

SOLID LIPID NANO PARTICLES: A NOVEL APPROACH IN DRUG DELIVERY

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ABSTRACT

Solid lipid nano particles are one of the promising drug delivery systems for poorly bioavailable drugs as well as for controlling the release of the drugs. In this review we discussed the applications, methods of manufacturing and methods of evaluation of solid lipid nanoparticles. In addition to that the article covers the applications of SLNs in transdermal, topical, gene vector carrier, targeting cancer cells in breast cancer and lymph node metastasis. The discussion includes sterilization of the dosage form and invitro drug release from the dosage form.

1.INTRODUCTION

Colloidal particles ranging in size between 10 and 1000 nm are known as nanoparticles. They are manufactured from synthetic/natural polymers and ideally suited to optimize drug delivery and reduce toxicity. Over the years, 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. However, the scarcity of safe polymers with regulatory approval and their high cost have limited the wide spread application of nanoparticles to regulatory approval and their high cost have limited the wide spread application of nanoparticles to clinical medicine (Scheffel,1970). To overcome these limitations of polymeric nanoparticles, lipids have been put forward as an alternative carrier, particularly for lipophilic pharmaceuticals. These lipid nanoparticles are known as solid lipid nanoparticles (SLNs), which are attracting wide attention of formulators world-wide.

SLN's are attracting major attention as novel colloidal drug carrier for intravenous administration (Mullet,1993). Solid lipid nanoparticles are submicron colloidal drug carriers which are composed of physiological lipid, dispersed in an aqueous surfactant solution. They are made up of solid hydrophobic core having a monolayer of phospholipid coating. The solid core contains drug dissolved or dispersed in the solid high melting fat matrix. The hydrophobic chains of phospholipids are embedded in the fat matrix. They have potential for carrying lipophilic or hydrophilic drugs (Domb,1993).

Advantages of SLN's over other polymer nanoparticles, liposomes, lipid emulsions:

- Small size and relatively narrow size distribution
- Control release of active drug over a long period
- Protection of incorporated drug against chemical degradation
- Possible sterilization by autoclaving or gamma irradiation
- No toxic metabolites are produced
- Avoidance of organic solvents
- Cheaper and stable
- Ease of industrial scale production by hot dispersion techniques
- increase in bioavailability and are biocompatible

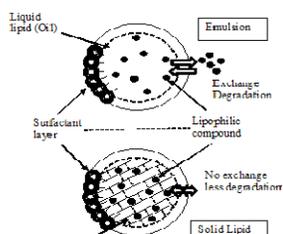


Figure 1: Solid lipid nanoparticle containing lipophilic drug in lipid core comparing with emulsion

Table1: Comparative study of properties of SLN's, polymer nanoparticles, liposomes, lipid emulsions (Schwarz,1994):

S.No	Property	SLN	Polymer nanoparticles	Liposomes	Lipid emulsions
1	Systemic toxicity	Low	Low	Low	Low
2	Cytotoxicity	Low	≥ to SLN	Low	Low
3	Residues from organic solvents	No	Yes	May or may not	No
4	Large scale production	No	No	Yes	Yes
5	Sterilization	Yes	No	Yes	No

Table2: Lipids and emulsifiers used for preparation of SLN'S (Sunil Kamboj,2010):

Lipids	Hard fats	Emulsifiers/co emulsifiers
Mineral oils	Witepsol W 35, S35, H 42, E 85	Soy lecithin
sucrose poly esters	Glyceryl monostearate	Egg lecithin
Triglycerides	Glyceryl behenate	Phosphatidyl choline
Tricaprin	Glyceryl palmitostearate	Poloxamer 188, 182, 407, 908.
Trilaurin	Cetyl palmitate	Tyloxapol
Trimyristin	Stearic acid	Polysorbate 20
Triopalmitin	Palmitic acid	Polysorbate 60
Tristearin	Deconic acid	Polysorbate 80
Diglycerides	Behenic acid	Sodium cholate
Fatty acids	Acidan N 12	Sodium glycocholate

Methods of preparation:

High shear homogenization: High shear homogenization technique was initially used for the solid lipid nanodispersions (Domb,1993). HSH method is used to produce SLN by melt emulsification. Homogenization is a fluid mechanical process that involves the subdivision of droplets or particles into micro- or nanosize to create a stable emulsion or dispersion. High pressure homogenizers push a liquid with high pressure (100–2000 bar) through a narrow gap (in the range of few microns) lipids used in this study include trimyristin, tripalmitin, a mixture of mono, di and triglycerides (Witepsol W35, Witepsol H35) with glyceryl behenate and poloxamer 188 used as stearic stabilizers (0.5% w/w). HPH method involves 2 processing procedures (Mukherjee). They are

a. Hot homogenization, b. Cold homogenization

a. Hot homogenization: This is applied to lipophilic and insoluble drugs. This technique does not suit for hydrophilic drugs into SLN because of higher partition of drug in water. Hot homogenization is carried out at temperatures above the melting point of the lipid and can therefore be regarded as the homogenization of an emulsion. Usually, lower particle sizes are obtained at higher processing temperatures because of lowered viscosity of the lipid phase (Lander,2000), although this might also accelerate the drug and carrier degradation. Better products are obtained after several passes through the high-pressure homogenizer (HPH), typically 3-5 passes. High pressure processing always increases the temperature of the sample (approximately 10° at 500 bars) (Jahnke,1998). In most cases, 3-5 homogenization cycles at 500-1500 bar are sufficient. Increasing the homogenization leads to an increase of the particle size due to particle coalescence, this occurs because of the high kinetic energy of the particles.

b. Cold homogenization: Cold homogenization technique is used for hydrophilic drugs. If the drugs have low aqueous solubility in the melted lipid, then surfactants can be used for solubilization of the drug. The solid particles are dispersed in an aqueous surfactant solution at a temperature below the lipid melting point, forming a 'pre-suspension'. The pre-suspension is then subjected to HPH below the lipid melting temperature to reduce the solid particle size. The advantage of this method is avoidance of or minimizes the melting process of lipid and hence it is suitable for thermo sensitive and thermo labile drugs relative to hot HPH, Cold HPH generally produces larger mean particle sizes and broader particle size distributions (Mehnert and Mader,2001).

Microemulsion based method: SLN's can be produced by micro emulsification method of molten lipids as the internal phase, and the subsequent dispersion of the microemulsion in an aqueous medium under mechanical stirring. They are made by stirring an optically transparent mixture at 65-70°C 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 mono octyl phosphate) and water. The hot microemulsion is dispersed in cold water under stirring. Typical volume ratios of the hot microemulsion to cold water are in the range of 1:25 to 1:50. Nanoparticles were produced only with solvents which distribute very rapidly into the aqueous phase (acetone), while larger particle sizes were obtained with more lipophilic solvents. The dilution process is critically determined by the composition of the microemulsion. According to the literature (Gasco,1997; Boltri,1993) the droplet structure is already contained in the microemulsion and therefore, no energy is required to achieve submicron particle size. The hydrophilic co-solvents of the microemulsion might play similar role in the formation of lipid nanoparticles as the acetone for the formation of polymer nanoparticles.

Multiple microemulsification: Multiple microemulsification is also used for production of SLN's. For the preparation of hydrophilic loaded SLN, a novel method based on solvent emulsification-evaporation has been used (Cortesi,2002). Here the drug is encapsulated with a stabilizer to prevent drug partitioning to external water phase of w/o/w double emulsion. But it has inherent instabilities due to coalescence of the internal aqueous droplets with in the oil phase, coalescence of droplets and rupture of the oil layer on the surface of the internal droplets (Florence and Whitehill,1982).

Journal of Chemical and Pharmaceutical sciences

SLN preparation by using supercritical fluid: This is a relatively new technique for SLN production and has the advantage of solvent-less processing (Chen,2006; Kaiser,2001). 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 method. Carbon dioxide (99.99%) was the good choice as a solvent for this method (Gosselin,2003).

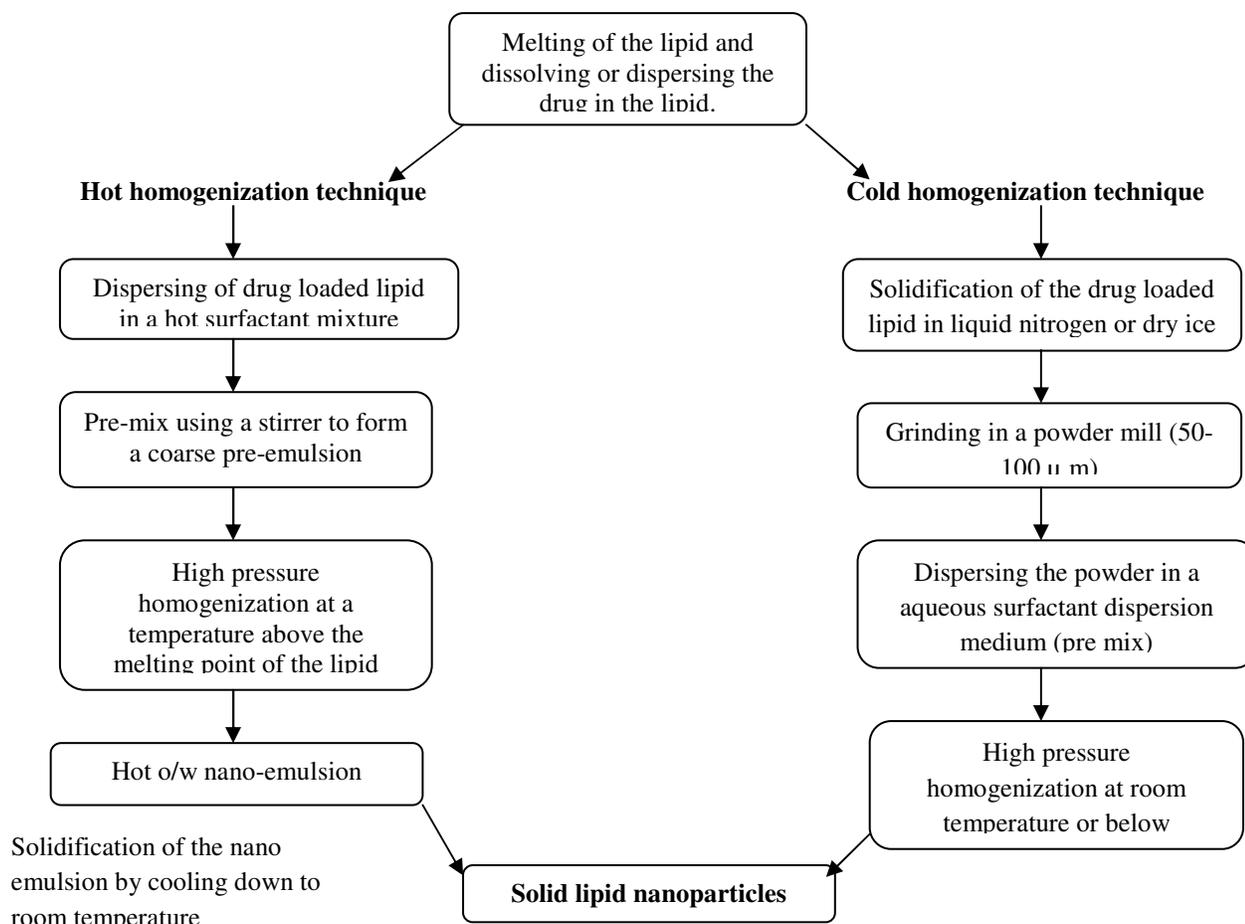


Figure 2: Flow chart diagram showing different steps in cold homogenization and hot homogenization techniques in the manufacturing of solid lipid nanoparticles

Spray drying method: It is 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 Muller (1998) recommends the use of lipid with melting point $>70^{\circ}$ for spray drying. The best result was obtained with SLN concentration of 1% in a solution of Trehalose in water or 20% Trehalose in ethanol-water mixtures (10:90% v/v).

Characterization solid lipid nanoparticles: Characterization of solid lipid nanoparticles is prerequisite for quality control.

The parameters to be characterized are

- Particle size, size distribution kinetics (zeta potential)
- Degree of crystallinity
- 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
- Surface morphology

The particle size/size-distribution may be studied using

- photon correlation spectroscopy (PCS)
- transmission electron microscopy (TEM)

Journal of Chemical and Pharmaceutical sciences

- scanning electron microscopy (SEM)
- atomic force microscopy (AFM)
- scanning tunneling microscopy (STM)
- freeze fracture electron microscopy (FFEM)

Rheology: SLN particles exhibit non Newtonian flow. This can be studied with the help of Von Brookfield viscometer.

Lipophilic Loading in lipid particles: Drugs, including agents for treating cancer, AIDS, fungal infections, high blood pressure, mental illness, skin disease, and imaging have been loaded into solid lipid nanoparticles. For efficiency and efficacy reasons, the amount of drug that can be loaded is very important. Calculated as the ratio of drug weight to the sum of drug and lipid weight, loading capacity typically ranges from 1-5% (Muller,2000).

Using HPH, Westesen obtained loading capacities up to 50% for Ubidecarenone, 20% for Tetracaine and etomidate, and 25% for cyclosporine (Schwarz and Mehnert; Westesen1997; Iscan,1999; Schwarz,1995). For HPH, Muller suggests that capacity is determined by the drug solubility in the melted lipid, the miscibility of the melted drug and melted lipid, and the physicochemical structure of the solid lipid.

Protein incorporation in SLN: The SLN production is based on solidified emulsion (dispersed phase) technologies. Therefore, due to their hydrophilic nature most proteins are expected to be poorly microencapsulated into the hydrophobic matrix of SLN, tending to partition in the water phase during the preparation process, which is further, enhanced by the use of surfactants as emulsion stabilizers. In addition, SLN can present an insufficient loading capacity due to drug expulsion after polymorphic transition during storage, particularly if the lipid matrix consists of similar molecules. However, lipids are versatile molecules that may form differently structured solid matrices, such as the nanostructured lipid carriers (NLC) and the lipid drug conjugate nanoparticles (LDC), that have been created to improve drug loading capacity (reviewed by Wissing and Muller (2004), Since the mid 1990's, authors have regularly published promising results concerning the incorporation of several peptides and proteins in solid lipid particulate carriers. Therapeutically relevant peptides (e.g. calcitonin, cyclosporine A, insulin LHRH, somatostatin), protein antigens (e.g. hepatitis B and malaria antigens) and model protein drugs (e.g. bovine serum albumin and lysozyme) have been investigated for incorporation into SLN form.

Drugs can be incorporated into SLN's

Compound	Clinical use
Timolol	High blood pressure and recurrent heart diseases
Decorticosterone	Hormone replacement therapy
Doxorubicin	Various cancers
Idarubicin	Leukemia
Pilocarpine	Glaucoma
Thymopentin	AIDS
Diazepam	Anxiety and epilepsy
Hydrocortisone	Asthma, skin conditions
Paclitaxel	Ovarian, breast cancers
Cortisone	Inflammation and arthritis
Magnetite	Magnetic targeting
Nimesulide	Anti aging
Tetracaine	Ophthalmic treatment
Acyclovir	Viral infections
Cyclosporine	Prophylaxis in organ transplants
Coenzyme Q 10	Cardiovascular diseases
Prednisolone	Inflammation

Determination of incorporated drug: It is of prime importance to measure the amount of incorporated drug in SLN. Since it influence the release characteristics. The amount of the drug incorporated encapsulated per unit weight of the nanoparticles is determined after separation of the free drug and solid lipids from aqueous medium. This separation can be carried out using ultracentrifugation, centrifugation filtration or gel permeation chromatography.

In the centrifugation filtration the filters such as ultra free –MC (Millipore) or ultras art -10 (Sartorius) are used along with classical centrifugation techniques. The degree of encapsulation can be assessed indirectly by determining the amount of drug remaining in the supernatant after centrifugation or filtration of SLN suspension or alternatively by dissolution of the sediment in an appropriate solvent and subsequent analysis.

Journal of Chemical and Pharmaceutical sciences

Standard analytical techniques such as spectrophotometry, spectrofluorophotometry, HPLC, or liquid scintillation can be used to assay the drug (Magenhei and Benita,1991). In gel permeation chromatography Sephadex^R and Sepharose^R gels are used for the removal of free drug from SLN preparations.

Sterilization of solid lipid nanoparticles: For parenteral administration, sterility of SLN is of utmost importance. Aseptic production, filtration, gamma irradiation and autoclaving are commonly used to achieve sterilization. Filterative sterilization of the dispersed system needs high pressure and is not applicable to nanoparticles. However the filtration method using nylon66 UTTIPOR^R 0.45mm membrane with CWST (critical water surface tension) value>80dynes/cm may be used if mean size is less than 0.4nm. Such membranes can be obtained by treating nylon66 membrane with acrylates possessing –OH groups in their side change.

Aseptic procedures can be applied in order to produce sterile SLN's. But they are very complex and expensive. Gamma sterilization has been chosen, but this treatment has been reported to cause unacceptable breakdown of bilayer components in phospholipid based drug carriers such as liposomes. The most popular and convenient method is sterilization by autoclaving at 121° for at least 15 min. But there are some disadvantages like high temperature reached during sterilization cause a hot o/w microemulsion to form in the autoclave and hence change in size of the hot particles on subsequent slow cooling, the SLN reform but nanoparticles may coalesce producing larger SLN than the initial ones. During heat sterilization chemical composition of drug may also undergo changes and the incorporated drug leaks out of the carrier, hence it is essential to study the stability of SLN's following sterilization.

Stability: The shelf life or stability of SLNs can be very good. Lipids can be chosen that do not hydrolyze in aqueous suspension. The very small particle size and density close to unity of SLN's means the gravity has little effect on particles in dispersion and Brownian motion is sufficient to maintain colloidal dispersions without creaming or sedimentation. For SLN's made with natural lipids and not made by an aseptic process, they can be prepared with long term stability against biological growth using standard preservatives when tolerable.

Stealth nanoparticles: 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 labeled 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 (Wang and Wu,2006).

Invitro drug release studies: *In-vitro* drug release studies are used for quality control studies as well as for the prediction of *in-vivo* kinetics. In this SLN's due to very small size of the particles, the release rate observed in-vivo can differ greatly from the release obtained in buffer solution. Hence *in-vitro* release studies remain useful for quality control as well as for evaluation of influence of process parameters on release rate of active components.

Release rate of SLN's dispersion is carried in dialysis tubing or without tubing. In dialysis, the SLNs dispersion is introduced into prewashed dialysis tubing, which is then hermetically sealed. The dialysis sac is dialyzed against dissolution medium at constant temperature with continuous stirring. The released drug diffuses through the dialysis membrane. Samples from dissolution medium are taken at specified time intervals, centrifuged and assayed for drug content. The sink conditions are maintained during release studies. Washington(1989) proposed this method and claimed that perfect sink conditions are not maintained during release studies, since SLN dispersions are not directly diluted in the dissolution medium. As a result, the rate of appearance of drug in the dissolution medium does not reflects its real release rate, but rather the concentration gradient between the continuous phase of the SLN dispersion medium and the dissolution medium.

Levy and Bentia (1990) reported a technique which avoids the enclosure of the colloidal drug carrier in a dialysis sac. This technique is based on REVERSE DIALYSIS. In this technique the SLN dispersion is directly diluted in the release medium, so that the perfect sink conditions are maintained. A number small dialysis sacs containing buffer are then suspended in the release medium for the monitoring of the amount of drug released. Disadvantage of this method is it is not sensitive enough to characterize rapid release rate of drug from colloidal carrier.

Applications:

Transdermal application: The smallest particle sizes are observed for SLN dispersions with low lipid content (up to 5%). Both the low concentration of the dispersed lipid and the low viscosity are disadvantageous for dermal administration. In most cases, the incorporation of the SLN dispersion in an ointment or gel is necessary in order to achieve a formulation which can be administered to the skin. The incorporation step implies a further reduction of the lipid content of the SLN dispersion resulting in semisolid, gel-like systems, which might be acceptable for direct application on the skin (Prow,2006).

SLNs as gene vector carrier: SLN can be used in the gene vector formulation (Rudolph). 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 acids (Hayes,2006; Pedersen,2006). The lipid nucleic acid nanoparticles were prepared from a liquid anaphase containing water and a water miscible organic solvent where both lipid and DNA are separately dissolved by removing the organic

Journal of Chemical and Pharmaceutical sciences

solvent, stable and homogeneously sized lipid-nucleic acid nanoparticle (70-100 nm) were formed. It is called genospheres. It is targeted specific by insertion of an antibody-lipo polymer conjugated in the particle.

SLNs for topical use: SLNs and NLCs have been used for topical application for various drugs such as tropolide (Lai,2006), imidazole, antifungals, anticancers (Mei and Wu2005), vitamin A (Chen,2006), isotretinoin (Jenning,2000), ketoconazole (Liu,2007), DNA (Choi,2006), flurbiprofen (Jain,2005) and glucocorticoids (Santos,2002). The penetration of phodphyllotoxin-SLN into stratum corneum along with skin surface leads to the epidermal targeting (Lai,2006). By using glyceryl behenate, vitamin A-loaded nanoparticles can be prepared. The methods are useful for the improvement of penetration with sustained release (Wong,2006). The isotretinoin-loaded lipid nanoparticles were formulated for topical delivery of drug. The soya bean lecithin and Tween 80 are used for the hot homogenization method for this. The methodology is useful because of the increase of accumulative uptake of isotretinoin in skin (Pandey,2005). Polyacrylamide, glycerol and water were used for the preparation of this type of SLN gel (Wang and Wu,2006).

SLN and NLC are very attractive colloidal carrier systems for skin applications due to their various desirable effects on skin besides the characteristics of a colloidal carrier system. They are well suited for use on damaged or inflamed skin because they are based on non-irritant and non-toxic lipids (Wissing and Muller,2003). Researchers have reported intensively on the topical application of SLN. During the last few years, SLN and NLC have been studied with active compounds such as vitamin E (Dingler,1999), tocopherol acetate (Wissing and Muller,2001), retinol (Jenning,2000), ascorbyl palmitate (Uner,2005), clotrimazole (Souto,2004), triptolide (Mei,2003), phodphyllotoxin (Chen,2006) and a nonsteroidal antiandrogen RU 58841 (Munster,2005) for topical application.

SLNs as cosmeceuticals: 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 (Wissing and Muller,2001). SLN and NLCs have proved to be controlled release innovative occlusive topical (Mei and Wu,2005). Better localization has been achieved for vitamin A in upper layers of skin with glyceryl behenate SLNs compared to conventional formulations (Wissing,2001).

SLNs as a targeted carrier for anticancer drug to solid tumors: SLNs have been reported to be useful as drug carriers to treat neoplasms (Lai,2006). 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 (Wong,2006). Tumor targeting has been achieved with SLNs loaded with drugs like methotrexate (Dvorak,1998) and camptothecin (Wang and Wu,2006).

SLNs in breast cancer and lymph node metastases: 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 has enhanced its efficacy and reduced breast cancer cells.

Oral SLNs in antitubercular chemotherapy: Antitubercular drugs such as rifampicin, isoniazid, pyrazinamide-loaded SLN systems, were able to decrease the dosing frequency and improve patient compliance (Pandey,2005). 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 (Dvorak,1998).

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