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Solubility Enhancement by Solid Lipid Nanoparticle

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ABSTRACT

Solid lipid nanoparticles were developed in the early 1990s 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. Due to their unique size-dependent properties, lipid nanoparticles offer the possibility to develop new therapeutics. The ability to incorporate drugs into nanocarriers offers a new prototype in drug delivery that could be used for secondary and tertiary levels of drug targeting. Hence, solid lipid nanoparticles hold great promise for reaching the goal of controlled and site-specific drug delivery and hence have attracted the wide attention of researchers. In this review, the latest research developments of the Solid lipid nanoparticles (SLNs) according to the recent relevant literature are focused. Different production methods which are suitable for large-scale production and applications of solid lipid nanoparticles are described. Characterization techniques of SLN like particle size and zeta potential, nuclear magnetic resonance, electron microscopy etc. has also been discussed.

INTRODUCTION

Solid lipid nanoparticles (SLN) introduced in 1991 represent an alternative carrier system to tradition colloidal carriers such as emulsions, liposomes and polymeric micro and nanoparticles[1]. Nanoparticles made from solid lipids are attracting major attention as a 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 an aqueous surfactant solution. Generally, they are made up of a 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[2].

Advantages of SLN

1. SLNs have better stability and ease of upgradability to production scale as compared to the liposome.
2. In SLNs the lipid matrix is made from a physiological lipid which decreases the danger of acute and chronic toxicity.
3. Very high long-term stability.
4. It is easy to manufacture than bipolymeric nanoparticles.
5. Better control over release kinetics of encapsulated compound.
6. SLNs can be enhancing the bioavailability of entrapped bioactive.
7. Chemical protection of labile incorporated compound.
8. Raw material which is to be required is same as that of the emulsion.
9. Large scale production is possible.
10. High concentration of functional compound can be achieved.
11. Lyophilization possible.

Disadvantage

1. Poor drug loading capacity.
2. Drug expulsion after polymeric transition during storage.

3. Relatively high water content of the dispersions (70-99.9%).
4. The low capacity to load hydrophilic drugs due to partitioning effects during the production process.

PREPARATION METHODS OF SLNs:

1. High shear homogenization technique:

High shear homogenization techniques were initially used for the production of solid lipid Nano-dispersions [3]. This technique is easy to handle. Dispersion quality is often compromised by the presence of microparticles [4]. β-carotene loaded solid lipid nanoparticles formulated by high shear homogenization process had been investigated on size distribution, stability, drug loading, and drug release [5]. Different process parameters like stirring rate and cooling condition on the particle size and zeta potential, emulsification time are investigated. Lipids used in this study are tripalmitin, a mixture of mono, di, triglycerides (WitepsolW35) with glycerol behenate and poloxamer 188 used as steric stabilizers (0.5% w/w). Higher stirring rates did not significantly affect the particle size, but slightly increased the polydispersity index.

2. High-pressure homogenization (HPH)

It is a reliable and powerful technique, which is used for the first time for 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) [6]. 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. HPH is of two types-hot homogenization and cold homogenization [7]. In both cases, a preparatory step involves the drug incorporation into the bulk lipid by dissolving or dispersing the drug in the lipid melt.

2.1 Hot homogenization technique

Melting of the lipid

↓

Dissolution of the drug in the molten lipid

↓

Mixing of the preheated dispersion medium and drug-lipid melt

↓

Premix using a stirrer to form a coarse pre-emulsion

↓

High-pressure homogenization at a temperature above the lipid melting point

↓

O/W – nano-emulsion

↓

Solidification of the nano-emulsion by cooling down to room temperature to form SLN.

The drug is dissolved or dispersed in melted solid lipid for SLN or in a mixture of liquid lipid (oil) and melted solid lipid for nanostructured lipid carrier. In the hot homogenization method, the lipid melt containing drug is dispersed in a solution of the hot surfactant at the same temperature (5–10^0C) above the melting point of the solid lipid or lipid blend by high-speed stirring. This pre-emulsion is then passed through a high-pressure homogenizer adjusted to the same temperature generally applying three cycles at 500 bar or two cycles at 800 bars. The hot homogenization technique can be used for lipophilic and insoluble drugs. As the exposure time to high temperature is relatively short, many heat sensitive drugs can be safely processed. The technique is not suitable for incorporation of hydrophilic drugs into SLN because a higher portion of drugs in water during homogenization results in low entrapment efficiency [5, 6].

2.2. Cold homogenization technique

Melting of the lipid

↓

Dissolution /solubilization of the drug in the molten lipid

↓

Solidification of the drug loaded lipid in liquid nitrogen or dry ice

↓

Grinding in a powder mill (50-100 micrometer particles)

↓

Dispersion of the lipid in the cold aqueous dispersion medium

↓

Solid lipid nanoparticles

The first step of preparation is same as that of hot homogenization which includes dispersion or dissolving or solubilisation of the drug in the molten lipid. Then the drug-lipid mixture is rapidly cooled either by means of liquid nitrogen or dry ice. The drug containing solid lipid is milled by means of mortar or ball mill to micron size (50-100 micron) and these microparticles are dispersed in chilled emulsifier solution yielding a pre-suspension. Then this pre-suspension is subjected to high-pressure homogenization at room or below room temperature, where the cavitation force is strong enough to break the microparticles to SLNs. This process avoids or minimizes the melting of lipid and therefore minimizing loss of hydrophilic drug to aqueous phase. Another method to minimize the loss of hydrophilic drug to aqueous phase is to replace water with other media (e.g. oil or PEG 600) with low solubility for the drug. In comparison to hot homogenization, in cold homogenization particle size and polydispersity index are more. The cold homogenization only minimizes the thermal exposure of drug, but it does not avoid completely it due to melting of the lipid/drug mixture in the first step of preparation [(7)]. High-pressure homogenization increases the temperature of the sample (e.g. 10-20ºC for each homogenization cycle). In most of the cases, 3-5 homogenization cycles at 500-1500 bar are sufficient to prepare SLN. Increasing the number of homogenization cycle or the homogenization pressure resulted in an increase of particle size due to particle coalescence which resulted from the high kinetic energy of particles.

Advantages

1. Low capital cost.
2. Demonstrated at lab scale.

Disadvantages

1. Energy intensive process.
2. Demonstrated at lab scale biomolecular damage.
3. Polydisperse distributions.
4. Unproven scalability.

3. Ultrasonication and high-speed homogenization

SLNs are also prepared by ultrasonication or high-speed homogenization techniques. For smaller particle size combination of both ultrasonication and high-speed homogenization is required. It reduces shear stress but has some disadvantages like potential metal contamination, physical instability like particle growth upon storage. In this probe sonicator or bath sonicator is used[8,9].

Advantages

1. Reduced shear stress.

Disadvantages

1. Potential metal contamination.
2. Physical instability like particle growth upon storage.

4. Micro-emulsion technique

The use of micro-emulsion in SLN production was described first by Gasco et al. [12]. It should be noted, however, that the term 'micro-emulsion' is controversial and there is a variety of opinions in the scientific circles on the structure and dynamics of micro-emulsion. The matter was discussed in detail by Moulik & Paul (13). In order to obtain micro-emulsion with lipids in solid state at room temperature, the process temperature must be higher than lipid melting point. Lipids (e.g. fatty acids and/or triglycerides) are melted and the mixture of water, emulsifiers and

co-emulsifiers is heated to the temperature of the lipids and blended under mild conditions. If the procedure runs correctly, we will obtain transparent, thermodynamically stable complex. The hot micro-emulsion is then dispersed in chilled water (2÷3°C) by smooth mechanical stirring, which ensures that the small particle size results from precipitation and not the mechanical stirring. The volume ratio of hot micro-emulsion to cold water should be from 1:25 to 1:50. The most popular emulsifiers are polysorbate 20, polysorbate 60 and soya lecithin. The most frequently used co-emulsifiers are usually alcohols, e.g. butanol. Technically, the precipitation of lipid particles in water is equivalent to diluting the complex, which leads to decrease in solid substance content in SLN dispersion. Due to diluting stage, the achievable lipid content is lower than in formulations obtained through HPH [10, 11].

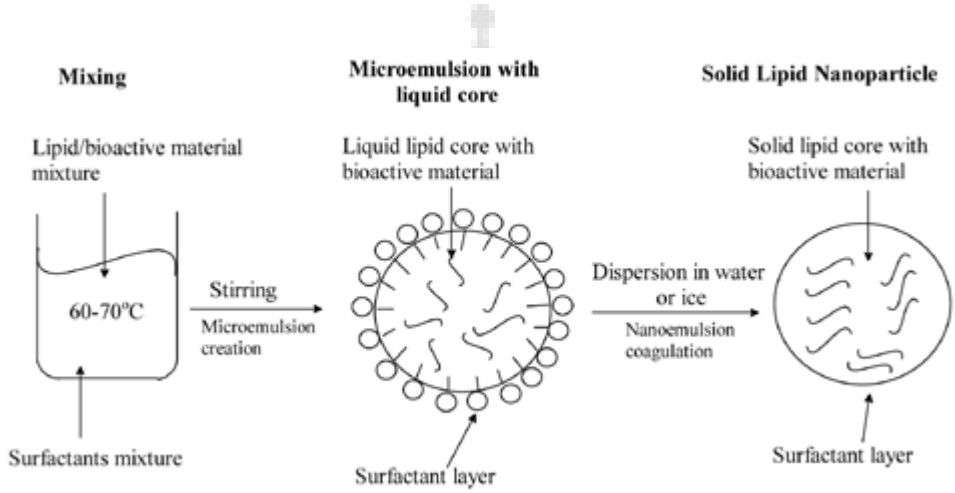

Figure 1: Schematic diagram showing the structures formed during the production of SLN by micro-emulsion technique

Advantages

1. Low mechanical energy input.
2. Theoretical stability.

Disadvantages

1. Extremely sensitive to change.
2. Labor intensive formulation work.
3. Low nanoparticle concentrations.

5. SLN preparation by using supercritical fluid:

This is a relatively new technique for SLN production and has the advantage of solvent-less processing [14, 15]. 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[16].

Advantages

1. Avoid the use of solvents.
2. Particles are obtained as a dry powder, instead of suspensions.
3. Mild pressure and temperature conditions.
4. Carbon dioxide solution is the good choice as a solvent for this method.

6. Spray drying method:

It is an alternative procedure to lyophilization in order to transform an aqueous SLN dispersion into a drug product. It is a cheaper method than lyophilization. This method causes particle aggregation due to high temperature, shear forces and partial melting of the particle. Freitas and Mullera[17] recommends the use of lipid with melting point >70 0 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).

7. Double emulsion method:

For the preparation of hydrophilic loaded SLN, a novel method based on solvent emulsification-evaporation has been used [18]. 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.

8. Precipitation method

The glycerides are 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 [19].

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 the diffuser at last, the SLN with the little and uniform particle size is formed [20].

10. Solvent evaporation

SLNs can also prepare by solvent evaporation method. The lipophilic material is dissolved in a water-immiscible organic solvent (e.g. cyclohexane) that is emulsified in an aqueous phase. Upon evaporation of the solvent, nanoparticles dispersion is formed by precipitation of the lipid in the aqueous medium by giving the nanoparticles of 25 nm mean size. The solution was emulsified in an aqueous phase by high-pressure homogenization. The organic solvent was removed from the emulsion by evaporation under reduced pressure (40–60 mbar) [21].

Advantages

1. Scalable.
2. Mature technology.
3. Continuous process.
4. Commercially demonstrated.

Disadvantages

1. Extremely energy intensive process.
2. Poly-disperse distributions.
3. Biomolecule damage.

EVALUATION PARAMETERS FOR SLNS [22, 23, 24]

1 Measurement of particle size and zeta potential

Photon correlation spectroscopy (PCS) and laser diffraction (LD) are the most powerful techniques for routine measurements of particle size. PCS (also known as dynamic light scattering) measures the fluctuation of the intensity of the scattered light which is caused by particle movement. This method covers a size range from a few nanometers to about 3 microns. PCS is a good tool to characterize nanoparticles, but it is not able to detect larger microparticles. Electron Microscopy provides, in contrast to PCS and LD, direct information on the particle shape. The physical stability of optimized SLN dispersed is generally more than 12 months. Zeta potential measurements allow predictions about the storage stability of the colloidal dispersion.

2. Photon Correlation Spectroscopy (PCS)

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

3. Electron Microscopy

Electron Microscopy methods such as Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) are used to measure the 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.

4. Atomic Force Microscopy (AFM)

It is an advanced microscopic technique which is applied as a new tool to image the original unchanged shape and surface properties of the particles. AFM measures the force acting between

the surface of the sample and the tip of the probe, when the probe is kept in close proximity to the sample which results in a spatial resolution of up to 0.01 nm for imaging.

5. Determination of Incorporated Drug

Amount of drug incorporated in SLNs influences the release characteristics hence it is very important to measure the amount of incorporated drug. The amount of drug encapsulated per unit weight. of nanoparticles is determined after separation of the free drug and solid lipids from the aqueous medium and this separation can be done by ultracentrifugation, centrifugation filtration or gel permeation chromatography. The drug can be assayed by a standard analytical technique such as a spectrophotometer, a spectrofluorophotometer, HPLC or liquid scintillation counting.

6. *In-vitro* drug release

6.1 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.

6.2 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.

7. Rheology

Rheological measurements of formulations can perform by Brookfield Viscometer, using a suitable spindle number. The viscosity depends on the dispersed lipid content. As the lipid content increases, the flow becomes non-Newtonian from Newtonian.

8. 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.

9. 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.

10. X-ray diffraction (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 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.

ROUTES OF ADMINISTRATION AND BIODISTRIBUTION OF SLNs:

The *in-vivo* behavior of the SLN particles will mainly depend on the following points: Interactions of the SLN with the biological surroundings including distribution processes (Adsorption of biological material on the particle surface and desorption of SLN components into biological surroundings) and enzymatic processes. Various administration routes are [25,26].

1 Parenteral administration

Peptide and proteins drugs are usually available for parenteral use in the market. Since their conventional oral administration is not possible due to enzymatic degradation in GI tract. Parenteral application of SLN reduces the possible side effects of drug incorporated with the increased bioavailability. These systems are very suitable for drug targeting.

2 Oral administration

Controlled release behavior of SLNs is reported to enable the bypass of gastric and intestinal degradation of the encapsulated drug, and their possible uptake and transport through the intestinal mucosa. However, the assessment of the stability of colloidal carriers in GI fluids is essential in order to predict their suitability for oral administration.

3 Rectal administration

When the rapid pharmacological effect is required, in some circumstances, parenteral or rectal Administration is preferred. This route is used for pediatric patients due to easy application.

4 Nasal administration

Nasal route is preferred due to its fast absorption and rapid onset of drug action also avoiding degradation of labile drugs in the GIT and insufficient transport across epithelial cell layers

5 Respiratory delivery

Nebulisation of solid lipid particles carrying anti-tubercular drugs, anti-asthmatic drugs, and anti-cancer was observed to be successful in improving drug bioavailability and reducing the dosing frequency for better management of pulmonary action.

6 Ocular administration

Biocompatibility and mucoadhesive properties of SLN improve their interaction with ocular mucosa and prolong the corneal residence time of the drug, with the aim of ocular drug targeting.

7 Topical administration

SLN 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.

APPLICATIONS OF SLN:

Solid lipid Nanoparticles possesses a better stability and ease of upgradability to production scale as compared to liposomes. This property may be very important for many modes of targeting. SLNs form the basis of colloidal drug delivery systems, which are biodegradable and capable of being stored for at least one year. They can deliver drugs to the liver *in-vivo* and *in-vitro* to cells which are actively phagocytic. There are several potential applications of SLNs some of which are given below [(27)].

1. SLNs as gene vector carrier

SLN can be used in the gene vector formulation. In one of the 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 (Rudolph C et al., 2004). The lipid-nucleic 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-nucleic 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.

2. SLNs for topical use

SLNs and NLCs have been used for topical application for various drugs such as tropolide, imidazole antifungals, anti-cancers, 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, vitamin A-loaded nanoparticles can be prepared. The methods are useful for the improvement of penetration with sustained release. The iso-tretinoin-loaded lipid nanoparticles were formulated for topical delivery of drug. The soya bean lecithin and Tween 80 were used for the hot homogenization method for this. The methodology is useful because of the increase of accumulative uptake of isotretinoin in skin.

3. 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. 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.

4. SLNs for 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 a surfactant.

5. SLNs as a targeted carrier for anticancer drug to solid tumors

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

6. 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 is enhanced its efficacy and reduced breast cancer cells.

7. Oral SLNs in antitubercular chemotherapy

Anti-tubercular drugs such as rifampicin, isoniazid, 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 were prepared. The nebulization in an animal by incorporating the above drug in SLN also reported for improving the bioavailability of the drug.

8. 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.

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