehnert, W. 'Solid lipid nanoparticles for controlled drug delivery - drug release and release mechanism', Eur J Pharm Biopharm, vol.45. 1998 pp.149 -155.

9. Allen, DD. Lockman, PR. Oyewumi, MO. Koziara, JM. Roder, KE. Mumper, RJ. 'Brain uptake of thiamine-coated nanoparticles', J Control Release, vol.93, 2003 pp.271–282.

10. Dingler, A. Feste. 'Lipid-Nanopatikel als kolloidale wirkstofftragersysteme zur dermalen Applikation', in PhD thesis. 1998: Berlin.

11. Cavalli, R. Bargoni, A. Podio, V. Muntoni, E. Zara, GP. and Gasco, MR. 'Duodenal administration of solid lipid nanoparticles loaded with different percentages of tobramycin', J Pharm Sci, vol.92, 2003 pp.1085–1094.

12. Tabatt, K. 'Transfection with different colloidal systems: comparison of solid lipid nanoparticles and liposomes', J Contr Rel, vol.97, 2004 pp.321-332.

13. Muller, RH. Radtke, M. Wissing, SA. 'Nanostructured lipid matrices for improved microencapsulation of drug', Int J Pharm, vol.242, 2002 pp.121-8.

14. Jenning, V. Gysler, A. 'Schafer-Korting M, Gohla S: Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin', Eur J Pharm Biopharm, vol.49. 2000 pp.211-8.

15. Diederichs, JE. Muller, RH. 'Liposomes in cosmetics and pharmaceutical products'. Pharm Ind, vol.56, no.3, 1994 pp.267–275.

16. Freitas, C. Muller, RH. 'Effect of light and temperature on zeta potential and physical stability in solid lipid nanoparticles (SLN) dispersions', Int J Pharm, vol.168, 1998 pp.221–229.

17.Gohla, SH. and Dingler, A. 'Scaling up feasibility of the production of solid lipidnanoparticles (SLN)', Pharmazie, vol.56, 2001 pp.61-63.

18.Chen, DB. Yang, TZ. Lu, WL. and Zhang, Q. 'In vitro study of two types of long circulating solid lipid nanoparticles containing paclitaxel', Chem & Pharm Bulletin, vol.49, 2001 pp.1444-1447.

19.Fundarò, A. 'Non-stealth and stealth solid lipid nanoparticles carrying doxorubicin: pharmacokinetics and tissue distribution after i.v. administration to rats. Pharmacological Research', vol.42, 2000 pp.337-343.

20.Vishvajit Kamble, A. Deepali, M. Jagdale and Vilasrao Kadam, J. 'SOLID LIPID NANOPARTICLES AS DRUG DELIVERY SYSTEM', International Journal of Pharma and Bio Sciences, vol.1, no.3, 2010

21.Radtke, M. Muller, RH. 'Comparison of structural properties of solid lipid nanoparticles (SLN) versus other lipid particles', Proc Int Symp Control Rel Bioact Mater, vol.27, 2000 pp.309- 10.

22.Uner, M. 'Preparation, characterization and physico-chemical properties of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC): their benefits as colloidal drug carrier systems', Pharmazie, vol.61, 2006 pp.375-86.

23.Muller, RH. Runge, SA. 'Solid lipid nanoparticles (SLN) for controlled drug delivery. In: Benita S, editor. Submicron emulsions in drug targeting and delivery', Amsterdam: Harwood Academic Publishers, 1998 pp.219-34.

24.Jenning, V. Gysler, A. Schafer-Korting, M. Gohla, S. 'Vitamin A loaded solid lipid nanoparticles for topical use: Occlusive properties and drug targeting to the upper skin', Eur J Pharm Biopharm, vol.49, 2000 pp.211-8.

25.Muller, RH. Radtke, M. Wissing, SA. 'Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations', Adv Drug Deliv Rev, vol.54, 2002 pp.131-55.

26. Pragati, S. Kuldeep, S. Ashok, S. Satheesh, M. 'Solid Lipid Nanoparticles: A Promising Drug Delivery Technology', International Journal of Pharmaceutical Sciences and Nanotechnology, vol.2, 2009.

27. ZHOU Shi-wen: New research on development of solid lipid nanoparticles. Journal of Medical Colleges of PLA, vol.22, no.6, 2007.

28.Muller, Maaben, S. Weyhers, H. Specht, F. Lucks, JS. 'Cytotoxicity of magnetite loaded polylactide, polylactide/glycolide particles and solid lipid nanoparticles (SLN)', Int J Pharm, vol.138, 1996 pp.85-94.

29.Maaben, S. Schwarz, C. Mehnert, W. Lucks, JS. Yunis-Specht, F. Muller, BW. Muller, RH. Comparison of cytotoxicity between polyester nanoparticles and solid lipid nanoparticles (SLN), Proc. Int Symp Control Release Bioact Mater, vol.20, 1993 pp.490-491.

30.Yang, SC. Lu, LF. Cai, Y. Zhu, JB. Liang, BW. Yang, CZ. 'Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain', J Control Release, vol.59, 1999 pp.299-307.

31.Fundaro, A. Cavalli, R. 'Bargoni A, Vighetto D, Zara GP, Gasco MR: Non-stealth and stealth solid lipid nanospheres carrying doxorubicin: pharmacokinetics and tissue distribution after i.v. administration to rats', Pharm Res, (1999) submitted.

32.Lippacher, A. Muller, RH and Mader, K. 'Investigation on the viscoelastic properties of lipid based colloidal drug carriers', Int J Pharm, vol.196, 2000 pp.227-230.

33. Muhlen, A and Mehnert, W. 'Drug release and release mechanism of prednisolone loaded solid lipid nanoparticles', Pharmazie, vol.53, 1998 pp.552-555.

34. Lander, R. Manger, W. Scouloudis, M. Davis, AKC and Lee, A. 'Gaulin homogenization: a mechanistic study', Biotech nol Prog, vol.16, 2000 pp.80-85.

35. Wolfgang Mehnert. Karsten Mader. 'Solid lipid nanoparticles Production, characterization and applications, Advanced Drug Delivery Reviews, vol.47, 2001 pp.165–196.

36.Siekmann, B. Westesen, K. 'Investigations on solid lipid nanoparticles prepared by precipitation in o/w emulsions', Eur J Pharm Biopharm, vol.43, 1996 pp.104-9.

37. Freitas, C, Mullera, RH. 'Spray-drying of Solid lipid nanoparticles (SLN TM)', Eur J Pharm Biopharm, vol.46, 1998 pp.145-51.

38. Cavalli, R. Marengo, E. 'Rodriguez L, Gasco MR: Effects of some experimental factors on the production process of solid lipid nanoparticles', Eur J Pharm Biopharm, vol.43, 1996 pp.110-5.

39.Chen, YJ. Jin, RX. Zhou, YQ. Zeng, J. Zhang, H. Feng, QR. 'Preparation of solid lipid nanoparticles loaded with Xionggui powder-supercritical carbon dioxide fluid extraction and their evaluation in vitro release', Zhongguo Zhong Yao Za Zhi, vol.31, 2006 pp.376-9.

40.Krishna Sailaja, A. Amareshwar, P. Chakravarty, P. 'Formulation of solid lipid nanoparticles and their applications', Current Pharma Research, vol.1, no.2, 2011 pp.197-203.

41. Gasco, MR. 'Solid lipid nanospheres from warm microemulsions', Pharm Tech Eur,1997 pp.52-8.

42. Boltri, L. Canal, T. Esposito, PA. Carli, F. 'Lipid nanoparticles: Evaluation of some critical formulation parameters', Proc Intern Symp Control Rel Bioact Mater, vol.20, 1993 pp.346-7.

43. De Labouret, A. Thioune, O. Fessi, H. Devissaguet, JP. Puiseieux, F. 'Application of an original process for obtaining colloidal dispersions of some coating polymers: Preparation, Charecterization, industrial scaling up', Drug Develop Ind Pharm, vol.21, 1995 pp.229-41.

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

45.Yang, S. Zhu, J. Lu, Y. Ling, B. Yang C. 'Body distribution of camptothecin solid lipid nanoparticles after oral administration', Pharm Res, vol.16, 1999 pp.751-7.

46. Pandey, R. Sharma, S. Khuller, GK. 'Oral SLN Based antitubercular chemotherapy', Tuberculosis (Edinb), vol.85, 2005 pp.415-20.

47. Meyer, E. Heinzelmann, H. 'Scanning force microscopy. In: Wiesendanger R, Guntherodt HJ, editors.Scanning tunneling microscopy II, Surface science. New York: Springer Verlag;1992 pp.99-149.

48.Drake, B. Prater, CB. Weisenhorn, AL. Gould, SAC. Albrecht, TR. Quate, CF. 'Imaging crystals polymers and process in water with the AFM', Science, vol.243, 1989 pp.1586-9.

49.Rainer Muller, H. Karsten Mader. Sven Gohla. 'Solid lipid nanoparticles (SLN) for controlled drug delivery-a review of the state of the art', European Journal of Pharmaceutics and Biopharmaceutics, vol.50, 2000 pp.161-177.

50.Utreja, S. Jain, NK. 'Solid Lipid Nanoparticles. Advances in Controlled and Novel Drug Delivery, CBS Publihers & Distributors, New Delhi, 2001 pp.408–425.

51.Chen, Y. Lu, LF. Cai, Y. Zhu, JB. Liang, BW. Yang, CZ. 'Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain', J Control Release, vol.59, 1999 pp.299–307.

52.Ahlin, P. Kristl, J. Kristl, A. Vrecer, F. 'Investigation of polymeric nanoparticles as carriers of enalaprilat for oral administration', Int J Pharm, vol.239, 2002 pp.113–120.

53.Manjunath, K. Venkateswarlu, V. 'Pharmacokinetics, tissue distribution and bioavailability of clozapine solid lipid nanoparticles after intravenous and intraduodenal administration', Journal of Controlled Release, vol.107, 2005 pp.215–228.

54.Venkateswarlu, V. Manjunath, K. 'Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles', Journal of Controlled Release, vol.95, 2004 pp.627– 638.

55.Muller, RH. Dingler, A. Weyhers, H. Zur Muhlen, A. Mehnert, W. 'Solid Lipid Nanoparticles (SLN)-ein neuartiger Wirkstoff-Carrier fur Kosmetika und Pharmazeutika. III. Langzeitstabilitat, Gefrierund Spruhtrocknung, Anwendung in Kosmetika und Pharmazeutika', Pharm Ind, 1997 pp.614-619.

56.Pinto, JF. Muller, RH. 'Pellets as carriers of solid lipid nanoparticles (SLNe) for oral administration of drugs', Die Pharmazie, 1999 pp.506-509.

57.Demirel, M. Yazan, Y. Muller, RH. Kilic, F. Bozan, B. 'Formulation and in vitro-in vivo evaluation of piribedil solid lipid particles', J Microencapsulation. 2000 submitted.

58.Runge, SA. Feste. 'Lipid-Nanopartikel (SLN) als kolloidaler ArzneistofftraÈger fuÈr Cyclosporin A', Ph.D. thesis, Free University of Berlin. 1998.

59.Penkler, L. Muller, RH. Runge, SA. Ravelli, V. 'Pharmaceutical cyclosporin formulation with improved biopharmaceutical properties, improved physical quality and greater stability, and method for producing said formulation', WO 99/56733 (1999).

60.Zur Muhlen, A. Mehnert, W. 'Drug release and release mechanism of prednisolone loaded Solid lipid nanoparticles', Pharmazie, vol.53, 1998; pp.552-5.

61.Rudolph, C. Schillinger, U. Ortiz, A. Tabatt, K. Plank, C. Muller, RH. 'Application of novel Solid lipid nanoparticles (SLN)- gene vector formulations based on a diametric HIV-1 VAT – peptide in vitro and in vivo', Pharmaceutic Res, vol.21, no.9, 2004 pp.1662-9.

62.Pedersen, N. Hansen, S. Heydenreich, AV. Kristensen, HG. Poulsen, HS. 'Solid lipid nanoparticles can effectively bind DNA, streptavidin and biotinylated ligands', Eur J Pharm Biopharm, vol.62, 2006 pp.155-62.

63.Mei, Z. Wu, Q. 'Triptolide loaded solid lipid nanoparticle hydrogel for topical application', Drug Develop Ind Pharm, vol.31, 2005 pp.161-8.

64.Chen, H. Chang, X. Du, D. Liu, W. Liu, J. Weng, T. 'Podophyllotoxin-loaded solid lipid nanoparticles for epidermal targeting', J Control Release, vol.110, 2006 pp.296-306.

65.Jenning, V. Schafer-Korting, M. Gohla, S. 'Vitamin A-loaded solid lipid nanoparticles for topicaluse: drug release properties', J Control Release, vol.66, 2000 pp.115-26.

66.Liu, J. Hu, W. Chen, H. Ni, Q. Xu, H. Yang, X. 'Isotretinoin-loaded solid lipid nanoparticles with skin targeting for topical delivery', Int J Pharm, vol.328, 2007 pp.191-5.

67.Santos, MC. Mehnert, W. Schaller, M. 'Drug targeting by solid lipid nanoparticles for dermal use', J Drug Target, vol.10, 2002 pp.489-95.

68.Wissing, S. Lippacher, A. Muller, R. 'Investigations on the occlusive properties of solid lipid nanoparticles (SLN)', J Cosmet Sci, vol.52, 2001 pp.313-24.

69.Murthy, RSR. 'Solid lipid nanoparticles as carriers for anti-cancer drugs to solid tumours', Drug Deliv, vol.12, 2005 pp.385-92.

70.Ruckmani, K. Sivakumar, M. Ganeshkumar, PA. 'Methotrexate loaded solid lipid nano particles (SLN) for effective treatment of carcinoma', J Nanosci Nanotechnol, vol.6, 2006 pp.2991-5.

71.Lu, B. Xiong, SB. Yang, H. Yin, XD. Chao, RB. 'Solid lipid nanoparticles of mitoxantrone for local injection against breast cancer and its lymphnode metastases', Eur J Pharm Sci, vol.28, 2006 pp.86-95.

72.Wong, HL. Rauth, AM. Bendayan, R. 'A new polymer-lipid hybrid nanoparticle system increases cytotoxicity of doxorubicin against multidrug-resistant human breast cancer cells', Pharm Res, vol.23, 2006 pp.1574-85.

73.Dvorak, HF. Wagy, AJ. Dvorak, JT. Dvorak, AM. 'Stealth liposomes', J Pathol, vol.133, 1998 pp.95-108.

74.Wang, Y. Wu, W. 'In situ evading of phagocytic uptake of stealth solid lipid nanoparticles by mouse peritoneal macrophages', Drug Deliv, vol.13, 2006 pp.189-92.

75.Cavalli, R. Gasco, MR. Morel, S. 'Behaviour of timolol incorporated in lipospheres in the presence of a series of phosphate esters', S.T.P. Pharma Sci, vol.2, 1992 pp.514-518.

76.Gasco, MR. Cavalli, R. Carlotti, ME. 'Timolol in lipospheres', Pharmazie, vol.47, 1992 pp.119-121.

77.Gasco, MR. Morel, S. Carpignano, R. 'Optimization of the incorporation of deoxycorticosterone acetate in lipospheres', J Pharm Biopharm, vol.38, 1992 pp.7-10.

78.Cavalli, R. Caputo, O. Gasco, MR. 'Solid lipospheres of doxorubicin and idarubicin', Int J Pharm, vol.89, 1993 pp.9-12.

79.Cavalli, R. Morel, S. Gasco, MR. Chetoni, P. Saettone, MF. 'Preparation and evaluation in vitro of colloidal lipospheres containing pilocarpine as ion pair', Int J Pharm, vol.117, 1995 pp.243-246.

80.Morel, S. Ugazio, E. Cavalli, R. Gasco, MR. 'Thymopentin in solid lipid nanoparticles', Int J Pharm, vol.132, 1996, pp.259-261.

81.Cavalli, R. Caputo, O. Carlotti, ME. Trotta, M. Scarnecchia, C. Gasco, MR. 'Sterilisation and. freeze-drying of drug-free and drugloaded solid lipid nanoparticles', Int J Pharm, vol.148, 1997 pp.47-54.

82.Cavalli, R. Peira, E. Caputo, O. Gasco, MR. 'Solid lipid nanoparticles as carriers of hydrocortisone and progesterone complexes with b-cyclodextrins', Int J Pharm, vol.182, 1999 pp.59-69.

83.Cavalli, R. Caputo, O. Gasco, MR. 'Preparation and characterization of solid lipid nanospheres containing paclitaxel', Eur J Pharm Sci, 2000 in press.

84.Jenning, V. Gysler, A. Schafer-Korting, M. Gohla, S. 'Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug penetration into porcine skin', Eur J Pharm Biopharm, 2000 in press.

85.Jenning, V. Schafer-Korting, M. Gohla, S. 'Vitamin A loaded solid lipid nanoparticles for topical application: drug release properties', J Control Release, 2000 in press.

Corresponding Author:

P.Shanmukhi*

Email: *shanmukhi.pharmacy@gmail.com*