Chapter 13
Solid Lipid Nanoparticles

Akhlesh Kumar Jain and Suresh Thareja

13.1 Introduction

The area of Novel Drug Delivery System is getting wider day by day in expanded area of biomedical science, bioengineering and nanotechnology (Ekambaram et al. 2012). Most of the latest delivery techniques explore nanosize-based particles, i.e. nanocarriers having the API (Shah et al. 2011). Few important drug carriers developed using nanotechnology-based approaches are nanoemulsion, nanosuspension, nanocrystals, nanoparticles and solid lipid nanoparticles (Jain 1997). Recent advances in the development of nanocarriers have started a new era in Formulation Science. Solid lipid nanoparticles (SLNs) were reported in 1991 as an unconventional carrier system to typical colloidal carriers such as emulsions, microemulsions, self micro-emulsifying drug delivery system, micellar systems, liposomes, polymeric microparticles and nanoparticles (Ramteke et al. 2012).

SLNs mingle advantages of the conventional carriers along with circumventing some of their major disadvantages. SLNs showed potential applications in drug, gene and vaccine delivery along with controlled and site-specific drug targeting. SLNs are effortlessly made nanoparticles composed of biodegradable polymers of high stability devoid of significant toxicity as well as commercially economic and could incorporate wide variety of drugs for effective targeting. SLNs are novel lipid-based formulations constituted exclusively of biodegradable lipids such as highly purified triglycerides, monoglycerides, complex glyceride mixtures, hard fats or even waxes, which turn solid at room temperature. Solid lipid nanoparticles are nanometre-sized particles that range from 50 to 200 nm and made of solid hydrophobic core which are suspended in aqueous phase containing surfactant.

A. K. Jain (✉) · S. Thareja
School of Pharmaceutical Sciences, GGV Central University, Bilaspur, Chhattisgarh, India

I. Bhushan et al. (eds.), *Nanomaterials and Environmental Biotechnology*,
Nanotechnology in the Life Sciences,
https://doi.org/10.1007/978-3-030-34544-0_13

The drug is dissolved or dispersed in solid core contains the solid high melting fat matrix. Both kinds of lipophilic or hydrophilic therapeutics and diagnostics could be incorporated into the SLN (Shah et al. 2011). SLNs not only unite the advantages of emulsion, liposomes and solid polymeric nanocarriers together but also eliminate few of their disadvantages. Major advantages included are biocompatibility and biodegradability, avoidance of drug leakage, stability against coalescence, nontoxicity, hydrolysis, physical stability and being an excellent carrier for lipophilic drugs (Cavalli et al. 2002). Lipid emulsion and liposomes are entirely different. Oil core made of a neutral lipid, covered by monolayer of amphiphilic lipid, makes lipid emulsion, whereas liposomes are bilayer lipid vesicles made of amphiphilic phospholipid having an interior aqueous cavity (Jain 1997). On the other hand, SLNs are designed from solid lipids and stabilized with an aqueous suspension of emulsifying agents. They look a lot like nanoemulsion; the only difference is that liquid lipid is replaced with a solid lipid, hence providing an outstanding opportunity for controlled drug release as solid lipid lowers the movement of encapsulated drug drastically compared to liquid oil phase (Martins et al. 2007). Also, encapsulation in solid lipids improves the stability of incorporated chemically sensitive lipophilic ingredients in contrast to liquid lipids of nanoemulsion. These prospective benefits of physicochemical properties associated with the physical state of the lipid phase are as follows:

(i) Movement of reactive radicals in solid material is slower compared to liquid medium and hence limits the degradation pathways.
(ii) Phase partition of the API and lipid phase into the solid lipid thus prevents leaching of drugs at the surface of SLN.
(iii) Enhanced absorption of inadequately absorbed drugs is reported after administration using SLN.

Large-scale production of SLNs could be achieved out in a cost-effective and relatively simple manner using high-pressure homogenization technique. Another approach for the production of SLNs is microemulsions or simply suspending liquid lipid in a solution of surfactant with stirring and sonication. SLNs made using various methods are present in suspension form; hence storing for prolonged period of time showed instability due to hydrolysis reactions. However conversion of SLNs into dry powder which can be reconstituted in order to improve stability of SLNs, with the help of lyophilization or spray drying, is an excellent way (Sinha et al. 2010). SLNs provide an excellent opportunity as an advanced drug carrier for oral delivery, topical administration, pulmonary administration, parenteral administration, gene delivery and potential adjuvant for vaccines. In a nutshell, they propose an extremely versatile platform for second- and third-order targeting of drugs.

The major advantages associated by SLNs are as follows:

(a) Suitable for controlled drug release and drug targeting.
(b) Suitable for delivery of both hydrophilic and lipophilic drugs.
(c) Reduced toxicity compared to polymeric nanoparticles as SLNs are made of biocompatible lipids.

(d) Provide protection to labile drugs from chemical, photochemical and oxidative degradation.
(e) Water-based technology (organic solvents can be avoided).
(f) SLN could be administered through various routes such as oral, pulmonary, intravenous, ophthalmic and dermal (Rabinarayan and Padilama 2010).

13.2 Composition of Solid Lipid Nanoparticles

Common ingredients used for preparation of solid lipid nanoparticles are solid lipid(s), stabilizers mainly surfactants and water. The word lipid is having broader sense here and also inclusive of fatty acids (e.g. stearic acid), steroids (e.g. cholesterol), triglycerides (e.g. tristearin), partial glycerides (e.g. Imwitor) and waxes (e.g. cetyl palmitate). All the varieties of emulsifying agents, irrespective of their molecular weight and net charge, could provide the desired stability to the SLN suspension. Sometimes, single surfactant unable to stop particle agglomeration effectively hence the combination of emulsifiers might be required for proper stabilization. The selection of emulsifiers is generally made as per the application and mode of administration of SLN. An obvious benefit of SLN is that the lipid mixture contains physiological lipids and hence limits the toxicity. Most of the additives used for preparation of SLNs are not harmful and falls in the generally recognized as safe (GRAS) category list of the Food and Drug Administration (FDA). SLN is composed of lipids and of stabilizers.

13.2.1 Lipids

In the last two and half decades, SLN proved to be a runaway success among nanocarriers because SLN not only "combine the few benefits of fatty emulsifying agents, liposomes and polymeric nanoparticles, but also have the ability to overcome major drawbacks of these carriers" (Muller and Runge 1998). Few problems associated with SLNs such as crystallinity and low entrapment have been overcome by nanostructured lipid carriers (NLC) (Patlolla et al. 2010). Lipids composed of mainly oily or waxy materials (fatty acids, their alcohols, their glycerol esters, i.e. phospholipids, glycolipids and sphingophospholipids) are having low solubility in polar solvents and freely miscible in organic solvents. The core of the SLN comprises of the lipid; this core itself determines pharmaceutical properties of the particles such as drug stability and drug release.

Generally, two or more lipid combinations are useful because it allows manipulation of encapsulation efficiency and release kinetics and also the physical characteristics, i.e. melting point, crystallinity and polymorphism. A list of various types of lipids can be chosen for development of SLN, and NLC is reported in Table 13.1.

Table 13.1 Commonly used lipids in the preparation of SLN and NLC

S. no.	Triglycerides	Free fatty acids	Free fatty alcohols	Waxes	Miscellaneous
1	Glyceryl tristearate	Behenic acid	Stearyl alcohol	Cetyl palmitate	Castor oil
2	Glyceryl tripalmitate	Palmitic acid and palmitoleic acid	Cetyl alcohol	Beeswax	Hydrogenated castor oil
3	Glyceryl trimyristate	Oleic acid	Myristyl alcohol	Carnauba wax	Hydrogenated palm oil
4	Medium chain triglycerides	Myristic acid	Lauryl alcohol	–	Anhydrous milk fat
5	Glyceryl trioleate	Stearic acid	–	–	Cacao butter
6	Tricaprylin	Arachidic acid and arachidonic acid	–	–	Goat fat
7	Tricaprin	Lauric acid	–	–	–
8	Trilaurin	Linoleic acid or linolenic acid	–	–	–

13.2.2 Surface-Active Compounds (SACs)

Once two phases are immiscible into each other (e.g. oil and aqueous) and mixed together, one phase is dispersed into the other phase, and an interfacial boundary is formed. Surface-active agents reduce this interfacial tension and stabilize the dispersion. Nevertheless, surfactant properties are associated by their chemical structure, and they could reduce the surface tension only up to a specific level which is sometimes not enough to stabilize the dispersion. Additional decrease can be carried out by the addition of cosurfactants (Solans and Sole 2012). The climbing of temperature diminishes the surface tension and hence could be utilized to manufacture hot emulsions with small energy involvement (Webster and Cates 2001). In addition to main constituents, a variety of other additives can also be required to be added such as cryoprotectants (trehalose, sorbitol) (Soares et al. 2013) and stabilizers which act through other mechanisms (polyvinyl alcohol) (Xie et al. 2011). Table 13.2 shows a list of excipients used for preparation and stabilization of SLN.

13.3 Techniques Used for Preparation

13.3.1 High-Pressure Homogenization

The basic method used for production of SLN is high-pressure homogenization which is a consistent and dominant practice, first of all explored for synthesis of SLNs. High-pressure homogenization can be carried out at higher temperature (hot HPH technique) or at under room temperature (cold HPH technique) (Muller and Lucks 1996). In both techniques, the lipid and drug are melted approximately

Table 13.2 List of various excipients used for preparation and stabilization of SLN

S. no.	Nonionic				Anionic	Cationic	Amphoteric	Cosurfactant
	Polyoxyethylene sorbitan fatty esters	Polyoxyethylene alkyl/aryl ethers	Poloxamers	Others				
1	Polysorbate 20	Tyloxapol	Poloxamer 407	Polyglyceryl-6 distearate	Sodium lauryl sulphate	Dimethyldioctadecy-lammonium bromide	L-α-phosphatidylcholine	1-Butanol
2	Polysorbate 60	Polyoxyethylene (20) stearyl ether	Poloxamer 188	Polyglyceryl-3 methylglucose distearate	Sodium dehydrocholate	Cetrimonium bromide	Egg lecithin	Diethylene glycol monoethyl ether
3	Polysorbate 80	Polyoxyethylene (20) cetyl ether	–	PEG caprylic/capric triglycerides	Sodium taurocholate	DOTAP	Soya lecithin	Low molecular weight PEG
4	Polysorbate 85	Polyoxyethylene (20) isohecadecyl ether	–	Macrogol (15)-hydroxystearate	Sodium cholate	Chlorhexidine salts	–	Propylene glycol
5	–	–	–	Polyoxyethylene glyceryl monostearate	Sodium glycocholate	DOTMA	–	Sorbitan monostearate

5–10 °C above the melting point of the lipid. High-pressure homogenizers push liquid through a narrow gap at high pressure (100–2000 bar) which accelerates the liquid particles to extremely higher speed (over 1000 km/h). Such higher forces and cavitation stress break the lipid droplets to the nanometric level. Usually, 5–10% lipid ratio is good enough to prepare the SLN, but up to 40% lipid content can be processed using this technique.

13.3.1.1 Hot Homogenization

In hot homogenization technique, the lipid melt containing the drug is dispersed under stirring in aqueous solution of surfactant at similar higher temperature to the melt. The resulted pre-emulsion is homogenized through a temperature-directed high-pressure homogenizer; usually three cycles (500 bar) are sufficient, followed by cooling of hot O/W nanoemulsion to room temperature, which leads to recrystallization of lipids in the form of solid lipid nanoparticles. Figure 13.1 provides a graphical illustration of SLN production using hot homogenization method. Generally, hot homogenization method can be used for hydrophobic and poorly soluble drugs. As the exposure period to elevated temperature is comparatively low, hence, majority of thermolabile drugs could be encapsulated into the SLNs before being degraded. However, this technique is not suitable for hydrophilic drugs because higher partitioning of drugs in to the aqueous phase during homogenization

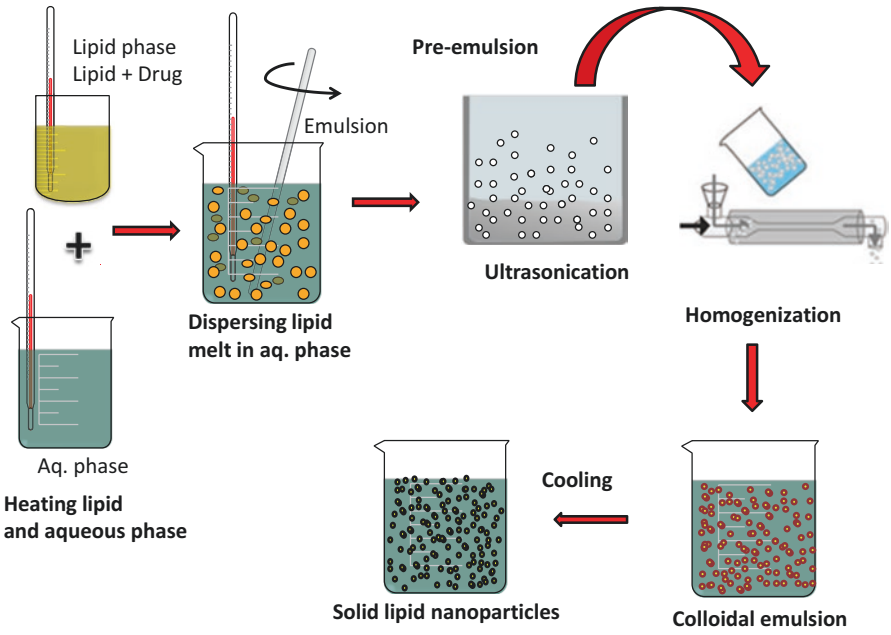

Fig. 13.1 Schematic depiction of various steps involved in the hot homogenization technique

process results in low entrapment efficiency (Gohla and Dingler 2001; Schwarz et al. 1994).

The properties of the final product are affected by the conditions at which pre-emulsion is processed, and it is desirable to obtain droplets in submicron range. Usually, higher temperatures produce smaller particle size because of the reduced viscosity of the internal phase. Nevertheless, elevated temperatures fasten up the degradation process of the drug as well as of matrix. Also, homogenization process can be repeated several times. In majority, 3–5 homogenization rounds at 500–1500 bar are enough to obtain the desired size. It should be remembered that the high homogenization pressure or greater number of cycles often results in an increase of the particle size because of the occurrence of particle coalescence due to high kinetic energy of the particles. Moreover, high-pressure homogenization produces a rise in the temperature of the sample (approximately 10 °C for 500 bar).

13.3.1.2 Cold Homogenization

The cold HPH is a suitable technique for processing temperature-sensitive drugs and/or hydrophilic drugs. During cold homogenization method, the lipid mixture containing the drug is cooled down to the melting temperature of the lipids; therefore, the solid lipid ground to lipid microparticles which are dispersed in a cold surfactant solution yield a pre-suspension. Pre-suspension of microparticles is homogenized at or below room temperature; the cavitation forces produced by the homogenization process are strong enough to rupture the particles into solid lipid nanoparticles. Strict control of temperature is required in order to make sure that the lipid remains in the unmolten state even after increase in temperature during homogenization. Initial step is similar to hot homogenization that comprises the dispersing or solubilization of the medicament into the lipid mixture. Second step is rapidly cooling of the melt which leads to uniform pattern of API in the lipid particles. Also, reduced temperatures enhance the delicateness of the lipid and ultimately particle comminution. Figure 13.2 provides a stepwise production of SLNs using cold homogenization method. Another way to minimize the loss of hydrophilic drug to aqueous phase is to replace water with other suitable media in which the drug is having low solubility (e.g. oil or PEG 600) (Rabinarayan and Padilama 2010).

Cold homogenization process has an edge over hot homogenization as it eliminates or limits the three major problems associated with the hot homogenization technique.

(a) Heat-induced drug degradation.
(b) Partitioning of the drug into the aqueous phase during homogenization.
(c) Crystallization step of the nanoemulsion leads to complexity and several modifications.

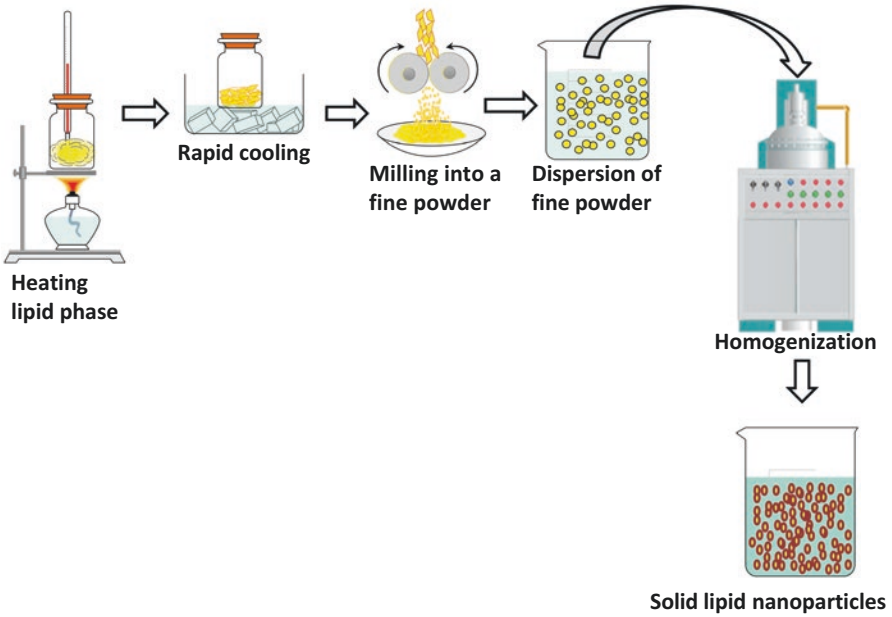

Fig. 13.2 Schematic depiction of various steps involved in the cold homogenization technique

13.3.2 Ultrasound Dispersion/Ultrasonication

Ultrasonication depends on the cavitations in hydrophilic phase that break down lipid matrix into finer particles. The acquired hot microemulsion is allowed to cool to solidify the droplets. The drug is added to the melted lipid; the heated aqueous phase is added to the melted lipid and subjected to high-speed stirring to prepare a nanoemulsion. Pre-emulsion of liquified lipid and heated surfactant mixture is formed, and ultrasound is supplied through probe which is in direct touch of the liquid. SLNs of lower size are formed using ultrasonication and high-speed homogenizer techniques. This technique has the possibility for scale up with flow cells, without moving elements (ease in cleaning), and allows to manage the procedure by changing the energy supplied by sonication. Major shortcoming is the chances of metal adulteration that goes higher with prolonged sonication times and physical instability due to growing solid particles while stirring the SLNs.

13.3.3 Solvent Emulsification/Evaporation

In this technique, water-immiscible organic solvents like cyclohexane, dichloromethane, toluene and chloroform are used for dissolving the hydrophobic drug and the lipid components. Further, the mixture is emulsified with a hydrophilic solution using a high-speed homogenizer. In order to get smaller-sized SLN, the coarse

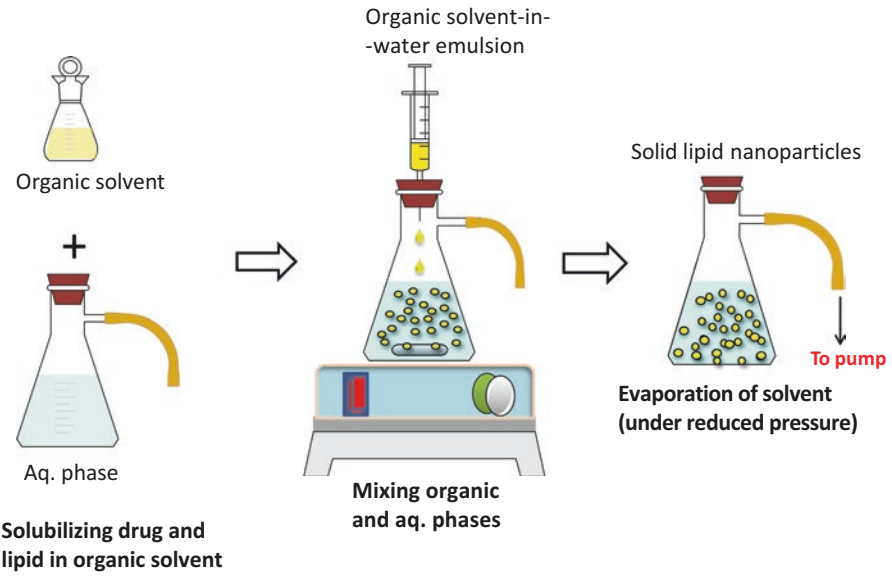

Fig. 13.3 Schematic depiction of various steps involved in the solvent evaporation technique

emulsion is immediately processed through the microfluidizer which produces nanoemulsion. Later on, this nanoemulsion was mechanically stirred at room temperature using rotary evaporator which evaporates the organic liquid evaporation under reduced pressure (40–60 mbar) and leaves lipid precipitates of SLNs (Siekmann and Westesen 1996). Figure 13.3 provides a stepwise demonstration of various aspects involved in the solvent evaporation method. The particle size of the SLNs can be modified by varying the concentration of lipid in organic phase. Generally, finer particle size could be obtained by keeping the lipid load below (Pinto and Muller 1999). Avoidance of high heat during the method is advantageous, and the produced SLN suspension can be centrifuged to collect the SLN.

13.3.4 Microemulsion-Based Technique

This method is based on the dilution of microemulsions. Microemulsions are clear or transparent biphasic liquid composed of a lipid phase and aqueous phase which are made stable using surfactant and also a cosurfactant in most of the cases. They are made by mixing optically transparent mixture composed of fatty acid (i.e. stearic acid), an emulsifier (i.e. polysorbate 20), co-emulsifiers (i.e. butanol) and water at 65–70 °C. The lipid chosen should have a low melting point. Additives like butanol are less preferred with respect to regulatory aspects. The microemulsions show properties of emulsions as well as a real solution. Addition of a microemulsion to water leads to the formation of fine particles and hence could be exploited as method of preparation of SLN (Gasco 1993). Because of the involvement of a dilution step,

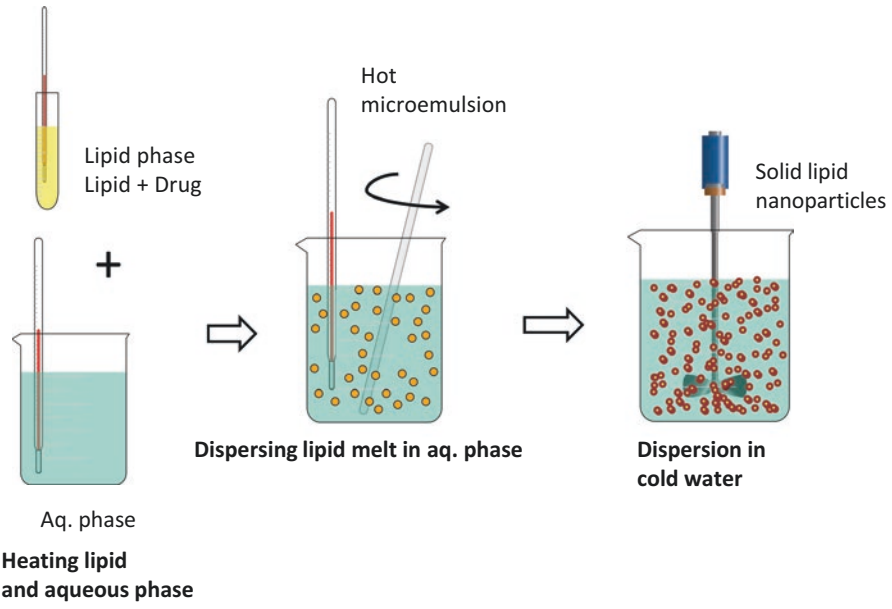

Fig. 13.4 Schematic depiction of various steps involved in the microemulsion-based technique

noticeably inferior concentration of SLN has been produced compared to HPH method. First of all, a hot microemulsion is prepared by stirring 10% molten solid lipid, 15% surfactant and up to 10% of cosurfactant with water. This hot microemulsion is diluted with cold water (1:50) through an especially designed thermostated syringe. The mixture is kept on stirring while dilution which leads to nanoprecipitation of lipids. Figure 13.4 provides a stepwise illustration of production of SLNs using microemulsion-based method. Large-scale production of SLN is possible using microemulsion technique. The microemulsion is produced in a large tank which is heated through a temperature-controlled device. Later on, microemulsion is diluted by transferring it to a cold water tank where lipid precipitation takes place (Carli 1999). Excess water could be taken by ultrafiltration or by lyophilization in order to make concentrated SLN. Also, microwave-assisted technique for formation of microemulsion is another way of preparing SLN based on microemulsion technique (Fig. 13.5).

13.3.5 Double Emulsion Method

So far, numerous methods have been developed to prepare SLN, for example, high-speed homogenization, ultrasound divergence and high-pressure homogenization. In the last few years, microemulsion method has emerged quickly due to its simple operations and so on, compared to previous method. Nevertheless, SLNs prepared

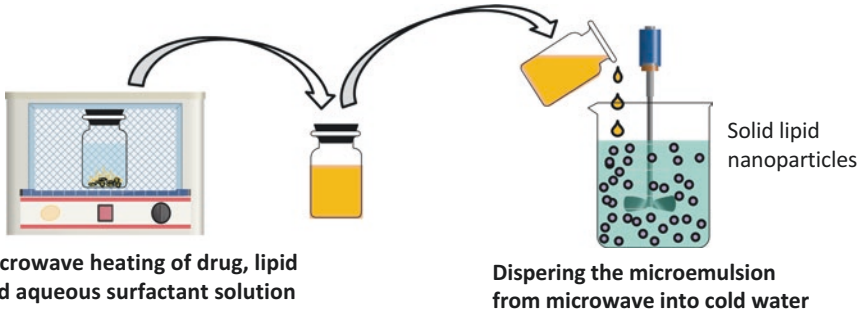

Fig. 13.5 Schematic depiction of various steps involved in microwave-assisted technique of microemulsion formation for SLN production

by microemulsion method have few disadvantages, particularly limited loading capacity of some water-soluble drugs because water-soluble drugs diffuse out from oil phase to aqueous phase while in emulsification process (Shi et al. 2011). The double emulsion technique was established to solubilize agents having high aqueous solubility in the internal aqueous phase of a W/O/W emulsion, with the aid of a stabilizer to prevent partitioning of the aqueous soluble drug to the external aqueous phase during solvent evaporation.

In this method the drug is dissolved into an aqueous solution which is emulsified with melted lipid mixture in order to form primary W/O emulsion stabilized with suitable excipients (e.g. gelatin, poloxamer 407). Further, the primary W/O emulsion is re-emulsified with hydrophilic solution of emulsifying agents to produce a double W/O/W emulsion. Stirring of W/O/W emulsion allows formation of SLNs which are isolated by filtration. Using this method, relatively large particles are produced, however, allow incorporation of higher quantity of hydrophilic molecules and also provide the opportunity of surface alterations (e.g. with PEGs). Double emulsion method can also keep away the need of melting the lipid for the development of peptide/protein-loaded lipid particles.

13.3.6 Membrane Contactor Technique

This method based on simple step of passing lipid through pores of a membrane with pressure at above the melting point of the lipid leads to formation of small droplets. The holes in the membrane work as parallel capillaries for entrance of the lipid phase. Simultaneously, hydrophilic phase is stirred constantly and flows tangentially inside the membrane module and brush off the droplets being produced at the hole openings. SLNs were produced by the cooling of the formulation at the RT. Initially, materials were kept in the thermostated bath to uphold the desired temperature, and N_2 was purged to generate the pressure for the liquid phase. Figure 13.6 provides a stepwise graphical view of SLN production using membrane

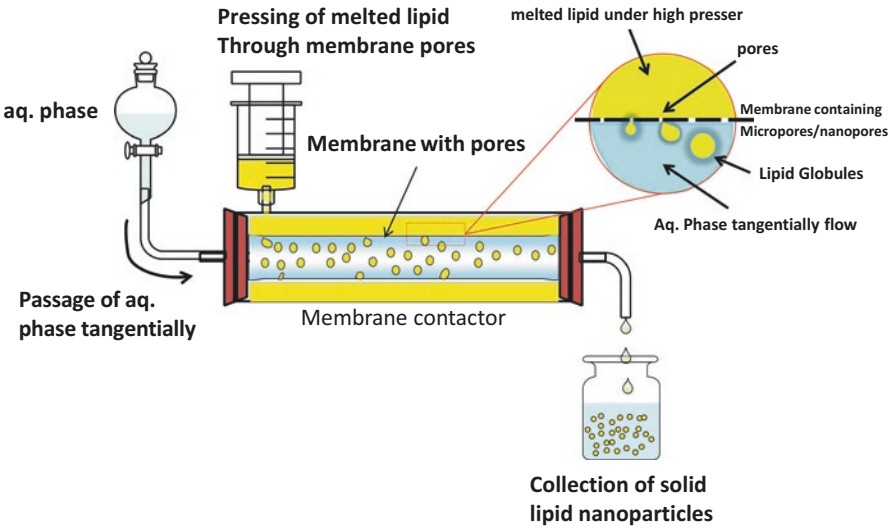

Fig. 13.6 Schematic depiction of various steps involved in the in the membrane contactor-based technique

contactor. The various process parameters (aqueous and lipid phase temperature, the lipid phase pressure, aqueous phase cross flow velocity, lipid phase amount and membrane pore size) affect the size of SLN formed.

The membrane contactor method can also be used for the preparation of polymeric nanoparticles, by methods involving a polymerization of dispersed monomers (interfacial polymerization method) or a dispersion of preformed polymers (nanoprecipitation method). The advantage of membrane contactor method is to manage the size of SLN by a suitable selection of membrane pore size and process parameters as well as it's scaling-up ability (Rabinarayan and Padilama 2010). Higher lipid content not only increases the mean particle diameters but also deteriorates membrane performance. The temperature and flow speed of the hydrophilic phase affect the diameter of SLN (Charcosset et al. 2005). The selection of emulsifying agents and their concentration influences the lipid flux and the diameter of SLNs (El-Harati et al. 2006).

13.3.7 Supercritical Fluid (SCF) Technology

Supercritical fluid (SCF) technique is a comparatively novel method used for preparation of SLN and however has been used for preparation of microparticles and drug powder (Yasuji et al. 2008). It has been proved to be useful alternatives for drying protein formulations as it has major advantage of solvent-less processing in addition to its low toxicity, relatively low critical temperature as well as cost-effectiveness. A fluid is considered as supercritical at the stage once its temperature and pressure go

above their correspondence critical value. A gas does not liquefy by increasing the pressure above the critical temperature. Upon increasing the pressure, the density of the gas raises lacking any noteworthy enlargement in viscosity, whereas the capability of the fluid to solubilize the agents too enhances.

The gas which has minute solubility property to dissolve a compound under ambient condition can behave as super solvent for no. of compound under supercritical state. Various gases, i.e. CO2, ethane CH2FCF3 and ammonia, were investigated and tried, but CO2 has proved itself as the best for SCF technique. The principle behind SCF technology is precipitating out of drug or particles by compressed anti-solvent, i.e. supercritical carbon dioxide (Yang and Ciftci 2016). The lipid and drug are dissolved in a solvent; the supercritical fluid, selected to act as anti-solvent to the solutes, is completely immiscible or incompletely soluble with the solvent. The dissolved solid in solvent is sprayed into the flowing SCF and leads to precipitation of solid solute particles (Byrappa et al. 2008).

Another method of SLN production using SCF method is considered as "supercritical extraction of emulsions" (SFEE) (Chattopadhyay et al. 2007). The lipoidic substances and the drug are solubilized in an lipophilic liquid like chloroform. The organic solution containing surfactant is suspended with a hydrophilic solvent containing cosurfactant. The resultant product is subjected to high-pressure homogenizer in order to produce O/W emulsion which enters from the top of the extraction column at a steady speed, and countercurrently the SCF is entered at a steady flow rate. Solid lipid nanoparticles are produced as dispersion into the SCF.

13.4 Characterization of Solid Lipid Nanoparticles

As discussed previously, SLNs are generally produced by homogenizing a suspension of lipids and aqueous phase. Characterization of SLN is a challenge due to the smaller size of the particles and the complexity of the system. Many important aspects have to be considered which might affect the stability and drug release kinetics of SLN. Particle size, size distribution and components of the nanocarriers, morphology and minute structure are vital characteristics of SLNs which affect encapsulation efficiency, stability and release pattern. All these factors point out that the performance of lipid nanoparticles—drug incorporation, release and stability—is influenced by particle size, shape and structure.

13.4.1 Physical Properties

13.4.1.1 Size and Its Distribution

Particle size is a main factor which ensue the in vivo fate of SLNs. Particles larger than 5 μm are restricted for parenteral route as these might block capillary and ultimately can cause embolisms. Clearance of particles from the circulation by the

reticuloendothelial organs is also a size-based phenomenon (Wu et al. 2012). Formulation-related variables (lipid nature, dispersing medium, surfactant, cosurfactant and other additives) as well as process variables (preparation method, extent of homogenization and sonication, temperature, equipment) are generally referred to as quality constraints. Size determination study of SLNs is carried out by scattering-based sizing techniques like photon correlation spectroscopy and/or laser diffraction. Particle size and morphology, therefore, may affect the entrapment and release kinetics of the therapeutics from the lipid nanoparticles.

Photon Correlation Spectroscopy

Photon correlation spectroscopy (PCS) or dynamic light scattering (DLS) is the mostly preferred technique to study the particle diameter of solid lipid nanoparticles in a suspension (Obeidat et al. 2010). It measures the fluctuation of the intensity of the scattered light which is caused by particle movement. PCS generally requires a tiny amount of sample, and that does not require any laborious sample preparation. It is nondestructive, faster technique which can determine the size range from 3 to 3000 nm approximately. PCS analyses the Brownian motion or random thermal of the particles in the suspended state in a dispersion medium. Particles in dispersion are irradiated by a laser light, and the changes in the intensity of scattered light are measured after multiple collisions of particles as a function of time. The PCS device has a laser source, which falls on the dispersion in a sample cell (temperature controlled), and scattered light is detected using a photomultiplier. Smaller particles cause high-intensity fluctuations compared to larger particles, because of their high diffusion coefficient. A statistical relationship is applied to correlate the scattering intensity vs time curve to obtain particle diameter and its distribution.

The technique gives the particle size, in terms of z-average diameter and polydispersity index (PDI) that is an indicative of the total length of the particle size distribution. The lower PDI is considered to be narrow particle size distribution, i.e. monodisperse suspension. Higher PDI values indicated poor uniformity of size distribution pattern. Most of the scientists recognize PDI values less than 0.3 as optimum values; however, 0.5 value of PDI is also sometimes accepted. Even though PCS is the extensively established technique, it is based on the translational diffusion coefficient D of the particles; hence it is an indirect method for measurement of particle size. Although PCS is a consistent method for analysing particles having tight distribution pattern, it is not recommended for multimodal size distributions. As presence of larger aggregates or microparticles may hinder the movement of nano range particles hence have a noteworthy effect on measurement process. SLN suspension is generally diluted in order to decrease manifold scattering effects. In addition PCS assumes that all the particles are of spherical shape; however, this is of least importance for SLNs but is a matter of concern for other colloidal carriers.

Laser Diffraction (LD) Spectroscopy

LD is an effective tool which has a broader detection range (20 nm–2000 μm). Laser diffraction (LD), which is also known as laser light scattering, can be used alone or in combination with PCS to obtain an entire population of particle size from tiny ones to large particles. LD is depending on the composite phenomenon of Fraunhofer, Mie and Rayleigh scattering from an illuminated particle in dispersion state. Principle behind LD is that once a laser light falls on a sample having suspended particles, the bigger particles scatter light at narrow angles whereas the minor ones at broader angles (Stoye et al. 1998). Nevertheless the technique deficient in precision for particle dispersions has drastically smaller diameter compared to laser wavelength.

In addition, laser diffractometer provides a good estimation of polydispersity of particles; hence LD covers a broader size range from nanometre to 100 micron. The main limitation of this theory is that its application to SLN demands knowledge refractive index at the measurement wavelength as results are strongly dependent on these optical parameters. The development of PIDS technology (Polarization Intensity Differential Scattering) greatly enhanced the sensitivity of LD to smaller particles (Jores et al. 2004). This technique merges wavelength dependence and polarization effects together and hence significantly increases the accurateness of LD towards tiny nanoparticles.

13.4.2 Microscopic Methods

13.4.2.1 Shape and Surface Morphology

Microscopic techniques such as electron microscopy (EM) and atomic force microscopy can also be used to get information on particle size in the nanometric range. In contrast to light scattering-dependent method, individual particles are measured by the Coulter counter and microscope techniques; hence these provide the benefit of a closer straight analysis. In addition, the results generated by PCS and LD could be affected by the anisometric morphology of the particles. Therefore, the data produced by PCS and LD could be corroborated with EM to determine the real size of the SLNs.

Morphology usually narrates to the external structure and surface pattern of the particle, while ultrastructure narrates to the internal structure of the particle. Ultrastructure indicates the existence and orientation of the different portions of the formulation and nanoparticle as a whole as well as associated structures like micelles, a core-shell structure which may or may not coexist. Generally, spheres have the minimum likely surface area out of other possible shape and, hence, could be stabilized with minimum quantity of surfactant. In addition, sphere particles have the greatest diffusion length and allow the opportunity of sustained and predetermined release of encapsulated therapeutics. In contrast, anisometric shape desires a

larger quantity of surfactant for stabilization. It is highly required that the drug is to be entrapped into the surfactant layer or adsorbed onto outer layer.

Electron Microscopy

Electron microscopy provides the direct information on the particle shape. However, special concern is possible artefacts which may arise by the sample preparation procedures. For example, solvent removal may influence the particle shape. These techniques utilize the electron beam as an alternative of light to visualize nanoparticles.

Scanning electron microscopy (SEM) measures electrons sprinkled from the outer layer of the sample to largely create the surface structure, whereas transmission electron microscopy (TEM) measures electrons transmitted through the sample and allows to look beyond the surface. Both techniques have some drawbacks associated with processing conditions (e.g. vacuum, heating) as well drawbacks associated with sample preparation, i.e. dehydration, conductive coating staining, etc.; sometimes changes can be induced within the nanoparticles, while sample preparation and analysis might produce invalid results.

TEM is also being used to determine the morphology and ultrastructure of SLNs (Friedrich et al. 2010). Sample preparation steps involve negative staining, freeze-fracture and vitrification to study the nanocarriers by TEM. Negative staining is carried out by utilizing heavy metal salt solutions on to the colloidal lipid dispersions (Blasi et al. 2013; Silva et al. 2011). A tiny droplet of SLN dispersion is kept on a TEM, grid stained with osmium tetroxide, phosphotungstic acid or uranyl acetate. Also, the resulting TEM photomicrographs generally may not be fair representations of the whole SLN population. Mainly TEM techniques provide information of two-dimensional projection of SLN; hence in order to get three-dimensional anisometric particles and to get additional information on the ultrastructure, freeze-fracture technique is utilized. The TEM micrographs of SLN after coating with uranyl acetate is shown in Fig. 13.7, which shows that SLNs have spherical shape and size from 80 to 200 nm (Li et al. 2006).

Atomic Force Microscopy

Atomic force microscopy is a superior method which can be used to study the realistic shape and surface morphology of the SLN. AFM offers few additional advantages compared to TEM and SEM. As vacuum is not required, the sample needs not to be coated by a conductive substance; however, the sample should be immobilized prior to scanning which can be easily done by removal of the aqueous media from SLN dispersion. Precaution needs to be taken as process of water removal can bring

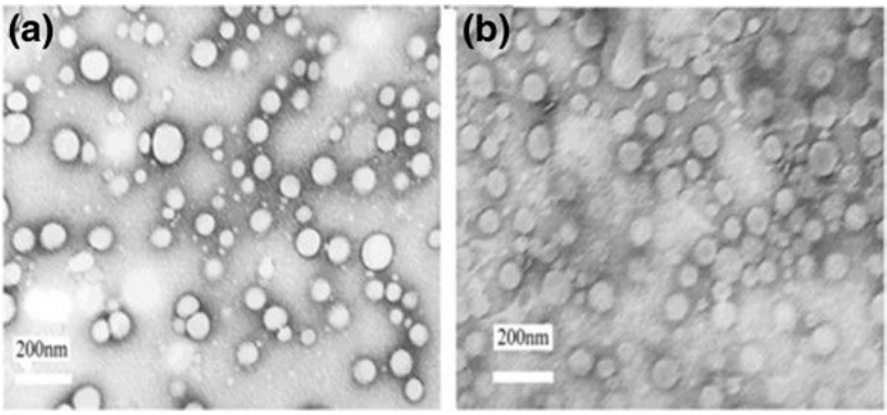

Fig. 13.7 Transmission electron microscopy photographs of solid lipid nanoparticles after staining with 1% uranyl acetate solution. (**a**) 5-FU loaded Cetyl palmitostearate SLNs (CP) (**b**) 5-FU loaded glyceryl monostearate (GMS) SLNs (Reprinted from Shenoy et al. (2013), with permission from Springer)

alterations linked with shrinkage, clustering and crystallization of the lipid (Shakesheff et al. 1994). Another strong benefit of AFM is that it can also provide the important details about soft surfactant layering surrounding the particles that is not possible with other similar techniques (Gref et al. 1994).

13.4.3 Surface Charge

The measurement of electric charge permits forecasting the long-term storage stability of SLNs. Generally, particle clustering occurs slightly for charged particles (high zeta potential) because electrical repulsion takes place between them. Though it is not tightly followed by the systems stabilized with steric stabilizers as adsorption of steric stabilizer around the particles diminishes the zeta potential but prevents aggregation (Müller 1996), zeta potential provides an idea about the amplitude of the electric attraction, or repulsion takes place among SLNs. Generally, greater zeta potential (e.g. more than +30 mV or less than −30 mV) stabilizes the SLN dispersion (Saupe et al. 2006) which prevents the contact between the particles. Particle cell interactions can also be affected by surface. However, colloidal particles having zeta potential closer to zero can express good long-term stability if they are sterically stabilized with the help of stabilizers (Okonogi and Riangjanapatee 2013). Zeta potential is affected by the pH, type and concentration of ions present; hence, the effect of pH and electrolyte on the stability of SLNs has been studied (Choi et al. 2014).

13.4.4 Drug Encapsulation and Loading Capacity

Huge varieties of drugs have been incorporated into the SLN. The most critical parameter to analyse the appropriateness of a drug carrier is its loading capacity which is generally expressed in percent related to the lipid phase. The various factors which influence the loading capacity of drug in the lipid are drug solubility in melted lipid, mixing efficiency of lipid and polymorphs.

The precondition to attain a considerable drug encapsulation is an adequate solubility in the lipid matrix, and drug solubility should be greater than desired as it diminishes upon cooling down the lipid matrix and could be lesser in the solid lipid. To encourage drug solubilization, solubilizers can be added to the lipid melt. More complex lipids rather simple mixtures of mono-, di- and triglycerides and also containing fatty acids of various chain lengths produce crystals with many imperfections allowing space to accommodate the drugs (Westesen and Siekmann 1997). Crystalline structure although property of the chemical nature of the lipid is of vital importance in determining whether a drug will tightly incorporate for the long-term or expel out easily.

13.4.4.1 Determination of Incorporated Drug

Quantity of encapsulated agents in SLNs manipulates the release kinetics; therefore it is of utmost crucial importance to determine the encapsulated agents. The quantity of drug entrapped per unit wt. of SLN is estimated after removal of the free drug. Separation of free drug can be carried out by ultracentrifugation and extensive dialysis. The concentration of drug after separation of free drug is determined by using standard analytical technique such as spectrophotometer, spectroflourophotometry or HPLC. Alternatively, encapsulation efficiency can be assessed by dissolving the SLN in a suitable solvent followed by measurement using respective analytical method. Another way to determine encapsulation efficiency is by extraction of the drug into appropriate solvent (Huang et al. 2008). EE is calculated in %:

$$\text{EE\%} = \frac{\text{Amount of drug into the particles}}{\text{Amount of drug added to the formulation}} \times 100$$

13.4.5 Drug Localization and Drug Release

A major concern with lipid nanoparticles is the burst release observed with these systems. A prolonged drug release was first obtained when studying the incorporation of prednisolone. Even it is possible to alter the release profiles as a

function of lipid matrix, stabilizer concentration and production condition (e.g. temperature) (Muhlen and Mehnert 1998). It is possible to achieve in vitro drug release up to 5–7 weeks with SLN. Moreover, release profiles could be altered to avoid any burst release; however this burst effect could be useful as loading dose.

Even though the drug is place inside the SLN but not always distributed homogenously throughout the matrix (Ren et al. 2013) and can be discreted in different regions of SLNs as well as in associated structures i.e. micelles, liposomes, drug nanocrystals. Methods used to find out the release can inherently manipulate the in vitro drug release pattern of SLN/NLC; hence critical consideration of methodologies should be performed. Regrettably, release experiments are carried out through several ways such as separation by centrifugation and dialysis; hence it is difficult to make comparison. The SLN dispersion is filled in pretreated dialysis bag and immersed in an appropriate dissolution fluid at RT under sink or nonsink conditions with gentle stirring. In vitro release profiles of carvedilol from SLN composed of various lipids (Compritol, lecithin) are shown in Fig. 13.8 and found that SLN made of higher lecithin concentration has shown a faster drug release compared to SLN made of Compritol (Aboud et al. 2016).

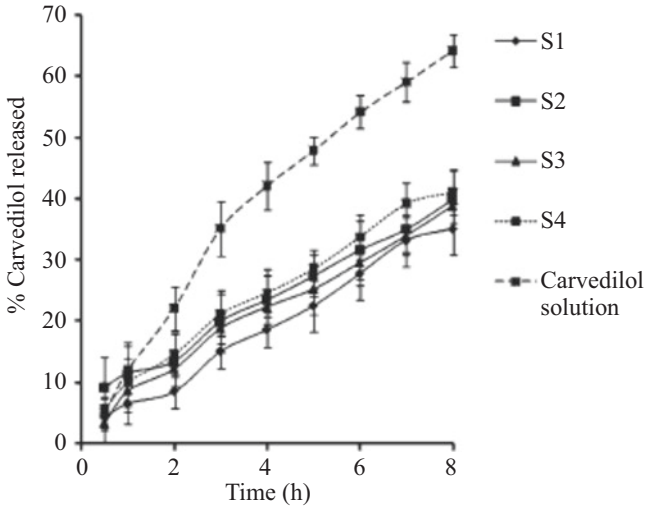

Fig. 13.8 In vitro release profiles of carvedilol from various SLNs made from different lipids. (Reproduced with kind permission from Springer, Aboud et al. (2016))

13.5 Applications of Solid Lipid Nanoparticles

13.5.1 Parenteral Delivery

SLNs are originally discovered to sort out few inherent difficulties of the parenteral nanoemulsions. During last two decades, SLN was explored for a diversity of novel applications and has been shown very useful to solve the problems associated to the faster drug leakage from the parenteral nanoemulsion. Modification of SLN surface of poloxamer- or poloxamine-stabilized SLN showed in vivo sustained and controlled effect (Scholer et al. 2001). Also, in vivo chemical stability of camptothecin increased after its encapsulation into the SLN (Joshi and Muller 2009). Many of the side effects of docetaxel have been reduced by parenteral administration of SLN-encapsulated docetaxel compared to plain drug (Liu et al. 2011). SLNs have proved to increase cellular uptake and targeting potential and ultimately improved the cancer therapy after encapsulation of cytotoxic drugs into the SLN (Liu et al. 2011). Also, SLNs have potential to overcome the challenges linked with parenteral administration of major diseases like malaria by increasing poor solubility of antimalarial drugs, for example, artemether and primaquine, after encapsulation in NLC by reducing haemolytic potential and increasing antimalarial response (Nayak et al. 2010; Mosallaei et al. 2013; Omwoyo et al. 2014). SLN increased drug levels in the lung, spleen and brain compared to plain drug solution which leads to higher distribution into the liver and kidneys.

13.5.2 Oral Delivery

Although the oral route is the most natural choice of drug administration, low solubility and bioavailability of diversity of therapeutics restrict its use. After orally administered, triglycerides (one of the components of SLN) are broken into mono- and diglycerides. These simple glycerides interact with bile salts present in the GIT and form micelles containing the drug which absorbed via chylomicron into the lymphatic system (Patil et al. 2012). Additionally, SLN may be taken up by M-cells of Peyer's patches (He et al. 2005). SLN may be administered orally as aqueous dispersions as well as traditional dosage forms such as tablets, pellets or capsules. Also, presence of food greatly affects SLN behaviour in GIT. The atmosphere of the stomach favours particle aggregation because of the presence of acid and high ionic strength. Improved bioavailability and sustained blood concentration of cyclosporine were found through lipid nanodispersions after per oral administration (Martins et al. 2007). In a latest study, SLNs have protected insulin against in vitro proteolytic enzymes upon encapsulation into the SLN (Almeida and Souto 2007). Also, SLNs have demonstrated the improvement in the bioavailability and protect the proteins against degradation after oral administration (Aungst 2000; Basu et al. 2010).

13.5.3 Transdermal and Topical Use

The use of SLN for transdermal and topical purpose expanded great attention. SLNs present higher chemical stability of the drugs, easily make film on skin surface, greatly hydrate skin and modulate drug release. Also, as they are made of nonirritant and nontoxic lipids, they can be applied to damaged and inflamed skin (Attama et al. 2009). Enhanced safety and therapeutic effectiveness after entrapment into the lipid particles have been reported for coenzyme *Q10* (Lin et al. 2010), celecoxib (Del Pozo-Rodriguez et al. 2010), ketoprofen (Obeidat 2012), vitamin A (Cirri et al. 2012) and many more. Also when systemic absorption is undesired after dermal application, SLN achieved epidermal targeting of podophyllotoxin which was confirmed with fluorescence microscopy (Dingler et al. 1999). Moreover SLN decreased the irritation effect of retinoic acid and also enhanced its stability (Chen et al. 2006). Additionally, SLNs have its application in the cosmetics as the incorporation of novel technologies has led to successful marketing. Various anti-ageing creams, sunscreen products and many more cosmetics have been using SLN and NLC and found that lipid particles in the form of cream formulation did not have irritant prospective with a greater hydration compared to traditional creams (Junyaprasert et al. 2009). Generally, smaller particle SLNs are more useful for dermal application. In the majority of cases, the SLN dispersion is added to an ointment or gel in order to design a formulation which can be easily applied to the skin. Enhancement of the SLN quantity in to the suspension comes out in a semisolid, gel-like consistency that is suitable for direct application on the skin surface (Farboud et al. 2011).

The greater advantage of using SLNs for topical products is that they protect the labile drugs against degradation, for example, retinol or vitamin C, which cannot be added directly into the creams because of the chemical instability which can be overcome by using SLN (Siddiqui et al. 2014).

13.5.4 Pulmonary, Nasal and Ocular Administration

SLNs are appropriate delivery system with excellent acceptability and higher safety for pulmonary treatment (Pardeike et al. 2010). However, SLNs as dry powders are not suitable for delivery to the lungs as the particle size is too small to be retained inside. Simple approach of aerosolization of aqueous SLN dispersions provides as an attractive approach. Also, tiny size of SLNs facilitates their incorporation into the microparticles and drops which can deliver the content to the alveoli. In this regard, SLN and NLC have improved the pharmacokinetic parameters of itraconazole (Pardeike et al. 2011), phenethyl isothiocyanate (Pilcer and Amighi 2010), celecoxib (Patlolla et al. 2010) and thymopentin (Jaafar-Maalej et al. 2011), after pulmonary administration (Pardeike et al. 2010). Also, incorporation of antituberculosis drugs into the SLN showed convincing findings after pulmonary administration (Liu et al. 2008).

Nasal administration is also proved to be promising using SLNs for variety of drugs, i.e. ropinirole (Chavan et al. 2013), alprazolam (Pardeshi et al. 2013) and budesonide (Joshi et al. 2012). Brain targeting is also reported using SLN after intranasal administration for risperidone and demonstrated enhanced brain bioavailability after nasal administration to the mice (Li et al. 2009). The major limitation of ophthalmologic formulation is low retention time into the eye cavity. Diclofenac encapsulated into the SLN demonstrated prolonged release and greater translocation to artificial corneal surface (Attama et al. 2008). Also, SLN-based tobramycin formulations proved to be more effective in the treatment of ocular infections (Zhang et al. 2006).

13.6 SLNs as a Carrier for Site-Specific Delivery

13.6.1 Application in Gene Delivery

In recent time, lipid-based nanocarriers have received greater interest in the area of gene therapy as convincing substitute of positively charged lipids as of protection ability of the incorporated agents against chemical degradation and their faster uptake by cells. In addition production method of SLNs requires low mechanical force; hence processing steps do not degrade DNA and RNA strands. However, cationic charge is a prerequisite for the efficient contact with the anionic cell surface and could be easily induced by incorporating suitable cationic surfactants into lipid matrix. Cationic lipid carriers have been investigated for transfection efficiency in African green monkey kidney fibroblast-like cells (Cos-1) and human bronchoepithelial cell (Olbrich et al. 2001) and found that SLNs efficiently bind and transfected plasmid DNA for the first time using solid lipid nanoparticles. In the recent past, several reports about successful incorporation of genetic materials such as DNA, plasmid DNA and other nucleic acid into the SLN have been published (Zhuang et al. 2010). Gene delivery of a diametric HIV-1 HAT peptide (TAT 2) by incorporation into SLN is demonstrated. Nucleic acid-encapsulated nanoparticles were prepared from a liquid phase composed of aqueous phase containing DNA and a water-miscible organic solvent containing lipid. The obtained particles are called genospheres (70–100 nm) which targeted specifically by insertion of an antibody-lipo polymer conjugated in the particle. Cationic SLNs have shown comparable in vivo transfection efficacy to the liposome made of similar cationic lipids. Recently Carrillo and coworkers used cationic SLN gene delivery to the brain (Carrillo et al. 2013).

13.6.2 SLN as Carriers for Peptides and Protein Drugs

Exception to most polymeric micro- and nanocarrier production techniques used for SLN does not require highly toxic organic liquids, which might be harmful for protein bioactives. Moreover, SLNs can be made to accumulate hydrophobic or water-soluble drugs for parenteral and non-parenteral routes.

SLN production is based on solidified emulsion (dispersed phase) methodologies; hence due to hydrophilic nature of proteins, they are expected to be poorly microencapsulated into the hydrophobic matrix of SLN. A large number of proteins and peptides, e.g. calcitonin, insulin, LHRH, somatostatin and protein antigens, have been studied for drug release kinetics, protein stability and in vivo performance after incorporation into the SLNs. Peptide ligand-modified SLNs are evaluated to increase oral bioavailability of proteins and found that absolute bioavailability of peptides was increased by 2.45 to 1.98 times compared to unmodified SLNs, indicating the scope and effectiveness for the improvement of the oral bioavailability of proteins (Fan et al. 2014). Another research group has prepared peptide-loaded SLNs by solvent diffusion method. The gonadorelin (a model peptide) was incorporated and evaluated for various parameters. The average diameter and zeta potential of SLN were found to be 421.7 nm and −21.1 mV, respectively. However, in vitro release followed biphasic pattern with slow release of gonadorelin (Hu et al. 2004). SLNs were prepared by solubilizing lysozyme (a model peptide) into the melted lipid phase. Results revealed solubility-dependent entrapment efficiency of the peptide in the lipid phase of the finished preparation.

13.6.3 Lipid Nanoparticle as a Carrier for Vaccine

Adjuvants are of great importance in vaccination in order to elicit enhanced immune response. Safer new subunit vaccines have poor immunization potential when used alone, and hence effective adjuvants are required (Copland et al. 2005). Improving the quantity of antigen transported is not a resolution as it enhances the vaccination cost drastically particularly for the developing countries. Conventional adjuvant such as alum is to be employed; however they suffer many disadvantages and have limited immunization to boost up potential. Alternatively various biodegradable polymeric microparticles, niosomes, liposomes and nanoparticles have been explored as an adjuvant for parenteral or mucosal administration routes (Eyles et al. 2003). However, most of the additives used for production of these colloidal carriers as well as manufacturing conditions have deterioration effect on vaccine stability. PLA/PLGA microspheres have shown potential as useful antigen delivery systems (Storni et al. 2005). Advantages of SLN as adjuvant compared to traditional adjuvants are the excellent tolerability by the body and biodegradation. The lipid portion of SLN degrades gradually allowing an extended contact of antigens to the immune

cells. Also, degradation of the antigen by the enzyme complexes is restricted due to steric stabilization by the surfactants which hamper the direct exposure (Olbrich and Muller 1999). First report of SLN as adjuvant displayed 43 and 73% effective protection (antibody levels) (Muller et al. 2000). Malaria protein antigen R32NS1 was incorporated into lipospheres and demonstrated increased specific IgG response in serum after intramuscular injection which extended for a minimum of 12-week period after primary immunization (Amsteel et al. 1992). Proteolytic degradation at various mucosae makes peptide or protein vaccines less effective for mucosal delivery; formulation into SLNs is a documented approach to enhance mucosal immunization. Intranasal administration of particles showed deposition of the particles into the lungs, and its lung mucosal layer takes a crucial part in generating immune response (Eyles et al. 2003).

13.7 Stability

SLN stability can be improved by removal of the water as the powder form is usually more stable than the suspensions. Lyophilization is a preferred and appropriate technique used to get rid of the water in the formulation. However, while in lyophilization the protection efficiency of the surfactant might be transformed as a result, the lyophilized SLNs may lead to bigger particles after redispersion (Del Pozo-Rodriguez et al. 2009). Drug leakage and modification in the surface charge are likely during lyophilization. Therefore, addition of appropriate cryoprotectant such as mannose, maltose and trehalose (10–15%) is essential before lyophilization (Cavalli et al. 1997). The properties of the redispersed powder are largely affected by the selection of the cryoprotectant.

Alternatively, spray drying is also another approach used to form powders from SLN dispersions. As heat is required in the process, hence lipids are preferred which have melting points higher than 70 °C. Sometimes it results in particle aggregation. Spray drying cannot be used for more diluted SLN dispersions. An additional method, of stabilizing SLN dispersions, is to make it viscous, as viscous dispersions have reduced rate of sedimentation and collision of the particles (Freitas and Muller 1998). In spite of all these problems observed during stabilization and storage of SLNs, these systems might be stable up to 3 years in dispersion state (Freitas and Muller 1999).

13.8 Conclusions

SLNs unite the advantage of polymeric nanoparticles, fatty emulsions as well as lipid vesicles. Moreover, possibility of accumulation of hydrophobic and aqueous soluble agents, enhanced stability, economy and simplicity of large-scale production process

makes them attractive carriers for drug delivery. Production techniques based on commercial production of emulsions and nutrition parenteral was primarily used for large-scale production of SLNs because necessary set of instrument simply existed and can be engaged at both lab and industrial scale. The scalability of methods of preparation of lipid nanoparticles leads to no difficulty in commercialization. In addition site-specific delivery and prolonged effect of drug could be healthier attained with SLNs. The other aspects of SLN related to commercial production such as sterilization, freeze drying and shelf life have already been developed to good standard.

References

Aboud HM, El Komy MH, Ali AA, El Menshawe SF, Elbary AA (2016) Development optimization, and evaluation of carvedilol-loaded solid lipid nanoparticles for intranasal drug. AAPS PharmSciTech 2016(17):1353–1365

Almeida AJ, Souto E (2007) Solid lipid nanoparticles as a drug delivery system for peptides and proteins. Adv Drug Deliv Rev 59:478–490

Amsteel S, Domb AJ, Laving CR (1992) Lipospheres as a vaccine carrier system: effects of size, charge, and phospholipids composition. Vaccin Res 1:383–395

Attama AA, Reichl S, Muller-Goymann CC (2008) Diclofenac sodium delivery to the eye: in vitro evaluation of novel solid lipid nanoparticle formulation using human cornea construct. Int J Pharm 355:307–313

Attama AA, Reichl S, Muller-Goymann CC (2009) Sustained release and permeation of timolol from surface-modified solid lipid nanoparticles through bioengineered human cornea. Curr Eye Res 34:698–705

Aungst BJ (2000) Intestinal permeation enhancers. J Pharm Sci 89:429–442

Basu B, Garala K, Bhalodia R, Joshi B, Mehta K (2010) Solid lipid nanoparticles :a promising tool for drug delivery system. J Pharm Res 3(1):84–92

Blasi P, Schoubben A, Romano G, Giovagnoli S, Di Michele A, Ricci M (2013) Lipid nanoparticles for brain targeting II. Technological characterization. Colloid Surf B 110:130–137

Byrappa K, Ohara S, Adschiri T (2008) Nanoparticles synthesis using supercritical fluid technology - towards biomedical applications. Adv Drug Deliv Rev 60:299–327

Carli F (1999) Physical chemistry and oral absorption of the nanoparticulate systems. Rencentre Pharmapeptides 5:158–160

Carrillo C, Hernandez N, Garcia-Montoya E, Perez-Lozano P, Sune-Negre JM, Tico JR, Sune C, Minarro M (2013) DNA delivery via cationic solid lipid nanoparticles (SLNs). Eur J Pharm Sci 49:157–165

Cavalli R, Caputo O, Carlotti ME, Trotta M, Scarnecchia C, Gasco MR (1997) Sterilization and freeze-drying of drug-free and drug-loaded solid lipid nanoparticles. Int J Pharm 148:47–54

Cavalli R, Gasco MR, Chetoni P, Burgalassi S, Saettone MF (2002) Solid lipid nanoparticles (SLN) as ocular delivery system for tobramycin. Int J Pharm 238:241–245

Charcosset C, El-Harati AA, Fessi H (2005) Preparation of solid lipid nanoparticles using a membrane contactor. J Control Release 108(1):112–120

Chattopadhyay P, Shekunov BY, Yim D, Cipolla D, Boyd B, Farr S (2007) Production of solid lipid nanoparticle suspensions using supercritical fluid extraction of emulsions (SFEE) for pulmonary delivery using the AERx system. Adv Drug Deliv Rev 59:444–453

Chavan SS, Ingle SG, Vavia PR (2013) Preparation and characterization of solid lipid nanoparticle-based nasal spray of budesonide. Drug Deliv Transl Res 3:402–408

Chen H, Chang X, Du D, Liu W, Liu J, Weng T, Yang Y, Xu H, Yang X (2006) Podophyllotoxin-loaded solid lipid nanoparticles for epidermal targeting. J Control Release 110:296–306

Choi KO, Aditya NP, Ko S (2014) Effect of aqueous pH and electrolyte concentration on structure, stability and flow behavior of non-ionic surfactant based solid lipid nanoparticles. Food Chem 147:239–244

Cirri M, Bragagni M, Mennini N, Mura P (2012) Development of a new delivery system consisting in drug–in cyclodextrin–in nanostructured lipid carriers for ketoprofen topical delivery. Eur J Pharm Biopharm 80:46–53

Copland MJ, Rades T, Davies NM, Baird MA (2005) Lipid based particulate formulations for the delivery of antigen. Immunol Cell Biol 83:97–105

Del Pozo-Rodriguez A, Solinis MA, Gascon AR, Pedraz JL (2009) Short- and long-term stability study of lyophilized solid lipid nanoparticles for gene therapy. Eur J Pharm Biopharm 71:181–189

Del Pozo-Rodriguez A, Delgado D, Solinis MA, Pedraz JL, Echevarria E, Rodriguez JM, Gascon AR (2010) Solid lipid nanoparticles as potential tools for gene therapy: in vivo protein expression after intravenous administration. Int J Pharm 385:157–162

Dingler A, Blum RP, Niehus H, Muller RH, Gohla S (1999) Solid lipid nanoparticles (SLN/Lipopearls)--a pharmaceutical and cosmetic carrier for the application of vitamin E in dermal products. J Microencapsul 16:751–767

Ekambaram P, Hasansathali AA, Priyanka K (2012) Solid lipid nanoparticles: a review. Sci Rev Chem Commun 2(1):80–102

El-Harati AA, Charcosset C, Fessi H (2006) Influence of the formulation for *solid lipid nanoparticles* prepared with a membrane contactor. Pharm Dev Technol 11(2):153–157

Eyles JE, Carpenter ZC, Alpar HO, Williamson ED (2003) Immunological aspects of polymer microsphere vaccine delivery systems. J Drug Target 11:509–514

Fan T, Chen C, Guo H, Xu J, Zhang J, Zhu X, Yang Y, Zhou Z, Li L, Huang Y (2014) Design and evaluation of solid lipid nanoparticles modified with peptide ligand for oral delivery of protein drugs. Eur J Pharm Biopharm 88:518–528

Farboud ES, Nasrollahi SA, Tabbakhi Z (2011) Novel formulation and evaluation of a Q10-loaded solid lipid nanoparticle cream: in vitro and in vivo studies. Int J Nanomedicine 6:611–617

Freitas C, Muller RH (1998) Spray-drying of solid lipid nanoparticles (SLN TM). Eur J Pharm Biopharm 46:145–151

Freitas C, Muller RH (1999) Correlation between long-term stability of solid lipid nanoparticles (SLN) and crystallinity of the lipid phase. Eur J Pharm Biopharm 47:125–132

Friedrich H, Frederik P, de With G, Sommerdijk N (2010) Imaging of self-assembled structures: interpretation of TEM and Cryo-TEM images. Angew Chem Int Ed 49:7850–7858

Gasco MR (1993) Method for producing solid lipid microspheres having a narrow size distribution. US Patent 5 250 236

Gohla SH, Dingler A (2001) Scaling up feasibility of the production of solid lipid nanoparticles (SLN). Pharmazie 56:61–63

Gref R, Minamitake Y, Peracchia MT, Trubetskoy V, Torchilin V, Langer R (1994) Biodegradable long-circulating polymeric nanospheres. Science 263:1600–1603

He J, Hou SX, Feng JF, Cai BQ (2005) Effect of particle size on oral absorption of silymarin-loaded solid lipid nanoparticles. China J Chin Mater Med 30:1651–1653

Hu FQ, Hong Y, Yuan H (2004) Preparation and characterization of solid lipid nanoparticles containing peptide. Int J Pharm 273:29–35

Huang G, Zhang N, Bi X, Dou M (2008) Solid lipid nanoparticles of temozolomide: potential reduction of cardial and nephric toxicity. Int J Pharm 355:314–320

Jaafar-Maalej C, Andrieu V, Elaissari A, Fessi H (2011) Beclomethasone-loaded lipidic nanocarriers for pulmonary drug delivery: preparation, characterization and in vitro drug release. J Nanosci Nanotechnol 11:1841–1851

Jain NK (1997) Controlled and novel drug delivery, 1st edn. CBS, New Delhi

Jores K, Mehnert W, Drechsler M, Bunjes H, Johann C, Mader K (2004) Investigations on the structure of solid lipid nanoparticles (SLN) and oil-loaded solid lipid nanoparticles by photon correlation spectroscopy, field-flow fractionation and transmission electron microscopy. J Control Release 95:217–227

Joshi MD, Muller RH (2009) Lipid nanoparticles for parenteral delivery of actives. Eur J Pharm Biopharm 71:161–172

Joshi AS, Patel HS, Belgamwar VS, Agrawal A, Tekade AR (2012) Solid lipid nanoparticles of ondansetron HCl for intranasal delivery: development, optimization and evaluation. J Mater Sci Mater Med 23:2163–2175

Junyaprasert VB, Teeranachaideekul V, Souto EB, Boonme P, Muller RH (2009) Q10-loaded NLC versus nanoemulsions: stability, rheology and in vitro skin permeation. Int J Pharm 377:207–214

Li Y, Dong L, Jia A, Chang S, Xue H (2006) Preparation and characterization of solid lipid nanoparticles loaded traditional Chinese medicine. Int J Biol Macromol 38:296–299

Li H, Zhao X, Ma Y, Zhai G, Li L, Lou H (2009) Enhancement of gastrointestinal absorption of quercetin by solid lipid nanoparticles. J Control Release 133:238–244

Lin YK, Huang ZR, Zhuo RZ, Fang JY (2010) Combination of calcipotriol and methotrexate in nanostructured lipid carriers for topical delivery. Int J Nanomed 5:117–128

Liu J, Gong T, Fu H, Wang C, Wang X, Chen Q, Zhang Q, He Q, Zhang Z (2008) Solid lipid nanoparticles for pulmonary delivery of insulin. Int J Pharm 356:333–344

Liu D, Liu Z, Wang L, Zhang C, Zhang N (2011) Nanostructured lipid carriers as novel carrier for parenteral delivery of docetaxel. Colloids Surf B 85:262–269

Martins S, Sarmento B, Ferreira DC, Souto EB (2007) Lipid-based colloidal carriers for peptide and protein delivery-liposomes versus lipid nanoparticles. Int J Nanomedicine 2:595–607

Mosallaei N, Jaafari MR, Hanafi-Bojd MY, Golmohammadzadeh S, Malaekeh-Nikouei B (2013) Docetaxel-loaded solid lipid nanoparticles: preparation, characterization, in vitro, and in vivo evaluations. J Pharm Sci 102:1994–2004

Muhlen Z, Mehnert W (1998) Drug release and release mechanism of prednisolone loaded solid lipid nanoparticles. Pharmazie 53:552

Müller RH (1996) Zetapotential und Partikelladung in der Laborpraxis – Einführung in die Theorie, praktische Meßdurchführung, Dateninterpretation. Wissenschaftliche Verlagsgesellschaft, Stuttgart, 254 S

Muller RH, Lucks JS (1996) Arzneistofftrager aus festen Lipidteilchen, Feste Lipidnanospharen (SLN). European Patent No. 0605497

Muller RH, Runge SA (1998) Solid lipid nanoparticles (SLN) for controlled drug delivery. In: Benita S (ed) Submicron emulsions in drug targeting and delivery. CRC, Amsterdam, pp 219–234

Muller RH, Mader K, Gohla S (2000) Enzymatic degradation of Dynasan 114 SLN – effect of surfactants and particle size. Eur J Pharm Biohgarm 50:161–170

Nayak AP, Tiyaboonchai W, Patankar S, Madhusudhan B, Souto EB (2010) Curcuminoids-loaded lipid nanoparticles: novel approach towards malaria treatment. Colloids Surf B Biointerfaces 81:263–273

Obeidat WM (2012) Investigation of temperature-induced physical instability of preserved coenzyme Q10-loaded (NLC): a comparative study at different temperatures. Afr J Pharm Pharmacol 6:2413–2423

Obeidat WM, Schwabe K, Muller RH, Keck CM (2010) Preservation of nanostructured lipid carriers (NLC). Eur J Pharm Biopharm 76:56–67

Okonogi S, Riangjanapatee P (2013) Potential technique for tiny crystalline detection in lycopene loaded SLN and NLC development. Drug Dev Ind Pharm 40(10):1–8

Olbrich C, Muller RH (1999) Enzymatic degradation of SLN-effect of surfactant and surfactant mixtures. Int J Pharm 180:31–39

Olbrich C, Bakowsky U, Lehr CM, Muller RH, Kneuer C (2001) Cationic solid-lipid nanoparticles can efficiently bind and transfect plasmid DNA. J Control Release 77:345–355

Omwoyo WN, Ogutu B, Oloo F (2014) Preparation, characterization, and optimization of primaquine-loaded solid lipid nanoparticles. Int J Nanomedicine 9:3865–3874

Pardeike J, Schwabe K, Muller RH (2010) Influence of nanostructured lipid carriers (NLC) on the physical properties of the Cutanova Nanorepair Q10 cream and the in vivo skin hydration effect. Int J Pharm 396:166–173

Pardeike J, Weber S, Haber T, Wagner J, Zarfl HP, Plank H, Zimmer A (2011) Development of an itraconazole-loaded nanostructured lipid carrier (NLC) formulation for pulmonary application. Int J Pharm 419:329–338

Pardeshi CV, Rajput PV, Belgamwar VS, Tekade AR, Surana SJ (2013) Novel surface modified solid lipid nanoparticles as intranasal carriers for ropinirole hydrochloride: application of factorial design approach. Drug Deliv 20:47–56

Patil S, Joshi M, Pathak S, Sharma S, Patravale V (2012) Intravenous β-artemether formulation (ARM NLC) as a superior alternative to commercial artesunate formulation. J Antimicrob Chemother 67:2713–2716

Patlolla RR, Chougule M, Patel AR, Jackson T, Tata PN, Singh M (2010) Formulation, characterization and pulmonary deposition of nebulized celecoxib encapsulated nanostructured lipid carriers. J Control Release 144:233–241

Pilcer G, Amighi K (2010) Formulation strategy and use of excipients in pulmonary drug delivery. Int J Pharm 392:1–19

Pinto JF, Muller RH (1999) Pellets as carriers of solid lipid nanoparticles (SLNk) for oral administration of drugs. Pharmazie 54:506–509

Rabinarayan P, Padilama S (2010) Production of solid lipid nanoparticles-drug loading and release mechanism. J Chem Pharm Res 2:211–227

Ramteke KH, Joshi SA, Dhole SN (2012) Solid lipid nanoparticle: a review. IOSR J Pharm 2(6):34–44

Ren J, Zou M, Gao P, Wang Y, Cheng G (2013) Tissue distribution of borneol-modified ganciclovir-loaded solid lipid nanoparticles in mice after intravenous administration. Eur J Pharm Biopharm 83:141–148

Saupe A, Gordon KC, Rades T (2006) Structural investigations on nanoemulsions, solid lipid nanoparticles and nanostructured lipid carriers by cryo-field emission scanning electron microscopy and Raman spectroscopy. Int J Pharm 314:56–62

Scholer N, Olbrich C, Tabatt K, Muller RH, Hahn H, Liesenfeld O (2001) Surfactant, but not the size of solid lipid nanoparticles (SLN) influences viability and cytokine production of macrophages. Int J Pharm 221:57–67

Schwarz C, Mehnert W, Lucks JS, Muller RH (1994) Solid lipid nanoparticles (SLN) for controlled drug delivery: I. Production, characterization and sterilization. J Control Release 30:83–96

Shah C, Shah V, Uphadhyay U (2011) Solid lipid nanoparticles: a review. Curr Pharm Res 1(4):351–368

Shakesheff KM, Davies MC, Domb A, Glasbey TO, Jackson DE, Heller J, Roberts CJ, Shard AG, Tendler SJB, Williams PM (1994) Visualizing the degradation of polymer surfaces with an Atomic Force Microscope. Proc Int Symp Control Release Bioact Mater 21:1343–1344

Shenoy VS, Gude RP, Murthy RSR (2013) In vitro anticancer evaluation of 5-fluorouracil lipid nanoparticles using B16F10 melanoma cell lines. Int Nano Lett 3:36

Shi L, Li Z, Yu L, Jia H, Zheng L (2011) Effects of surfactants and lipids on the preparation of solid lipid nanoparticles using double emulsion method. J Dispers Sci Tech 32(2):254–259

Siddiqui A, Alayoubi A, El-Malah Y, Nazzal S (2014) Modeling the effect of sonication parameters on size and dispersion temperature of solid lipid nanoparticles (SLNs) by response surface methodology (RSM). Pharm Dev Technol 19:342–346

Siekmann B, Westesen K (1996) Investigations on solid lipid nanoparticles prepared by precipitation in o/w emulsions. Eur J Pharm Biopharm 43:104–109

Silva AC, Gonzalez-Mira E, Garcia ML, Egea MA, Fonseca J, Silva R, Santos D, Souto EB, Ferreira D (2011) Preparation, characterization and biocompatibility studies on risperidone-loaded solid lipid nanoparticles (SLN): high pressure homogenization versus ultrasound. Colloid Surf B 86:158–165

Sinha VR, Srivastava S, Goel H, Jindal V (2010) Solid lipid nanoparticles (SLN'S) – trends and implications in drug targeting. Int J Adv Pharm Sci 1:212–238

Soares S, Fonte P, Costa A, Andrade J, Seabra V, Ferreira D, Reis S, Sarmento B (2013) Effect of freeze-drying, cryoprotectants and storage conditions on the stability of secondary structure of insulin-loaded solid lipid nanoparticles. Int J Pharm 456:370–381

Solans C, Sole I (2012) Nano-emulsions: formation by low-energy methods. Curr Opin Colloid Interface Sci 17:246–254

Storni T, Kundig TM, Senti G, Johansen P (2005) Immunity in response to particulate antigen-delivery systems. Adv Drug Deliv Rev 57:333–355

Stoye I, Schroder K, Muller-Goymann CC (1998) Transformation of a liposomal dispersion containing ibuprofen lysinate and phospholipids into mixed micelles – physico-chemical characterization and influence on drug permeation through excised human stratum corneum. Eur J Pharm Biopharm 46:191–200

Webster AJ, Cates ME (2001) Osmotic stabilization of concentrated emulsions and foams. Langmuir 17:595–608

Westesen K, Siekmann B (1997) Investigation of the gel formation of phospholipid-stabilized solid lipid nanoparticles. Int J Pharm 151:35–45

Wu M, Tia Z, Yuan S, Huang Z (2012) Magnetic and optical properties of the Aurivillius phase $Bi_5Ti_3FeO_{15}$. Mater Lett 68:190–192

Xie S, Zhu L, Dong Z, Wang Y, Wang X, Zhou W (2011) Preparation and evaluation of ofloxacin loaded palmitic acid solid lipid nanoparticles. Int J Nanomedicine 6:547–555

Yang J, Ciftci ON (2016) Formation of hollow solid lipid micro- and nanoparticles using supercritical carbon dioxide. Food Bioprod Process 98:151–160

Yasuji T, Takeuchi H, Kawashima Y (2008) Particle design of poorly water-soluble drug substances using supercritical fluid technologies. Adv Drug Deliv Rev 60:388–398

Zhang N, Ping Q, Huang G, Xu W, Cheng Y, Han X (2006) Lectin-modified solid lipid nanoparticles as carriers for oral administration of insulin. Int J Pharm 327:153–159

Zhuang CY, Li N, Wang M, Zhang XN, Pan WS, Peng JJ, Pan YS, Tang X (2010) Preparation and characterization of vinpocetine loaded nanostructured lipid carriers (NLC) for improved oral bioavailability. Int J Pharm 394:179–185