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Review Article

A concise review on preparation methods used for the development of solid lipid nanoparticles

Vasu Deva Reddy Matta

Dr. Reddy's Laboratories Ltd., Bachupally, Hyderabad, Telangana State, India

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*Address for Correspondence:

Vasu deva Reddy Matta, Dr. Reddy's Laboratories Ltd., Bachupally, Hyderabad, Telangana State, India

Abstract

Solid lipid nanoparticles (SLNs) are in submicron size range nanoparticles and are made of biocompatible and biodegradable materials (mainly composed of lipids and surfactants) capable of incorporating both lipophilic and hydrophilic drugs. SLNs are also considered as substitute to other colloidal drug systems, also used as controlled systems and targeted delivery. SLNs can be considered as an alternative for oral drug delivery vehicle to improve the oral bioavailability of drugs, associated reduction of drug toxicity and stability of drug in both GIT and plasma. There are different techniques used for the preparation of SLNs. Generally, the preparation of SLNs and any other nanoparticle system necessitates a dispersed system as precursor; otherwise particles are produced through the use of a particular instrumentation. This review provides the summary on the techniques or methods used for the development of SLNs of poorly water soluble drugs for improved drug delivery.

Keywords: Solid lipid nanoparticles, controlled delivery, precursor, techniques.

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Introduction

Administration by the oral route remains most prevalent way of drug delivery. Despite the popularity and versatility of the oral route, significant problems remain. Not all drug molecules possess the physical, chemical or biological characteristics necessary for the successful therapy by oral route[1,2]. Problems such as poor solubility or chemical stability in the location of the gastrointestinal tract, poor permeability over the biological membranes or compassion to metabolism are well known to result in the refusal of potential drug candidates as oral applied products[3-8]. Lipid based drug delivery systems have been proposed as a means of by-passing some of more resistant chemical or physical barriers associated with poorly absorbed drugs[9-13]. Hence, various alternative drug delivery systems are developed to enhance the oral BA of these drugs. The delivery systems include; enhancement of solubility through solid dispersions[14, 15], liquisolid compacts[16]; avoid first-pass metabolism through buccal delivery or nasal route[17-20]; increase the stability and prolonged residence time through floating systems[21-26], increase the mucoadhesive property[27-30]; lipid based delivery systems for by passing metabolism with solid lipid nanoparticles[31,32], transfersomes[33,34], nanostructured lipid carriers[35] and micronization for reducing particle size using nanosuspensions[36-38].

Colloidal carrier systems also protect sensitive drugs against the degradation in biological fluids. They offer protection of patient against gastric irritation and can also be the candidate for prolonged drug action due to sustained release. Colloidal particles as drug carriers are also promising candidates for drug targeting[39]. Colloidal carrier systems are mostly based on compositions similar to the physiological structures exhibit greater biological acceptance. Also, lipids are easily metabolized to nontoxic metabolites. Colloidal systems using biodegradable polymers have also been extensively investigated and proved to be ideal candidates for per oral drug administration[40,41].

Solid lipid nanoparticles (SLNs) are emerging as alternative carriers to colloidal drug systems, for controlled systems and targeted delivery. These are in submicron size range (50-1000 nm) and are made of biocompatible and biodegradable materials capable of incorporating lipophilic and hydrophilic drugs. SLNs combine the advantage of different colloidal carriers, for instance, like emulsions and liposomes, these are physiologically acceptable and like polymeric nanoparticles, controlled release of drug from lipid matrix can be anticipated[42-44].

SLNs are particles made from solid lipids (i.e., lipids solid at room temperature and also at body temperature) and stabilized by surfactant(s). By definition, the lipids can be highly purified triglycerides, complex glyceride mixtures or

even waxes. Through the work of various research groups, the SLN carrier system has been characterized intensively[45].

There are different techniques for the preparation of SLNs. Generally, the preparation of any nano carrier system requires a dispersed system as precursor, or else particles are produced through the use of a specific instrumentation. This review mainly provides the insights onto the different production techniques used for the development of SLNs.

Structure of solid lipid nanoparticles

SLNs consist of a core of solid lipid with the bioactives being a part of the lipid matrix (Figure 1). The particle is stabilized by a surfactant layer, which may consist of a single surfactant, but typically is composed of a mixture of surfactants. In general, the use of crystallized lipids instead of liquid lipids has been shown to increase control over release and stability of incorporated bioactive. This is because mobility of bioactives can be controlled by controlling the physical state of the lipid matrix[44].

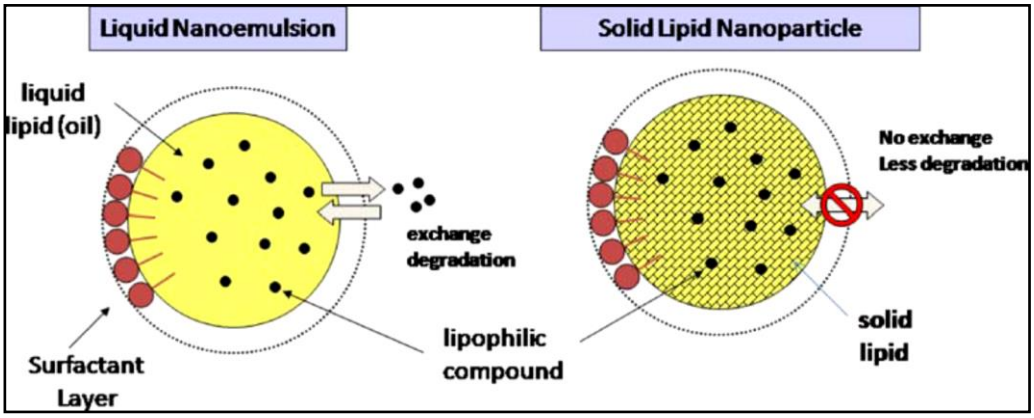

Figure 1: Structure of liquid nanoemulsions (left) and solid lipid nanoparticles (right) stabilized by a surfactant layer carrying a lipophilic bioactive

Preparation methods of SLNs

Based on the type of precursor and also type of process, SLNs preparation methods are classified as follows: emulsions as precursor, hot homogenization, melt dispersion or film formation technique, phase-inversion temperature method, solvent evaporation-diffusion from emulsions), microemulsions (microemulsion dilution and microemulsion cooling techniques), micellar solutions (coacervation technique). Some preparation techniques are based on supercritical fluids. The most important techniques which involve the use of particular instrumentation are: membrane contactor technique, spray-drying, spray-congealing and electrospray. Different methods used for the production of SLNs mentioned in Table 1.

Table 1: Preparation method of SLNs based on type of precursor and instrumentation used.

Type of precursor	Method used
Precursor as emulsion	hot homogenization technique
	melt dispersion technique
	phase-inversion temperature
	solvent evaporation-diffusion
	hot homogenization technique
Precursor as microemulsion	microemulsion dilution
	microemulsion cooling techniques
Precursor as micellar solutions	Coacervation technique
Use of particular instrumentation	membrane contactor technique,
	spray-drying,
	spray-congealing
	Electrospray.

High pressure homogenization (Hot and Cold)

High pressure homogenization (HPH) has emerged as a reliable and powerful technique for the preparation of SLN. Homogenizers of different sizes are commercially available from several manufacturers. The high pressure homogenization technique has been demonstrated to be the most effective technique due to some advantages such as narrow particle size distribution of the product with a low content of microparticles (> 5 μm is requested for iv injections), higher particle content in the dispersions, avoidance of organic solvents, acceptability of the homogenization equipment by the regulatory authorities (even for parenteral products), scale-up feasibility and the availability of homogenization lines in industry[46]. Depending on the size of production-scale homogenizers, a wide production range can be possible[47]. There are two general approaches within the homogenization technique, the hot and the cold homogenization. In both cases, a preparatory step involves the drug incorporation into the bulk lipid by dissolving or dispersing the drug in the lipid melt.

Hot homogenization technique

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. Pre-emulsion of the drug loaded lipid melt and the aqueous emulsifier phase (same temperature) is obtained by high-shear mixing device (Ultra-Turrax). The quality of the pre-emulsion affects the quality of the final product to a large extent and it is desirable to obtain droplets in the size range of a few micrometers. HPH of the pre-emulsion is carried out at temperatures above the melting point of the lipid. In general, higher temperatures result in lower particle sizes due to the decreased viscosity of the inner phase. However, high temperatures may also increase the degradation rate of the drug and carrier. The homogenization step can be repeated several times. It should always be kept in mind, that high pressure homogenization increases the temperature of the sample (approximately 10°C for 500 bar). In most cases, 3–5 homogenization cycles at 500–1500 bar are sufficient. Increasing the homogenization pressure or the number of cycles often results in an increase of the particle size due to particle coalescence which occurs as a result of the high kinetic energy of the particles[48].

The primary product of the hot homogenization is a nanoemulsion due to the liquid state of the lipid. Solid particles are expected to be formed by the following cooling of the sample to room temperature or to temperatures below[49]. Due to the small particle size and the presence of emulsifiers, lipid crystallization may be highly retarded and the sample may remain as a supercooled melt for several months.

The hot homogenization technique is also suitable for drugs showing some temperature sensitivity because the exposure to an increased temperature is relatively short. In case of highly temperature-sensitive compounds the cold homogenization technique can be applied.

A camptothecin loaded SLN suspension consisted of 0.1% (w/w) camptothecin, 2.0% (w/w) stearic acid, 1.5% (w/w) soybean lecithin and 0.5% (w/w) polyoxyethylene–polyoxypropylene copolymer (Poloxamer 188) was prepared by high pressure homogenization[50,51].

Cold homogenization technique

In contrast, the cold homogenization is carried out with the solid lipid and represents, therefore, a high pressure milling of a suspension. Effective temperature control and regulation is needed in order to ensure the un-molten state of the lipid due to the increase in temperature during homogenization. Cold homogenization has been developed to overcome the following three problems of the hot homogenization technique:

- Temperature-induced drug degradation
- Drug distribution into the aqueous phase during homogenization
- Complexity of the crystallization step of the nanoemulsion leading to several modifications and/or supercooled melts

The first preparatory step is the same as in the hot homogenization procedure and includes the solubilization or dispersing of the drug in the melt of the bulk lipid. However, the following steps are different. The drug containing melt is rapidly cooled (e.g.by means of dry ice or liquid nitrogen). The high cooling rate favors a homogenous distribution of the drug within the lipid matrix. The solid, drug containing lipid is milled to microparticles. Typical particle sizes obtained by means of ball or mortar milling are in the range of 50–100 microns. Low temperatures increase the fragility of the lipid and favor, therefore, particle comminution. The solid-lipid microparticles are dispersed in a chilled emulsifier solution. The pre-suspension is subjected to high pressure homogenization at or below room temperature. In general, compared to hot homogenization, larger particle sizes and a broader size distribution are observed in cold homogenized samples[49]. The method of cold homogenization minimizes the thermal exposure of the sample, but it does not avoid it due to the melting of the lipid /drug-mixture in the initial step.

Microemulsion method

Addition of a microemulsion to water leads to precipitation of the lipid phase forming fine particles. This effect is exploited in the preparation method for SLN developed by Gasco[45].

Considering incorporation of shear and temperature-sensitive compounds such as DNA, albumin and erythropoietin, the HPH is not suitable and therefore, other preparation techniques; such as precipitation from microemulsion have been developed. Microemulsions are thermodynamically stable colloid mixtures of two immiscible solvents stabilized by an adsorbed surfactant film at the liquid–liquid interface. They can be prepared spontaneously by mixing surfactant, co surfactant, oil and water. Thus, no energy is required to prepare microemulsion, and the simplest representation of the structure of microemulsion is the droplet model with small droplet diameter, generally below 100 nm. Synthesis of nanoparticles in microemulsions is an area of considerable current interest[49].

To form a microemulsion with a lipid being solid at room temperature, the microemulsion needs to be produced at a temperature above the melting point of the lipid. The lipid (fatty acids and/or glycerides) is molten, a mixture of water, co-surfactant(s) and the surfactant is heated to the same temperature as the lipid and added under mild stirring to the lipid melt. A transparent, thermodynamically stable system is formed when the compounds are mixed in the correct ratio for microemulsion formation. This microemulsion is then dispersed in a cold aqueous medium (2-3°C) under mild mechanical mixing, thus ensuring that the small size of the particles is due to the precipitation. Volume ratios of the hot microemulsion to cold water are in the range of 1:25 and 1:50. Rapid recrystallization of oil droplet on dispersion in cold aqueous medium produces SLNs. Surfactants and co-

surfactants include lecithin, bile salts, but also alcohols such as butanol. Excipients such as butanol are less favorable with respect to regulatory aspects[52].

Solvent emulsification method

SLNs have been produced by solvent emulsification technique by Siekmann[53]. Lipid matrix is dissolved in water immiscible organic solvent (chloroform or cyclohexane) that is emulsified in an aqueous phase. Upon evaporation of the solvent, nanoparticle dispersion is formed due to the precipitation of the lipid in the aqueous medium[54].

The advantage of this procedure over the cold homogenization process described before is the avoidance of any thermal stress. Residues of organic solvents used in this method create toxicity problems and is the major disadvantage of this method.

Solvent diffusion method

The first step in the production of lipid nanoparticles by the solvent diffusion technique is to prepare a solvent in water emulsion with a partially water miscible solvent containing the lipid. Low toxic, water miscible solvents such as benzyl alcohol or butyl lactate were used. Upon transferring a transient oil-in-water emulsion into water and continuous stirring, droplets of dispersed phase solidify as lipid nanoparticles due to diffusion of the organic solvent[55].

Double emulsion method

Recently, a novel method based on solvent emulsification–evaporation for the preparation of SLN loaded with hydrophilic drugs has been introduced. Here, the hydrophilic drug is encapsulated along with a stabilizer to prevent drug partitioning to the external water phase during solvent evaporation in the internal water phase of a w/o/w double emulsion. In double emulsion technique, the drug was dissolved in aqueous solution, and then was emulsified in melted lipid. This primary emulsion was stabilized by adding stabilizer (e.g. gelatin, poloxamer-407). Then this stabilized primary emulsion was dispersed in aqueous phase containing hydrophilic emulsifier[56]. Thereafter, the double emulsion was stirred and was isolated by filtration. Double emulsion technique avoids the necessity to melt the lipid for the preparation of peptide-loaded lipid nanoparticles and the surface of the nanoparticles could be modified in order to sterically stabilize them by means of the incorporation of a lipid/-PEG derivative. Sterical stabilization significantly improved the resistance of these colloidal systems in the gastrointestinal fluids.

This technique is mainly used to encapsulate hydrophilic drug (peptides). A major drawback of this technique is the formation of high percentage of microparticles. SLNs loaded with insulin-mixed micelles (Ins-MMs) were prepared by a novel reverse micelle-double emulsion method, in which sodium cholate and soybean phosphatidylcholine were employed to improve the lipid solubility of insulin, and the mixture of stearic acid and palmitic acid were employed to prepare insulin loaded solid lipid nanoparticles. Some of the formulation parameters were optimized to obtain high quality nanoparticles. The particle size, zeta potential, entrapment efficiency (EE %) and drug loading capacity (DL %) were 114.7±4.68 nm, −51.36±2.04mV, 97.78±0.37% and 18.92±0.07%, respectively[57].

Homogenization followed by Ultra sonication

It is a simple, sensitive and reproducible method used to prepare SLNs. In brief, drug, lipid, and emulsifier were dissolved in a common solvent and evaporated under reduced temperature to obtain solvent free drug dissolved or dispersed lipid phase. Drug loaded lipid melt was homogenized with hot aqueous surfactant in solution using homogenizer to get coarse emulsion. The coarse emulsion so obtained was ultrasonicated using ultrasonicator to obtain nanoemulsion. SLNs are formed upon cooling to room temperature [58-63].

Solvent injection method

The basic principle for the formation of SLNs is similar to the solvent diffusion method. However, SLNs are prepared by rapidly injecting a solution of solid lipids in water miscible solvents into water. Mixture of water miscible solvents can be used to solubilize solid lipids. Normally used solvents in this method are acetone, ethanol, isopropanol, and methanol[64].

Other methods

Supercritical fluid

This is new technique for preparation of SLN giving the advantage of solvent less processing. SLN can be produced by the rapid expansion of supercritical carbon dioxide solutions. Carbon dioxide with 99.99% is good solvent for preparation of SLN by this method[65].

Membrane contactor method

This is a new method of preparation of SLN using a membrane contactor. 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 and the aqueous phase circulates inside the membrane module, and sweeps away the droplets forming at the pore outlets. SLNs are formed by the following cooling of the preparation to room temperature.

In this method different process parameters (aqueous phase and lipid phase temperatures, aqueous phase cross-flow velocity and lipid phase pressure, membrane pore size) influence the size of SLNs[66].

Co-flowing microchannel technique

Zhang et al. research group investigated a new method of production of SLNs in a co-flowing microchannel. The microchannel system assembled with inner and outer capillaries. A lipid-solvent phase obtained by dissolving lipid in a water-miscible solvent is injected into the inner capillary, while an aqueous phase with surfactant is injected into the outer capillary at the same time. When these two fluids meet in the outer capillary, the solvent in the lipid phase diffuses rapidly into the aqueous phase, resulting in the local supersaturation of lipid and finally formation of SLNs. This is a simple and easy approach to produce SLNs with small diameters and slight narrow size distribution[67]. The particle diameter was influenced by several factors, the velocities of the lipid-solvent and the aqueous phases, the lipid concentration and the surfactant concentration. Some of the drugs loaded into SLNs using different methods showed in Table 2 and 3.

Table 2: Different methods of preparation and drugs investigated

Method of preparation	Drugs investigated
Hot homoginization technique	All trans retinol Ibuprofen Camptothecin Dexamethasone Ketaconazole Alpha lipoic acid Beta carotene Daidzein Paclitaxel Diclofenac sodium Clotrimazole
Cold homoginization technique	Vinorelbine bitartarate Prednisolone
Microemulsion technique	Tobromycin Idarubicin Tamoxifen Curcuminoid Tea polyphenols Cyclosporine A Doxorubicin, Cisplatin Atazanavir Sulphate
Solvent emulsification method	Vinpocitins Tashinone 2A
Solvent diffusion method	Adefovir Paclitaxel Clobetasol propionate, Peptide(Gonodorelin)
Homogenization followed by sonication	Clozapine Lovastatin Nitrendipine
(W/o/w) double emulsion method	Insulin

Table 3: Various SLNs formulations studied by different researcher to improve the oral bioavailability of drugs

Drug	Method used	Inference	Ref
Adefovir dipivoxil	solvent injection	Improved oral BA	68
Arteether	HPH	Improved oral BA	69
Baicalin	Coacervation	Enhanced bioavailability	70
Capecitabine	HH-US	Improved BA and tumor targeting	71
Carvedilol	Microemulsion	Improved BA	72
Felodipine	HH-US	Improved BA	73
Isradipine	HH-US	Improved BA	74
Idarubicin	Microemulsion	Improved BA, modifies the PK and tissue distribution	75
Ketoconazole	HH-US	Improved BA	76
Lacidipine	HH-US	Improved oral BA	77
Methotrexate	Solvent diffusion method	improved BA	78
Nimodipine	HPH	Enhanced bioavailability	79
Nisoldipine	HH-US	Improved BA	80
Nisoldipine	HH-US	Improved BA	81
Peptides/proteins	Cold homogenization	Improved stability and permeability	82
Puerarin	Solvent injection method	Improved BA	83
Quercetine	Emulsification-solidification	SLNs are carrier to enhance the absorption	84
Rifampicin, Isoniazid and Pyrazinamide	Emulsion-solvent diffusion	Improved BA and stability, Reducing dosing frequency	85
Rosuvastatin calcium	HH-US	Improved BA	86
Rosuvastatin calcium	HH-US	Improved BA	87
Ropinirole	HH-US	Improved BA and brain delivery	88
Raloxifene hydrochloride	Solvent emulsification/evaporation	Bioavailability enhanced	89
Simvastatin	Solvent injection	Improved BA	90
Tobramycin	Microemulsion	Improved BA, sustained drug release, lymphatic Targeting	91
Vinpocetine	Ultrasonic-solvent emulsification	Improved oral BA by increased saturated solubility and reduced metabolism	92
Zaleplone	HH-US	Improved BA	93

Conclusion

Different methods have been industrialized for the fabrication of SLNs with the possibility to obtain different size and shape. The size of the SLNs can affect the pharmacological properties of the particles, but it is not the unique parameter considered to compare the various techniques. From a technological point of view, the opportunity to scale-up the process is very vital, but also the viability of the method is appropriate, in fact, the usage of expensive and complex machine can obstruct the production on large scale manner.

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