

SOLID LIPID NANOPARTICLES: A MODERN FORMULATION APPROACH IN DRUG DELIVERY SYSTEM

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<p>*For Correspondence: MET's Bhujbal knowledge city, Institute of Pharmacy, Adgaon, Nasik, Maharashtra, India.</p>	<p>ABSTRACT Solid lipid nanoparticles were developed as an alternative to other traditional colloidal carriers like liposomes, polymeric nanoparticles and emulsions as they have advantages like controlled drug release and targeted drug delivery with increased stability. Solid lipid nanoparticles are at the forefront of the rapidly developing field of nanotechnology with several potential applications in drug delivery, clinical medicine and research as well as in other varied sciences. Hence, solid lipid nanoparticles hold great promise for reaching the goal of controlled and site-specific drug delivery and hence have attracted wide attention of researchers. This paper gives an overview about advantages, disadvantages of solid lipid nanoparticles, excipients and all the different methods of preparation, Aspects of SLN stability. KEY WORDS: Solid lipid nanoparticles, colloidal drug carriers, homogenization, Surfactants.</p>
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INTRODUCTION

S LNs introduced in 1991 represent an alternative and better carrier system to traditional colloidal carriers such as emulsions, liposomes and polymeric micro and nanoparticles. (Ekambaram et al., 2012). Nanoparticles made from solid lipids are attracting major attention as novel colloidal drug carrier for intravenous applications as they have been proposed as an alternative particulate carrier system. The system consists of spherical solid lipid particles in the nanometer ranges, which are dispersed in water or in aqueous surfactant solution. Generally, they are made of solid hydrophobic core having a monolayer of phospholipids coating. The solid core contains the 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 to carry lipophilic or hydrophilic drugs or diagnostics (Shah *et al.* 2011). 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 clinical medicine (Scheffel *et al.*, 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 (Jumaa *et al.* 2000). SLNs combine the advantages and avoid the drawbacks of several colloidal carriers of its class such as physical stability,

protection of incorporated labile drugs from degradation, controlled release, excellent tolerability (Sarathchandiran *et al.*, 2012). SLN formulations for various application routes (parenteral, oral, dermal, ocular, pulmonar, rectal) have been developed and thoroughly characterized in vitro and in vivo (Mulla *et al.*, 2010).

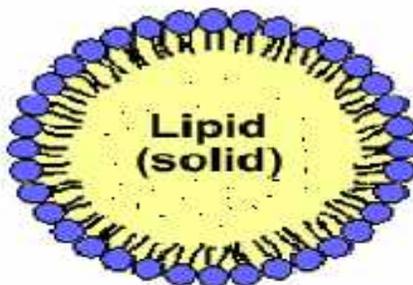


Fig. 1: Structure of solid lipid nanoparticle (SLN)

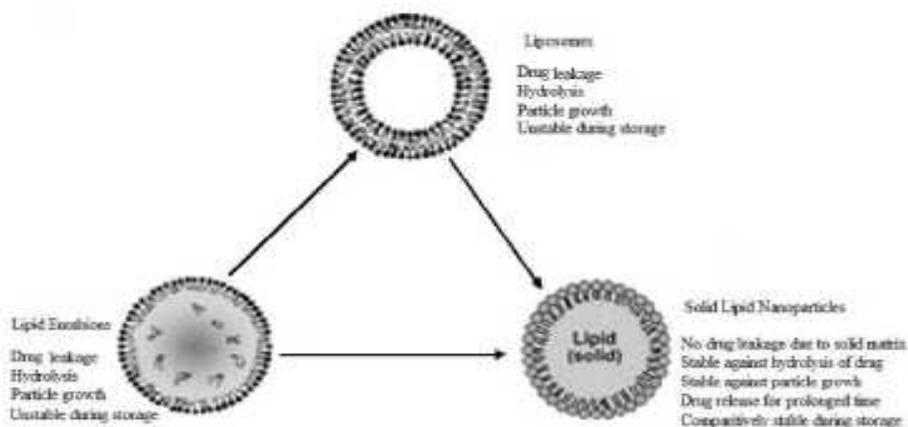


Fig. 2: A diagrammatic representation on SLN over emulsions and liposomes

Advantages of SLN (Garud *et al.* 2012):

1. Use of biodegradable physiological lipids which decreases the danger of acute and chronic toxicity and avoidance of organic solvents in production methods
2. Improved bioavailability of poorly water-soluble molecules
3. Site specific delivery of drugs, enhanced drug penetration into the skin via dermal application
4. Possibility of scaling up.
5. Protection of chemically labile agents from degradation in the gut and sensitive molecules from outer environment
6. SLNs have better stability compared to liposomes
7. Enhance the bioavailability of entrapped bioactive and chemical production of labile incorporated compound.

Disadvantages of SLN:

1. Poor drug loading capacity.
2. Drug expulsion after polymeric transition during storage.
3. Relatively high-water content of the dispersions (70-99.9%).

Solid lipid nanoparticles excipients:

The major problem for the SLNs to be introduced to the market is the use of excipients having no accepted status. For topical SLN, all excipients used in current topical cosmetic and dermal pharmaceutical products can be used. For oral administration of SLN, all excipients can be employed that are frequently used in traditional oral dosage forms such as tablets, pellets, and capsules. Even surfactants with cell membrane-damaging

potential, e.g. SDS, can be used. SDS is contained in many oral products and accepted as excipients by the regulatory authorities. In addition, substances with accepted Generally Recognized as Safe (GRAS) status can be used. The situation is different for parenteral administration as solid lipids have not yet been administered parenterally before-in contrast to liquid lipids (o/w emulsions for iv administration, prolonged release oil-based injectables for im administration). However, the glycerides used for SLN production are composed of compounds (glycerol, fatty acids) which are also present in emulsions for Parenteral nutrition (Yadav *et al.*, 2013). The general excipients used in any SLN formulation are solid lipids, emulsifiers, co-emulsifiers and water. The term lipid is used here in a broader sense and includes triglycerides (e.g. tristearin), partial glycerides (e.g. Imwitor), fatty acids (e.g. stearic acid), steroids (e.g. cholesterol) and waxes (e.g. cetyl palmitate). All classes of emulsifiers (with respect to charge and molecular weight) have been used to stabilize the lipid dispersion. It has been found that the combination of emulsifiers might prevent particle agglomeration more efficiently (Mehnert *et al.* 2001).

Table 1: Shows list of excipients used in SLN preparation (Verma *et al.* 2016)

LIPIDS	SURFACTANTS and CO-SURFACTANTS
Triacylglycerols: Tricaprin Trilaurin Trimyrustin Tripalmitin, Tristearin	Phospholipids: Soy lecithin Egg lecithin Phosphatidylcholine
Acylglycerols: Glycerol monostearate Glycerol behenate Glycerol palmitostearate	Ethylene oxide/propylene oxide copolymers: Poloxamer 188 Poloxamer 182 Poloxamer 407 Poloxamine 908
Fatty acids: Stearic acid Palmitic acid Decanoic acid Behenic acid	Sorbitan ethylene oxide/propylene oxide copolymers: Polysorbate 20 Polysorbate 60 Polysorbate 80
Waxes: Cetyl palmitate	Alkylaryl polyether alcohol polymers: Tyloxapol
Cyclic complexes: Cyclodextrin	Bile salts: Sodium cholate Sodium glycocholate Sodium taurocholate Sodium taurodeoxycholate Taurocholic acid sodium salt
Hard fat types: Witepsol W 35 Witepsol H 35	Alcohols: Ethanol Butanol (11)

Methods of preparation of solid lipid nanoparticles (Mehnert *et al.*, 2001).

1. High pressure homogenization
 - a. Hot homogenization
 - b. Cold homogenization
2. Ultrasonication /high speed homogenization
 - a. Probe ultrasonication
 - b. Bath ultrasonication
3. Solvent evaporation Method
4. Solvent emulsification-diffusion method
5. Supercritical fluid method
6. Microemulsion based method
7. Double emulsion method
8. Precipitation technique

9. Film-ultrasound dispersion
10. Solvent Injection Technique

11. Using Membrane Contractor

1. High pressure homogenization (HPH)

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. 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.

Table: 2 Hot Homogenization and Cold homogenization (Jaiswal *et al.*, 2013)

Steps	Hot Homogenization Technique	Cold Homogenization Technique
Step 1.	Melt lipid; dissolve or solubilize active ingredients in the lipid.	
Step 2.	Disperse melted lipid in hot aqueous surfactant solution.	Cooling and recrystallization of the active lipid mixture using liquid nitrogen or dry ice.
Step 3.	Preparation of a pre-emulsion by means of a rotorstator homogenizer.	Milling of the active lipid mixture by means of a ball mill or a jet mill.
Step 4.	High-pressure homogenization above the melting point of the lipid.	Disperse lipid microparticles in cold aqueous surfactant solution.
Step 5.	Cooling and recrystallization.	High-pressure homogenization at or below room temperature.

2] Ultrasonication or High-Speed Homogenization

This ultrasonication technique is a dispersing technique, which was initially used for the production of solid lipid nanodispersion. Ultrasonication based on the mechanism of cavitation. In first step, the drug was added to previously melt solid lipid. In second step, the heated aqueous phase (heated to same temperature) was added to the melted lipid and emulsified by probe sonication or by using high speed stirrer or aqueous phase added to lipid phase drop by drop followed by magnetic stirring. The obtained pre-emulsion was ultrasonicated using probe sonicator with water bath (at 0°C). In order to prevent recrystallization during the process, the production temperature kept at least 5°C above the lipid melting point. The obtained nanoemulsion (o/w) was filtered through a 0.45µm membrane in order to remove impurities carried in during ultrasonication (Eldem *et al.* 1991). Then they obtained SLN is stored at 4°C. To increase the stability of the formulation, was lyophilized by a lyophilizer to obtain freeze-dried powder and sometime mannitol (5%) was added into SLNs as cryoprotector.

3] Solvent evaporation Method

In solvent emulsification-evaporation method, the lipophilic material and hydrophobic drug were dissolved in a water immiscible organic solvent (e.g. cyclohexane, dichloromethane, toluene, chloroform) and then that is emulsified in an aqueous phase using high speed homogenizer. To improve the efficiency of fine emulsification, the coarse emulsion was immediately passed through the microfluidizer. Thereafter, the organic solvent was evaporated by mechanical stirring at room temperature and reduced pressure (e.g. rotary evaporator) leaving lipid precipitates of SLNs (Siekmann *et al.* 1996). Here the mean particle size depends on the concentration of lipid in organic phase. Very small particle size could be obtained with low lipid load (5%) related to organic solvent.

4] Solvent emulsification-diffusion method

In solvent emulsification-diffusion technique, the solvent used (e.g. benzyl alcohol, butyl lactate, ethyl acetate, isopropyl acetate, methyl acetate) must be partially miscible with water and this technique can be carried out either in aqueous phase or in oil. Initially, both the solvent and water were mutually saturated in order to ensure the initial thermodynamic equilibrium of both liquid. When heating is required to solubilize the lipid, the saturation step was performed at that temperature. Then the lipid and drug were dissolved in water saturated solvent and this organic phase (internal phase) was emulsified with solvent saturated aqueous

solution containing stabilizer (dispersed phase) using mechanical stirrer. After the formation of o/w emulsion, water (dilution medium) in typical ratio ranges from 1:5 to 1:10, were added to the system in order to allow solvent diffusion into the continuous phase, thus forming aggregation of the lipid in the nanoparticles. Here the both the phase were maintain at same elevated temperature and the diffusion step was performed either at room temperature or at the temperature under which the lipid was dissolved. Throughout the process constant stirring was maintained. Finally, the diffused solvent was liminated by vacuum distillation or lyophilization (Rabinarayan *et al.*2010).

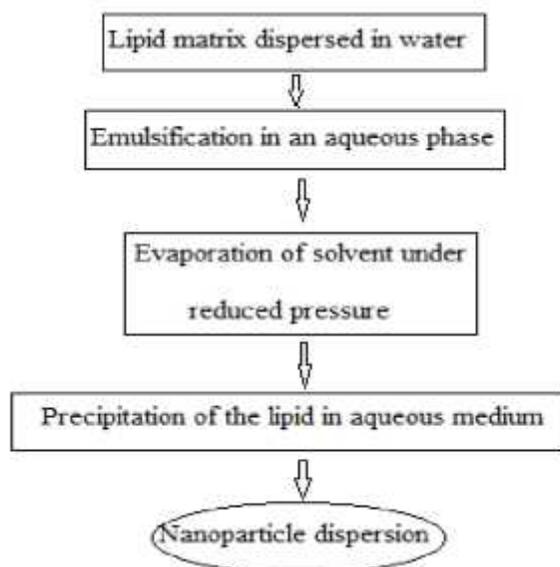


Fig.: 3 Systematic representation for emulsification-diffusion method

5] Supercritical fluid method

This is a novel technique which recently applied for the production of SLNs. A fluid is qualified as supercritical when its pressure and temperature exceed their respective critical value. Above the critical temperature, it is not possible to liquefy a gas by increasing the pressure. The supercritical fluid has unique thermo-physical properties. As the pressure is raised, the density of the gas increases without significant increase in viscosity while the ability of the fluid to dissolve compounds also increases. A gas may have little to no ability to dissolve a compound under ambient condition can completely dissolve the compound under high pressure in supercritical range. Therefore, its solvation power is altered by careful control of changes in temperature and pressure. Many gases like, CO₂, ammonia, ethane and CH₂FCF₃ were tried, but CO₂ is the best option for SCF technique because, it is generally regarded as safe, easily accessible critical point [31.5°C, 75.8 bar), does not causes the oxidation of drug material, leaves no traces behind after the process, is inexpensive, noninflammable, environmentally acceptable an easy to recycle or to dispose off. In the SCF phase or this technique generally use organic solvents (e.g. DMSO, DMFA) because they are fully miscible in SCF-CO₂. This technology comprises several processes for nanoparticles production such as rapid expansion of supercritical solution (RESS), particles from gas saturated solution(PGSS), gas/supercritical antisolvent (GAS/SAS), aerosol solvent extraction solvent (ASES), solution enhanced dispersion by supercritical fluid (SEDS), supercritical fluid extraction of emulsions (SFEE). Mainly SAS and PGSS were used for SLN preparation.

a. GAS/SAS

In this process SCF acts as antisolvent for processing solid that are insoluble in SCF. It exploits the ability of SCF to dissolve in organic solvent and reduce the solvation power of solid in solution, thus causing the solid to precipitate. At first, the near critical or supercritical fluid was introduced in a vessel containing an organic solvent in which the solid material to be crystallized was dissolved which causes the intimate mixing of the fluid and liquid resulting in liquid expansion and particle precipitation. A clear disadvantage of this technique is the lack of control on the particle formation. A modification of SAS technique was used to prepare lysozyme

spherical nanoparticles, which combines both the atomization and anti-solvent process, by using water/ethanol solution.

b. PGSS

In this process, the SCF was dissolved in liquid substrate, or a solution of substrate in solvent, or a suspension of substrate in solvent followed by a rapid depressurization of this mixture through a nozzle causing the formation of SLN. The great advantage of this process is that it produces particles of great variety of substance that need not be soluble in SCF-CO₂. Limitations are, care must be taken for thermolabile solute and the final product may contain microparticles. Insulin nanoparticles are produced by this process, in which the solvent used, was DMSO and the lipid mixture (tristearin, phosphatidylcholine, dioctylsulfosuccinate) were atomized to produce insulin SLN (<500nm) (Ramteke *et al.*2012).

6] Microemulsion based method

This method is based on the dilution of microemulsions. As micro-emulsions 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°C, which typically composed of a low melting fatty acid (e.g. stearic acid), an emulsifier (e.g. polysorbate 20), co-emulsifiers (e.g. butanol) and water. The hot microemulsion is dispersed in cold water (2-3°C) under stirring. SLN dispersion can be used as granulation fluid for transferring in to solid product (tablets, pellets) by granulation process, but in case of low particle content too much of water needs to be removed. High-temperature gradients facilitate rapid lipid crystallization and prevent aggregation. Due to the dilution step; achievable lipid contents are considerably lower compared with the HPH based formulations (Ekambaram *et al.*2012).

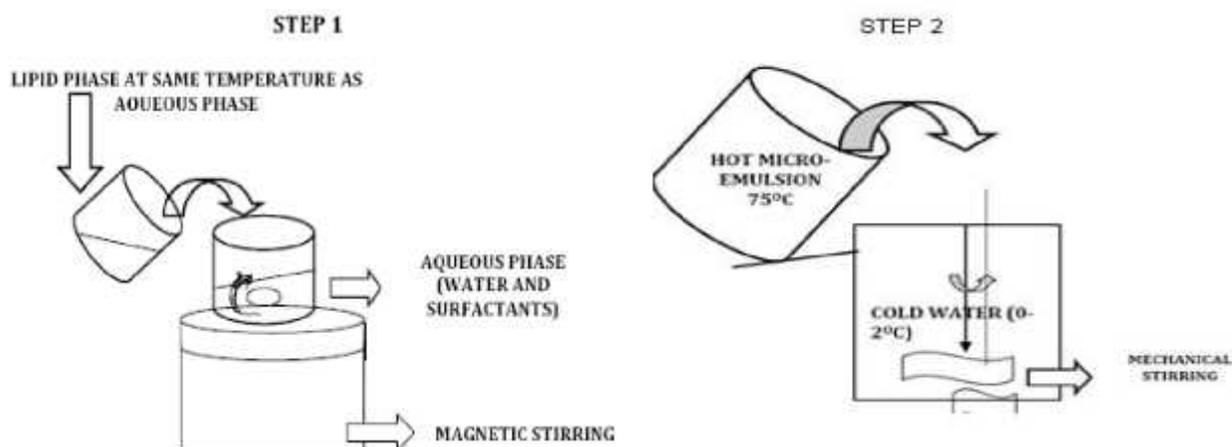


Fig.4: Microemulsion method

7] Double emulsion method

Warm w/o/w double microemulsion can be prepared in two steps. Firstly, w/o microemulsion is prepared by adding an aqueous solution containing drug to a mixture of melted lipid, surfactant and co-surfactant at a temperature slightly above the melting point of lipid to obtain a clear system. In the second step, formed w/o microemulsion is added to a mixture of water, surfactant and co-surfactant to obtain a clear w/o/w system. SLNs can be obtained by dispersing the warm micro double emulsions in cold then washed with dispersion medium by ultra-filtration system. Multiple emulsions have inherent instabilities due to coalescence of the internal aqueous droplets within the oil phase, coalescence of the oil droplets, and rupture of the layer on the surface of the internal droplets. In case of SLNs production, they have to be stable for few minutes, the time between the preparations of the clear double microemulsions and its quenching in cold aqueous medium, which is possible to achieve (Lv *et al.*2009).

8] Precipitation technique

Solid lipid nanoparticles can also be produced by a precipitation method which is characterized by the need for solvents. The glycerides will be dissolved in an organic solvent (e.g. chloroform) and the solution will be emulsified in an aqueous phase. After evaporation of the organic solvent the lipid will be precipitated forming nanoparticles (Yadav *et al.*2013).

9] Film-ultrasound dispersion

The lipid and the drug were put into suitable organic solutions, after decompression, rotation and evaporation of the organic solutions, a lipid film is formed, then the aqueous solution which includes the emulsions was added. Using the ultrasound with the probe to diffuser at last, the SLN with the little and uniform particle size is formed (Ekambaram et al., 2012).

10] Solvent Injection Technique

In solvent injection (or solvent displacement) method the lipid and the drug are dissolved in a water-miscible organic solvent (ethanol, acetone, isopropanol) and this solution is injected through a syringe needle in water under stirring: lipid precipitates as nanoparticles while contacting water, encapsulating the drug. Particle size can be influenced by lipid type, surfactant and solvent used, and from the viscosity of the outer phase.

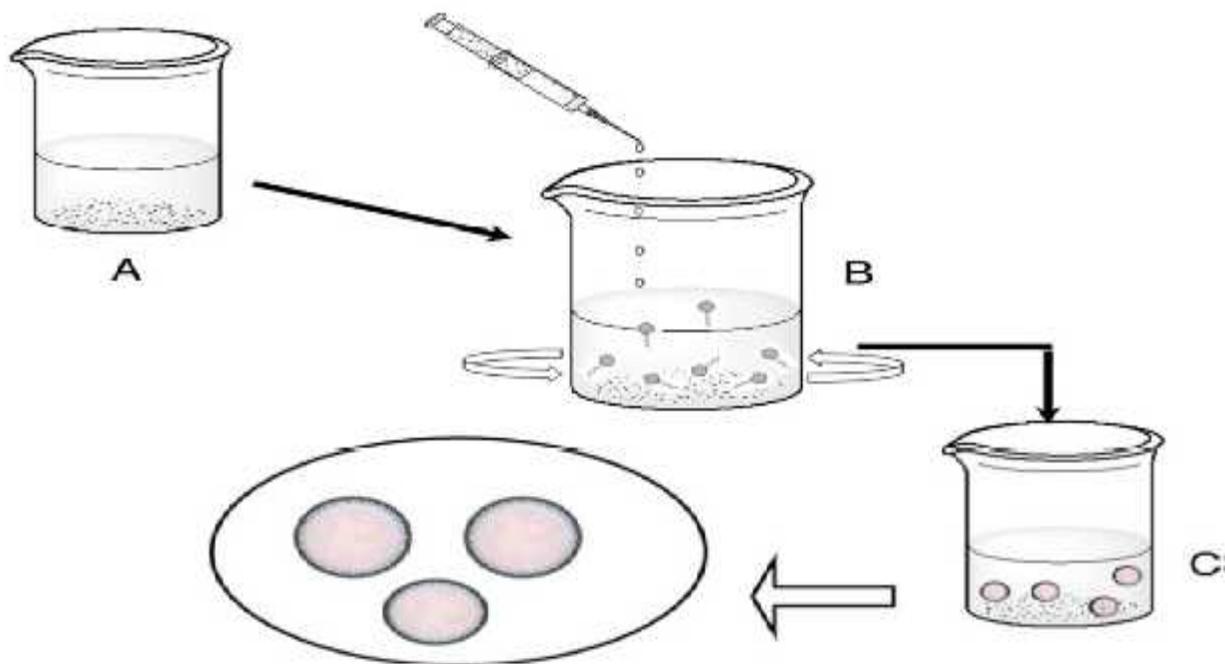


Fig.5 Solvent Injection Technique

11] Membrane contractor method

The present study investigates a new process for the preparation of SLN using a membrane contractor, to allow large scale production. A schematic drawing of the process is shown in Fig. 3. The lipid phase is pressed, at a temperature above the melting point of the lipid, through the membrane pores allowing the formation of small droplets. The aqueous phase circulates inside the membrane module, and sweeps away the droplets forming at the pore outlets. SLN are formed by the following cooling of the preparation to room temperature. The influence of process parameters (aqueous phase and lipid phase temperatures, aqueous phase cross-flow velocity and lipid phase pressure, membrane pore size) on the SLN size and on the lipid phase flux is investigated. Also, vitamin E loaded SLN are prepared, and their stability is demonstrated (Charcosset et al.2005).

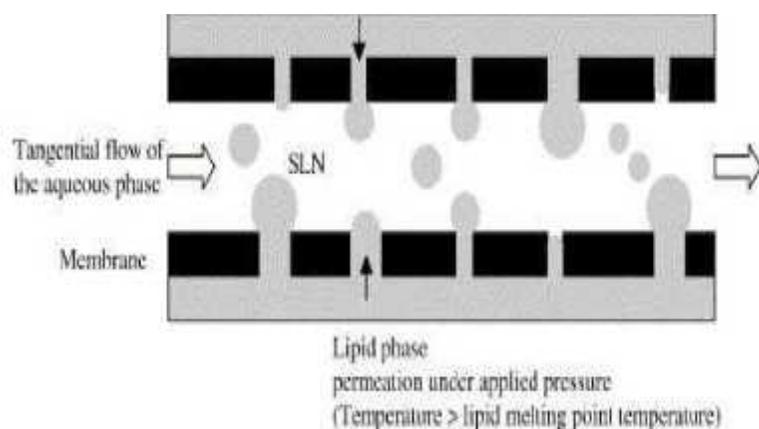


Fig. 6 Membrane contractor method

Table 3: Shows comparison of different formulation methods

S. No	Method	Advantages	Disadvantages
1a	Hot HPH	Versatile, avoid use of organic solvent, easy scalability, Short production time, instruments easily available and no regulatory problems	High temperature lead to degradation, conformational changes in protein, coalescence of particles, burst release due to high emulsifiers
1b	Cold HPH	Minimizes thermal exposure of the drug but does not avoid it completely. Useful in temperature labile drugs or hydrophilic drugs	Higher Polydispersity index
2	Emulsification-solvent evaporation	Avoidance of heat during production thus useful for thermolabile drugs. Simple procedure	solvent residues
3	Emulsification Solvent diffusion	Simple procedure, Fast drug release (drawback when slow release is required)	Low lipid content, Low EE and DL, organic solvent residue, Lack of scale up
4	Micro emulsion	No need for specialized equipment and energy for production	high concentrations of surfactants and co surfactants, presence of large amounts of water in system
5	Membrane Contractor	Simple method, Control of particle size by selection of process parameters, and its scaling-up abilities	-
6	PGSS	one step procedure, no need of organic solvent, low processing temperature conditions	frequent nozzle blockage with hydro-phallic drugs, machinery is costly
7	Multiple emulsion	No need to melt lipids, high loading of hydrophilic drugs, useful for protein loading	Use of solvent and surfactant
8	Solvent injection	no need for high pressure homogenization, easy handling, fast production process. No need for specialized equipment	Use of solvent and surfactant
9	film Ultra sonication dispersion	Simple, No need for specialized equipment	Metallic particle contamination, broader particle size
10	Phase inversion	Useful for thermolabile drugs, avoid organic solvent, No need for specialized equipment	-

Secondary production steps

Sterilization

Sterilization of the nanoparticles is desirable for parenteral administration and autoclaving which is applicable to formulations containing heat-resistant drugs. Effects of sterilization on particle size have been investigated and it was found to cause a distinct increase in particle size. Schwarz investigated the impact of different sterilization techniques (steam sterilization at 121°C (15 min) and 110°C (15min), g-sterilization) on SLN characteristics. The results indicate that particle aggregation might occur as a result of the treatment. Critical parameters include sterilization temperature and SLN composition. The correct choice of the emulsifier is of significant importance for the physical stability of the sample at high temperatures. Increased temperatures will affect the mobility and the hydrophilicity of all emulsifiers to a different extent. Steam sterilization will cause the formation of an o/w-emulsion due to the melting of the lipid particles. Solid particles are formed after recrystallization. Schwarz found that lecithin is a suitable surfactant for steam sterilization, because only a minor increase in particle size was observed. Experiments conducted by Freitas indicated that lowering of the lipid content (to 2%) and surface modification of the glass vials prevent the particle increase to a large extent

and avoid gelation. Additionally, it was observed by Freitas that purging with nitrogen showed a protective effect during sterilization. That observation suggests that chemical reactions could contribute to particle destabilization. γ -irradiation could be an alternative method to steam sterilization for temperature sensitive samples (Sinha *et al.*2010).

Lyophilization

Lyophilization is a promising way to increase the chemical and physical stability over extended periods of time. Lyophilization had been required to achieve long term stability for a product containing hydrolysable drugs or a suitable product for per-oral administration. Transformation into the solid state would prevent the Oswald ripening and avoid hydrolytic reactions. In case of freeze drying of the product, all the lipid matrices used, form larger solid lipid nanoparticles with a wider size distribution due to presence of aggregates between the nanoparticles. The conditions of the freeze-drying process and the removal of water promote the aggregation among SLNs. An adequate amount of cryoprotectant can protect the aggregation of solid lipid nanoparticles during the freeze-drying process (Ohshima *et al.*2009, Subedi *et al.*2009, Dong *et al.*2009).

Spray drying

It is an alternative and cheaper technique to the lyophilization process. This recommends the use of lipid with melting point more than 70°C. The best results were obtained with SLN concentration of 1% in a solution of trehalose in water or 20% trehalose in ethanol-water mixture. The addition of carbohydrates and low lipid content favor the preservation of the colloidal particle size in spray drying. The melting of the lipid can be minimized by using ethanol–water mixtures instead of pure water due to cooling leads to small and heterogeneous crystals, the lower inlet temperatures (Freitas *et al.*1998).

Storage stability of SLN (Qing *et al.*2009, Paliwal *et al.*2009)

The physical properties of SLN's during prolonged storage can be determined by monitoring Changes in zeta potential, particle size, drug content, appearance and viscosity as the function of time. External parameters such as temperature and light appear to be of primary importance for long – term stability. The zeta potential should be in general, remain higher than -60mV for a dispersion to remain physically stable.

4°C - Most favorable storage temperature.

20°C - Long term storage did not result in drug loaded SLN aggregation or loss of drug.

50°C - A rapid growth of particle size was observed.

CHARACTERIZATION PARAMETERS:

1. Particle Size and Shape

2. Measurement of zeta potential

3. Determination of incorporated drug (Loading Efficiency and Entrapment Efficiency)

4. Measurement of degree of crystallinity and lipid modification

1. Particle Size and Shape

SLNs are submicron sized, particle size and shape is determined by:

a) Photon Correlation Spectroscopy (PCS)

It is an established method which is based on dynamic scattering of laser light due to Brownian motion of particles in solution/suspension. This method is suitable for the measurement of particles in the range of 3 nm to 3 μ m. The PCS device consists of laser source, a sample cell (temperature controlled) and a detector. Photomultiplier is used as detector to detect the scattered light. The PCS diameter is based on the intensity of the light scattering from the particles.

b) Electron Microscopy

Electron Microscopy methods such as Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) are used to measure the physical characterization like overall shape and morphology of lipid nanoparticles. It permits the determination of particle size and distributions. SEM uses electrons transmitted from the surface of the sample while TEM uses electrons transmitted through the sample. TEM has a smaller size limit of detection (Tiwari *et al.*2015).

2. Measurement of zeta potential

Zeta potential is used to measure the charge on the particles. It allows prediction about the storage stability of colloidal dispersion because of repulsion between particles. Malvern Zetasizer is most widely used instrument

for measurement of Zeta potential. A zeta potential measurement can also be helpful in designing particles with reduced RES uptake. Zeta potential below -25 mV and above + 25mV are required for full electrostatic stabilization of the formulation.

Table 4: Shows main characteristics of particle size measurement methods

Method	Principle	Measured size	Limitation
LS	Light Interaction	50nm-1µm	Non-appropriated for very polydisperse populations Indirect method Great influence of aggregates or larger particles
LLD	Light Interaction	1-1000µm	High amount of sample required Indirect method
SEM, TEM	Microscopy	50nm-100µm	Time consuming Influence of the preparation sample
AFM	Microscopy	10nm-1µm	Sampling Non-automated Complexity of the set up Image treatment Subjective
ANUC FFF	Centrifugation Elution	20µm-1µm	Complex Data Processing Difficult to handle Optimization needed for each kind of particles
CE PCH, SEC	Electrophoresis Chromatography	20-500nm <100nm	Complexity of the set up Long steps of optimization Time consuming

LS, light scattering; LLD, laser light diffraction; SEM, scanning electron microscopy; TEM, transmission electron microscopy; AFM, atomic force microscopy; ANUC, analytical ultracentrifugation; FFF, field flow fractionation; CE, capillary electrophoresis; PCH, psched column hydrodynamic; SEC, size exclusion chromatography.

3. Determination of incorporated drug (Loading Efficiency and Entrapment Efficiency)

It is of prime importance to measure the amount of drug incorporated in SLNs, since it influences the release characteristics. The amount of drug encapsulated per unit wt. of nanoparticles is determined after separation of the free drug and solid lipids from the aqueous medium. This separation can be carried out using centrifugation, filtration or gel permeation chromatography. In centrifugation filtration, the filters such as U'trafree →MC (Milipore) or Utrasart→ 10 (Sartorius) are used along with classical centrifugation techniques. The degree of encapsulation can be assessed indirectly by determining in the supernatant after centrifugation filtration / ultracentrifugation of SLN suspension or alternatively by dissolution of the sediment in an appropriate solvent and subsequent analysis. Standard analytical techniques such as UVspectrophotometry, spectrofluorophotometry, high-performance liquid chromatography, or liquid scintillation counting can be used to assay the drug. In gel permeation chromatography Sephadex® and Sepharose® gels are used for removal of free drug from SLN preparations. First, preliminary calibration of column is carried out using SI. Ns and free drug. SLN preparations are applied to the column and washed with suitable buffer. Fractions containing SLNs can be Collected and analyzed for the actual drug content after dissolution/extraction with appropriate solvent. Drug content can also be determined directly in SLNs by extracting the drug with suitable solvent under optimum conditions and subsequent analysis of aqueous extract (Ye *et al.*2008, Huang *et al.*2008).

4. Measurement of degree of crystalline and lipid modification

Powder X - ray diffraction and differential scanning calorimetry (DSC)-

The geometric scattering of radiation from crystal planes within a solid allow the presence or absence of the former to be determined thus the degree of crystalline to be assessed. DSC can be used to determine the nature and the speciation of crystallinity within nanoparticles through the measurement of glass and melting point temperature. Thermodynamic stability, lipid packing density and quantification are a serious challenge due to the increase, while drug incorporation rates decrease in the following order: Super cooled melt < α-modification < β9-modification < β-modification. Due to the small size of the particles and the presence of emulsifiers, lipid crystallization modification changes might be highly retarded. Differential scanning

calorimetry (DSC) and X-ray scattering is widely used to investigate the status of the lipid. Infrared and Raman spectroscopy are useful tools for investigating structural properties of lipids. Their potential to characterize SLN dispersions has yet to be explored (Schubert *et al.* 2005).

***In vitro* and *ex vivo* methods for the assessment of drug release from SLN (Yi *et al.* 2006, Gande *et al.* 2007)**

A large number of drugs including very hydrophilic molecules have been postulated to be incorporated into SLN. Various methods used to study the *in vitro* release of the drug are:

- Side by side diffusion cells with artificial or biological membrane
- Dialysis bag diffusion technique.
- Reverse dialysis bag technique.
- Agitation followed by ultracentrifugation or centrifugal ultra-filtration.

In vitro drug release

Dialysis tubing

In vitro drug release could be achieved using dialysis tubing. The solid lipid nanoparticle dispersion is placed in pre-washed dialysis tubing which can be hermetically sealed. The dialysis sac 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 the drug content using a suitable analytical method.

Reverse dialysis

In this technique, a number of small dialysis sacs containing 1 mL of dissolution medium are placed in SLN dispersion. The SLN's are then displaced into the medium.

Ex vivo model for determining permeability across the gut (Qing *et al.* 2009)

In short, the rat jejunum (20 – 30 cm distal from the pyloric sphincter) was excised from the rats after sacrificing the animal used for the study or excised 10 cm long segments of duodenum (1 cm distal to pyloric sphincter); jejunum (15 cm to pyloric sphincter), ileum (20 cm proximal to cecum) and colon (2 cm distal to cecum) were immediately cannulated and ligated on both sides used for their permeability studies (Elnaggar *et al.* 2011)

Routes of administration:

Per oral administration

Per oral administration forms of SLN may include aqueous dispersions or SLN loaded traditional dosage forms, e.g. tablets, pellets or capsules. The microclimate of the stomach favors particle aggregation due to the acidity and high ionic strength. It can be expected, that food will have a large impact on SLN performance. The plasma levels and body distribution were determined after administration of CA-SLN suspension versus a CA solution (CA-SOL). Two plasma peaks were observed after administration of CA-SLN. The first peak was attributed to the presence of free drug; the second peak can be attributed to controlled release or potential gut uptake of SLN. These two peaks were also found in the total CA concentration-time profiles of all measured organs. It was also found that the incorporation into SLN protected CA from hydrolysis. The conclusion from this study was that SLN are a promising sustained release system for CA and other lipophilic drugs after oral administration. Increased bioavailability and prolonged plasma levels have been described after per oral administration of cyclosporine containing lipid nanodispersions to animals (Yadav *et al.* 2011).

Parenteral administration

SLN have been administered intravenously to animals. Pharmacokinetic studies of doxorubicin incorporated into SLN showed higher blood levels in comparison to a commercial drug solution after i.v. injection in rats. Concerning the body distribution, SLN were found to cause higher drug concentrations in lung, spleen and brain, while the solution led to a distribution more into liver and kidneys. Parenteral application is a very wide field for SLN. Subcutaneous injection of drug loaded SLN can be employed for commercial aspect, e.g., erythropoietin (EPO), interferon- β . Other routes are intraperitoneal and also intra-articular. Intraperitoneal application of drug-loaded SLN will prolong the release because of the application area. In addition, incorporation of the drug into SLN might reduce irritancy compared to injecting drug micro particles (Yadav *et al.* 2011).

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. An increase of the solid lipid content of the SLN dispersion results in semisolid, gel-like systems, which might be acceptable for direct application on the skin (Bhaskar *et al.* 2009).

Topical application

Regarding the regularity aspect, topical application is relatively unproblematic. The major advantages for topical products are the protective properties of SLN for chemically labile drugs against degradation and the occlusion effect due to film formation on the skin. Especially in the area of cosmetics there are many compounds such as retinol or vitamin C which cannot be incorporated because of the lack of chemical stability. Incorporation of retinol is only possible when applying certain protective measures during production (e.g. noble gasing) and using special packing materials (e.g. aluminium) (Lippacher *et al.* 2001).

Ophthalmic administration

Many investigations have been made to use nanoparticles for prolonged release of drugs to the eye. The basic problem of ophthalmologic formulation is the fast removal from the eye, which implies clearance of the applied drug through the nose. It could be shown for nanoparticles that an increased adhesiveness is available leading to higher drug levels at desired site of action. However, the basic problem was that the nanoparticles are of limited toxicological acceptance. It was shown by Gasco that SLN have a prolonged retention time at the eye. This was confirmed by using radiolabeled formulations and γ -scintigraphy. The lipids of SLN are easy to metabolize and open a new way for ophthalmological drug delivery without impairing vision. (Araujo *et al.* 2009).

Pulmonary administration

A very interesting application appears to be the pulmonary administration of SLN. SLN powders cannot be administered to the lung because the particle size is too small and they will be exhaled. A very simple approach is the aerosolization of aqueous SLN dispersions. The important point is that the SLN should not aggregate during the aerosolization. The aerosol droplets were collected by collision of aerosol with a glass wall of a beaker. This basically demonstrates that SLN are suitable for lung delivery. After localization into the bronchial tube and in the alveoli, the drug can be released in a controlled way from the lipid particles (Yadav *et al.* 2011).

Applications of SLN

There are several potential applications of SLNs some of which are given below:

SLN as potential new adjuvant for vaccines

Adjuvants are used in vaccination to enhance the immune response. The safer new subunit vaccines are less effective in immunization and therefore effective adjuvants are required. New developments in the adjuvant area are the emulsion systems. These are oil-in-water emulsions that degrade rapidly in the body. Being in the solid state, the lipid components of SLNs will be degraded more slowly providing a longer lasting exposure to the immune system.

Solid lipid nanoparticles in cancer chemotherapy

From the last two decades, several chemotherapeutic agents have been encapsulated in SLN and their *in-vitro* and *in-vivo* efficacy have been evaluated. Outcomes of these studies have been shown to improve the efficacy of chemotherapeutic drugs, simultaneously reduction in side effects associated with them. Improved stability of drugs, encapsulation of chemotherapeutic agents of diversified physicochemical properties, enhanced drug efficacy, improved pharmacokinetics and less *in-vitro* toxicity are the important features of SLN which make them a suitable carrier for delivering chemotherapeutic drugs. Several obstacles frequently encountered with anticancer compounds, such as normal tissue toxicity, poor specificity and stability and a high incidence of drug resistant tumor cells, are at least partially overcome by delivering them using SLN. The rapid removal of colloidal particles by the macrophages of the RES is a major obstacle to targeting tissues elsewhere in the body, such as bone marrow and solid tumors.

A) SLN as targeted carrier for anticancer drug to solid tumor

SLN have been to be useful as drug carriers. Tamoxifen is an anticancer drug incorporated in SLN to prolong the release of drug after IV administration in breast cancer. Tumor targeting has been achieved with SLN loaded with drugs like methotrexate and camptothecin.

B) SLN in breast cancer and lymph node metastases

Mitoxantrone SLN local injections were formulated to reduce the toxicity and improve the safety and bioavailability of the drug.

Solid lipid nanoparticles for delivering peptides and proteins (Ekambaram et al., 2012)

Solid lipid particulate systems such as solid lipid nanoparticles (SLN), lipid microparticles (LM) and lipospheres have been sought as alternative carriers for therapeutic peptides, proteins and antigens. The research work developed in the area confirms that under optimized conditions they can be produced to incorporate hydrophobic or hydrophilic proteins and seem to fulfill the requirements for an optimum particulate carrier system. Proteins and antigens intended for therapeutic purposes may be incorporated or adsorbed onto SLN, and further administered by parenteral routes or by alternative routes such as oral, nasal and pulmonary. Formulation in SLN confers improved protein stability, avoids proteolytic degradation, as well as sustained release of the incorporated molecules. Important peptides such as cyclosporine A, insulin, calcitonin and somatostatin have been incorporated into solid lipid particles and are currently under investigation. Several local or systemic therapeutic applications may be foreseen, such as immunisation with protein antigens, infectious disease treatment, chronic diseases and cancer therapy.

Solid lipid nanoparticles for targeted brain drug delivery

The extremely small particle size of solid lipid nanoparticles, which are less than 50 nm, might be beneficial with respect to drug targeting. Small carrier size generally favors reduced uptake by the reticuloendothelial system. Drug targeting might also be possible by surface modification of solid lipid nanoparticles. SLNs can improve the ability of the drug to penetrate through the blood-brain barrier and is a promising drug targeting system for the treatment of central nervous system disorders. In a study to overcome the limited access of the drug 5-fluoro-2'-deoxyuridine (FUdR) to the brain, 3',5'-dioctanoyl-5- fluoro-2'-deoxyuridine (DO-FUdR) was synthesized and incorporated into solid lipid nanoparticles (DOFUdR-SLN)

Solid lipid nanoparticles for parasitic diseases

Parasitic diseases (like malaria, leishmaniasis, trypanosomiasis) are one of the major problems around the globe. Antiparasitic chemotherapy is the only choice of treatment for these parasitic infections, the reason for this is that these infections do not elicit pronounced immune response hence effective vaccination may not be possible. Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) represent a second generation of colloidal carriers and have emerged as an effective alternative to liposomes mainly due to their better stability profile, ease of scalability and commercialization and relative cost efficacy. Moreover, SLN and NLC due to their particulate nature and inherent structure exhibit good potential in the treatment of parasitic infections. Recent reports including our investigation have validated their utility at least to some extent. However, the need of hour is to undertake extensive investigations on SLN and NLC matrices in order to extend their versatility with respect to encapsulation ability and target ability and to arrive at a versatile, effective and economical approach for the delivery of anti-parasitic drugs.

CONCLUSION

SLN as colloidal drug carrier combines the advantage of polymeric nanoparticles, fat emulsions and liposome; due to various advantages, including feasibility of incorporation of lipophilic and hydrophilic drugs, improved physical stability, low cost, ease of scale-up, and manufacturing. SLNs are prepared by various advanced techniques. The site specific and sustained release effect of drug can better be achieved by using SLNs. Nanoparticles have been used extensively for applications in drug discovery, drug delivery, and diagnostics and for many others in medical field. They are relatively novel drug delivery systems, having received primary attention from the early 1990s and future holds great promise for its systematic investigation and exploitation. We can expect many patented dosage forms in the form of SLNs in the future.

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