Chapter 3
Production Techniques

Abstract Besides composition and structure, an important factor which influences the performance and characteristics of lipid nanoparticles is their production method, and a variety of production techniques have been introduced since the inception of lipid nanoparticles as potential colloidal carriers. The production techniques can be categorized into two groups; techniques which require high energy for dispersion of the lipid phase (such as high pressure homogenization, high sheer homogenization, ultrasonication) and techniques which require precipitation of nanoparticles from homogenous systems (such as microemulsions, solvent-based techniques, membrane contactors and coacervation). The choice of an appropriate technique is based on the physicochemical properties of the drug, the physicochemical characteristics and stability of the colloidal formulation and the availability of equipment. This chapter gives an overview of the techniques used in the production of lipid nanoparticle dispersions and also provides a brief introduction to a novel technique based on the use of microwave energy developed by our group.

Keywords Production · Homogenization · Microemulsion · Solvent-based · Coacervation · Membrane contactor · Microwave

3.1 General Considerations

Several approaches for the preparation of lipid nanoparticle dispersions have been reported since these carriers were first described in early 1990s (Gasco 1993; Schwarz et al. 1994; Siekmann and Westesen 1992). The preparation technique has a significant role in the performance of the colloidal formulation. The choice of preparation technique for lipid nanoparticle dispersions may be influenced by:

- Physicochemical properties of the drug to be incorporated
- Stability of the drug to be incorporated
- Desired particle characteristics of the lipid nanoparticle dispersion

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- Stability of the lipid nanoparticle dispersion
- Availability of the production equipment

A brief description of a variety of production techniques is discussed here. Table 3.1 gives a brief outline of the mechanisms involved in lipid nanoparticle formation by various techniques, and the major advantages and disadvantages associated with those techniques.

3.2 Production of Lipid Nanoparticles

3.2.1 High Pressure Homogenization

Being the most reliable and influential technique, high pressure homogenization has emerged as the industry choice of production technique for preparation of lipid nanoparticle dispersions. The method was introduced by Siekmann and Westesen (1992) and Muller et al. (1993), and was later developed and patented for production of SLNs by Müller and Lucks (1996).

Production of SLNs by high pressure homogenization can be achieved by two approaches—the hot homogenization and the cold homogenization approaches.

3.2.1.1 Hot Homogenization

Hot homogenization is a well-established technique from its use in the preparation of fat (or parenteral) emulsions and in dairy processing. Parenteral emulsions are generally produced in temperature controlled cabinets and high temperature is essential for their preparation. Parenteral emulsions are prepared using liquid lipids. Lipid nanoparticles can be drawn from fat emulsions by substituting the liquid lipids (or oils) with solid lipids. Thus, existing production lines for fat emulsions can also be very well engaged in lipid nanoparticle production by the hot homogenization technique (Müller et al. 2000).

Figure 3.1 gives a schematic depiction of the steps involved in the hot homogenization technique. The hot homogenization technique is often regarded as the "homogenization of emulsions" because lipid nanoparticle dispersions are prepared at temperatures above the melting point of the lipid used (Mehnert and Mäder 2001). The solid lipids are melted and the drug is solubilized or dispersed in the molten lipid. The drug-contained lipid melt is dispersed in a hot aqueous surfactant solution, previously maintained at the same temperature, under high speed stirring. Subsequent ultrasonication produces a pre-emulsion. Usually, a piston-gap homogenizer or a jet-stream homogenizer is used to homogenize the pre-emulsion to produce a hot colloidal emulsion. The droplets of the hot colloidal emulsion are recrystallized by cooling the emulsion to room temperature in order to generate SLNs. In some exceptional cases, specific thermal treatment of emulsions such as cooling to refrigeration conditions or even sub-zero temperatures may be required (Bunjes et al. 1996; Lim and Kim 2002; Schwarz and Mehnert 1997; Unruh et al. 2001; Westesen et al. 1997).

Table 3.1 Mechanism, advantages and disadvantages of methods used in preparation of lipid nanoparticles

Production technique	Mechanism of particle formation	Advantages	Disadvantages
High pressure homogenization	High mechanical shear due to strong turbulent eddies Lowering of pressure across the valves of homogenizers Strong cavitation forces	*Hot homogenization* Well established technology Effective dispersion of particles Reproducible High lipid content Simple to scale-up	*Hot homogenization* Extremely high energy inputs (heat and shear forces) High polydispersity Temperature-induced degradation of drugs Complex crystallization; leads to several lipid modifications and occurrence of supercooled melts Inappropriate for hydrophilic drugs; readily distribute in the aqueous phase Reduction in homogenization efficiency at elevated temperatures
		Cold homogenization Effective dispersion of particles Suitable for thermo-sensitive drugs No complex lipid modifications Increased drug-loading due to rapid cooling Suitable for hydrophilic drugs; reduced lipid melting reduces drug loss Simple to scale-up	*Cold homogenization* Extremely high energy inputs Large particles with high polydispersity Drug expulsion on storage
Microemulsion technique	Lipid crystallization due to rapid solidification of microemulsion	Sophisticated equipment not required Low energy inputs Higher temperature gradients; faster lipid crystallization, avoids particle aggregation Simple to scale-up	Low lipid content

(continued)

Table 3.1 (continued)

Production technique	Mechanism of particle formation	Advantages	Disadvantages
Microwave-assisted micro-emulsion technique	Direct coupling of microwaves with molecules Lipid crystallization due to rapid solidification of microemulsion	Controlled microwave heating Rapid and efficient heating Low energy inputs Shorter duration of preparation Higher temperature gradients; faster lipid crystallization	Scalability issues
Solvent evaporation	Lipid crystallization due to solvent evaporation in an anti-solvent	Sophisticated equipment not required Highly suitable for thermo-sensitive drugs Small particle diameters Simple to scale-up	Toxicological issues due to use of organic solvents Particle aggregation in absence of rapid solvent evaporation Low lipid content
Double emulsion	Lipid crystallization due to solidification of emulsion	Sophisticated equipment not required Low energy inputs	Low lipid content
Solvent diffusion	Lipid crystallization due to diffusion of solvent from internal organic phase to external aqueous phase	Sophisticated equipment not required Pharmaceutically accepted organic solvents used; solvent recycling feasible Small particle diameters and low polydispersity Simple to scale-up	Although rare, risk of toxicological risks due to incomplete evaporation of organic solvents Low lipid content
Solvent injection (or displacement)	Lipid crystallization due to rapid diffusion of solvent from internal organic phase to external aqueous phase	Sophisticated equipment not required Pharmaceutically accepted organic solvents used; solvent recycling feasible Highly efficient and versatile technique Higher performance Simple to scale up	Solvent removal difficult; use of freeze-drying or evaporation-under-reduced-pressure Low lipid content

(continued)

Table 3.1 (continued)

Production technique	Mechanism of particle formation	Advantages	Disadvantages
High shear homogenization and/or ultrasonication	Shear between adjacent particles Formation, growth and implosive collapse of bubbles due to cavitation forces	Use of organic solvents can be avoided Use of large amounts of surfactants can be avoided Simple technique with lower production cost Higher energy inputs	Unsuitable for higher lipid contents High polydispersity Physical instability due to high shearing Metal contamination due to ultrasonication Poor encapsulation efficiency
Membrane contactor method	Lipid/oil phase infuses through membrane pores into the tangentially flowing aqueous phase to form droplets Oil droplets crystallize to form lipid nanoparticles	Controlled particle size with selection of membrane with correct pore size Simple to scale-up	Clogging of membrane pores; frequent replacement or cleaning procedures
Supercritical fluid extraction of emulsions	Parallel processes of supercritical fluid extraction (diffusion) of organic solvent from emulsions and lipid dissolution Expansion of organic phase; leads to lipid crystallization	Efficient Rapid and efficient solvent removal Monodispersity Removal of low molecular weight impurities is easy with supercritical fluids Supercritical fluid carbon dioxide causes plasticization of lipid structures; thermodynamically stable lipid nanoparticle dispersions Supercritical fluid lower melting point of lipids; suitable for thermo-sensitive drugs	Use of organic solvents Sophisticated equipment required

(continued)

Table 3.1 (continued)

Production technique	Mechanism of particle formation	Advantages	Disadvantages
Coacervation technique	Decrease in pH of micellar solution of an alkaline salts of fatty acids by acidification (coacervating solution) in presence of a polymeric stabilizer causes proton exchange an lipid precipitation (coacervation)	Suitable for lipophilic drugs (by solubilising in the micellar solution after coacervation) Suitable for hydrophobic ion pairs of hydrophilic drugs Solvent-free technique Use of sophisticated technique not required Monodispersity Simple to scale-up	Suitable for lipids that an form alkaline salts Not suitable for pH-sensitive drugs
Phase inversion temperature technique	Spontaneous inversion of o/w emulsion to w/o emulsion due to thermal treatment (subsequent heating-cooling cycles) Lipid crystallization as a result of emulsion breakage due to irreversible shock induced by rapid cooling	Solvent-free technique Use of large amounts of surfactants can be avoided Combines structural advantages of polymeric nanocapsules and liposomes; imparts stability to the system Suitable for thermo-sensitive drugs Shorter heating periods avoids drug degradation	Particle aggregation Excipients influence the phase inversion behavior Emulsion instability

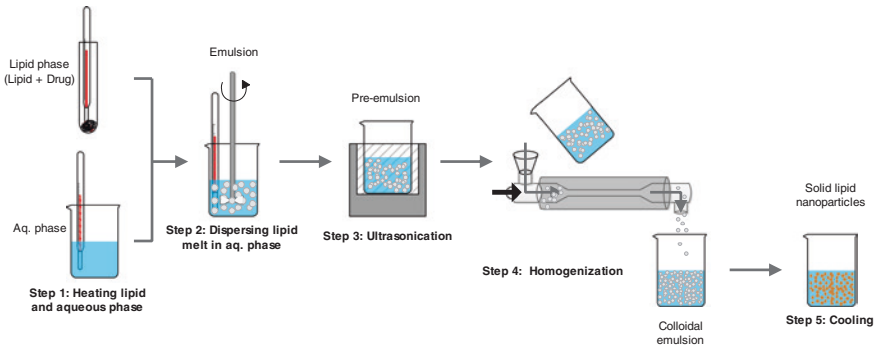

Fig. 3.1 Schematic depiction of steps involved in the hot homogenization technique

The particle size of nanoparticles usually depends on the composition of the dispersions (lipids, surfactants and the dispersion medium) and the homogenization parameters. Particle size can be decreased by increasing the emulsifier-to-lipid ratio, increasing the homogenization pressure, adjusting the homogenization time, increasing the homogenization temperature or adjusting the melt viscosity (Dingler and Gohla 2002; Jenning et al. 2002; Patravale and Ambarkhane 2003; Yang and Zhu 2002). The average particle diameter of SLNs prepared by high pressure homogenization techniques typically ranges from 50 to 400 nm (Blasi et al. 2013a, b; Doktorovova et al. 2014; Durán-Lobato et al. 2013; Dwivedi et al. 2014; Finke et al. 2012; Manjunath et al. 2011; Prombutara et al. 2012; Silva et al. 2011; Wang et al. 2012). Particle size is an important material property that decides the fate of the lipid nanoparticles in the biological system including its elimination from the system (Wu et al. 2011).

3.2.1.2 Cold Homogenization

Cold homogenization techniques are used in the preparation of lipid nanoparticles by passing the predispersed lipid matrix through a high pressure homogenizer at temperatures below the melting point of the lipid. As this technique involves grinding of solid lipids at high pressures, it is sometimes described as "high pressure milling of a lipid suspension" (Mäder and Mehnert 2005).

Figure 3.2 gives a schematic depiction of the cold homogenization technique. Similar to hot homogenization, the solid lipid is heated and drug molecules are incorporated into the matrix by dissolving or dispersing them in molten lipid. The drug-containing lipid melt is rapidly solidified by cooling with dry ice or liquid nitrogen. Rapid cooling favours homogenous distribution of the drug within the lipid material. The solid is then ground into a fine powder by milling into microparticles. The microparticles are subsequently dispersed in a cold aqueous surfactant solution. The dispersion is subjected to high pressure homogenization to generate SLNs.

Cold homogenization involves homogenization of solid lipids as opposed to a lipid melt in hot homogenization. This dispersion of solid lipids requires high

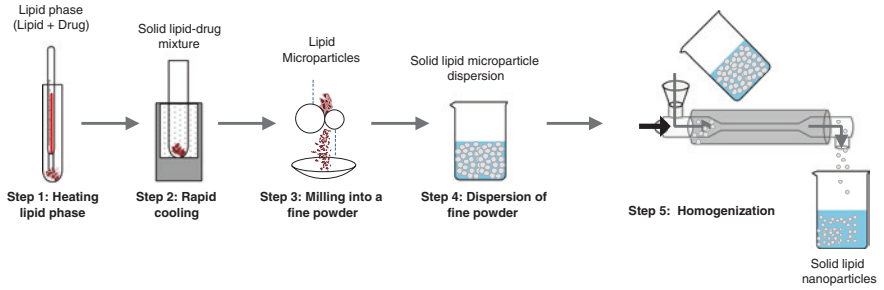

Fig. 3.2 Schematic steps involved in the cold homogenization technique

energy input which in turn requires harsh homogenization conditions. Thus homogenisation itself is more effective for the hot case, and smaller particles which are more monodisperse result (Mäder and Mehnert 2005).

3.2.2 Microemulsion Technique

The preparation of SLN dispersions by precipitation from a hot microemulsion was described by Gasco (1993). A microemulsion is a thermodynamically stable system comprising of water and oil, stabilized by surfactant (and a co-surfactant, if required) and is optically isotropic.

Figure 3.3 depicts the preparation of lipid nanoparticle dispersion by precipitation from a hot microemulsion. The lipid phase and aqueous surfactant/

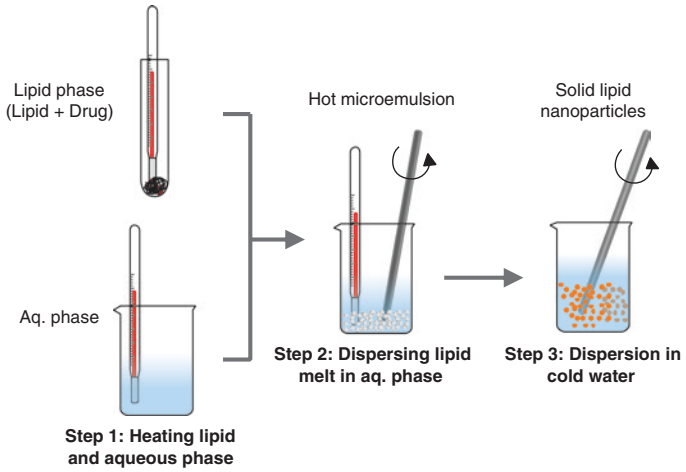

Fig. 3.3 Schematic steps involved in the microemulsion technique

co-surfactant system are separately heated to a temperature above the melting point of the solid lipid. The drug and the lipid are heated together to solubilize the drug in the molten lipid. The lipid melt is later emulsified in the hot surfactant/co-surfactant system under continuous stirring to yield a hot microemulsion, which is then dispersed in cold water (typically 2–4 °C), under mechanical stirring, to yield SLNs. Typically, microemulsion:aqueous phase ratios are 1:25 or 1:50.

3.2.3 Microwave-Assisted Microemulsion Technique

The use of microwave energy in chemical synthesis and processes has been widely investigated in recent years (Gawande et al. 2014). Microwave-assisted synthesis of compound libraries for generation and optimization of new drug candidates is well-established (Hayes 2004). Microwave-assisted drying of pharmaceutical products and long-term stability of solid dispersions of drugs is a widely reported application (Bergese et al. 2003; Moneghini et al. 2008, 2009). Only a few reported successes of the use of microwave energy in pharmaceutical formulation, such as that of polymeric nanoparticles, have been reported (An et al. 2006; Bergese et al. 2003; Waters et al. 2011). The authors of this book have developed a novel production technique for SLNs based on the use of microwave energy (Shah et al. 2014).

Figure 3.4 gives a schematic diagram of steps involved in the microwave-assisted microemulsion technique. The drug, lipid and aqueous surfactant/co-surfactant system are subjected to controlled microwave heating at a temperature above the melting point of the solid lipid. Constant stirring while heating the formulation components in a controlled microwave environment yields a hot microemulsion. Unlike the conventional microemulsion technique, all ingredients are heated in a single synthesis vessel. This step is therefore referred to as "single pot" production of microemulsion. The hot microemulsion obtained from the microwave is again then dispersed in cold water (at 2–4 °C) to generate SLNs.

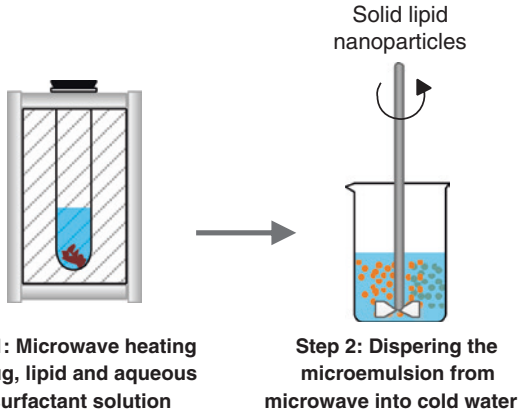

Fig. 3.4 Schematic steps involved in the microwave-assisted microemulsion technique

The use of a controlled environment is the key to successful development of the lipid nanoparticle formulation. The stearic acid lipid nanoparticles prepared by this technique produce particles of approximately 200–250 nm with good physical stability, encapsulation efficiency and drug loading (Shah et al. 2014).

3.2.4 Solvent Evaporation

Solvent evaporation is a well-established technique used in the preparation of pseudolatex (Vanderhoff et al. 1979). The technique has since been used, for example, to prepare lipid nanoparticle dispersions of cholesteryl acetate via precipitation from lecithin-stabilized solvent-in-water emulsions (Sjöström and Bergenståhl 1992).

Figure 3.5 illustrates the solvent evaporation method. The solid lipid is initially dissolved in an organic solvent and the lipophilic drug may also be dissolved in the organic solvent together with the solid lipid. Since their inception, lipid nanoparticle dispersions have been prepared using different organic solvents such as cyclohexane, chloroform and ethyl acetate (Cortesi et al. 2002; Siekmann and Westesen 1996; Sjöström and Bergenståhl 1992). The organic phase is emulsified in an aqueous solution of surfactant to yield an organic solvent-in-water emulsion. A lipid nanoparticle dispersion is then formed on complete evaporation, under reduced pressure, of the organic solvent.

3.2.5 Double Emulsion

The production of lipospheres by the double emulsion method was first described by Cortesi et al. (2002). The double emulsion method was introduced to solubilize

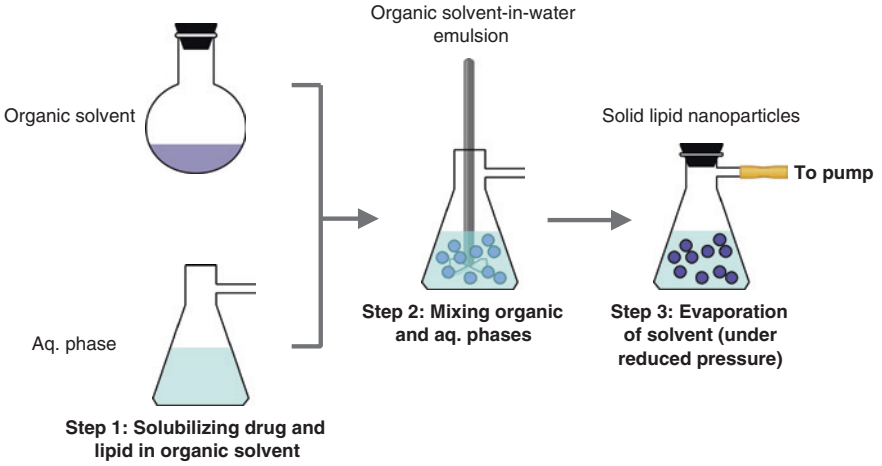

Fig. 3.5 Schematic steps involved in the solvent evaporation technique

hydrophilic drugs in the internal water phase of a w/o/w emulsion, with the aid of a stabilizer to prevent its loss to the external water phase during solvent evaporation.

In a w/o/w double emulsion procedure, an aqueous solution of drug is emulsified in molten lipid to give a primary w/o emulsion and stabilized by adding stabilizers such as gelatin or poloxamer in the aqueous phase. Subsequent dispersion of the primary emulsion in a second aqueous solution of stabilizer under constant stirring generates a w/o/w double emulsion. Constant stirring for longer periods leads to precipitation of lipid nanoparticles. Figure 3.6 illustrates the production of lipid nanoparticles by the double emulsion method.

Garcia-Fuentes et al. (2003) introduced a modified double emulsion technique that was an extension of the solvent evaporation method. The double emulsion was successfully applied to the production of tripalmitin nanoparticles and conveniently modified to prepare surface-coated nanoparticles. Briefly, an aqueous drug solution was emulsified in an organic phase, which had emulsifier and solid lipid previously solubilized into it. The primary emulsion was consequently emulsified in a second aqueous solution to give a double emulsion. The stabilizer in this aqueous solution forms the outer coating of the lipid nanoparticles. The complete removal of solvent was achieved under constant stirring over time. The mean diameter of tripalmitin nanoparticles was 200 nm, using lecithin as the emulsifier. Mean particle sizes of the surface modified nanoparticles ranged from 110–240 nm, depending on the emulsifier in the external water phase. The reproducibility of these results were confirmed by Garcia-Fuentes et al. (2005), who also prepared NLCs by modifying the inner structure with the incorporation of a liquid lipid, Miglyol® 812.

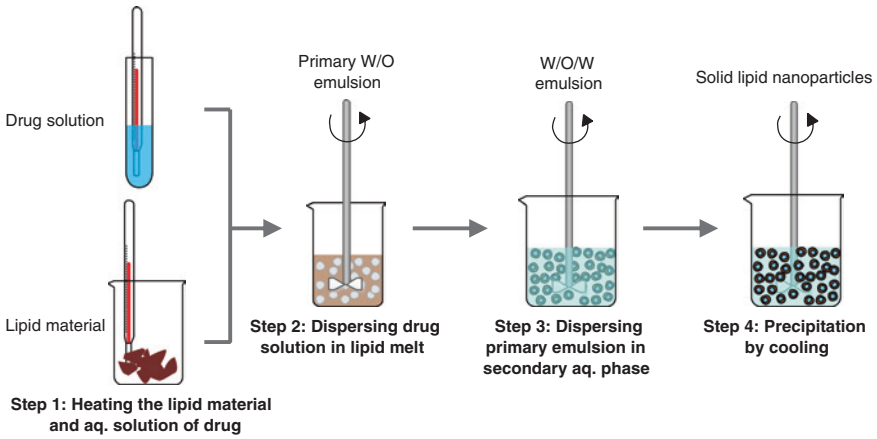

Fig. 3.6 Schematic steps involved in the double emulsion technique

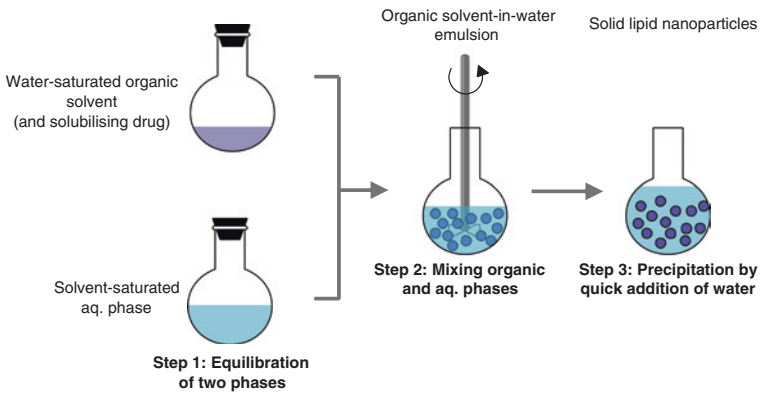

Fig. 3.7 Schematic steps involved in the solvent diffusion technique

3.2.6 Solvent Diffusion

The solvent diffusion method was first introduced in the production of polymeric nanoparticles by Leroux et al. (1995). The method was developed and patented by Quintanar-Guerrero et al. (1996). Hu et al. (2002) introduced a novel solvent diffusion technique in the preparation of lipid nanoparticles.

A schematic protocol for preparation of lipid nanoparticles using the solvent diffusion technique is shown in Fig. 3.7. Partially water-miscible solvents are used to solubilize the solid lipids. A number of solvents partially soluble in water such as benzyl alcohol, butyl lactate, isobutyric acid, isovaleric acid and tetrahydrofuran have been used in the preparation of lipid nanoparticles (Battaglia et al. 2007; Shahgaldian et al. 2003a, b, c; Trotta et al. 2003, 2005). Prior to lipid solubilization, the water-miscible solvents are saturated with water to ensure preliminary thermodynamic equilibrium between the two liquids. The drug may be added to the organic solvent phase. The organic phase is then emulsified with a solvent-saturated aqueous phase containing an emulsifier. Water is added to the primary emulsion to extract the solvent into the external water phase and this generate SLNs. Typical emulsion:water ratios are 1:5 or 1:10.

3.2.7 Solvent Injection (or Displacement)

Solvent injection is a well-established technique from its use in preparation of liposomes and polymeric nanoparticles (Batzri and Korn 1973; Fessi et al. 1989). Solvent injection is a modification of a solvent diffusion technique.

In the solvent injection technique, the lipid is solubilized in a semi-polar water-miscible solvent or water-soluble solvent mixture while the drug is dissolved in the organic phase. The organic phase is rapidly injected, under constant stirring,

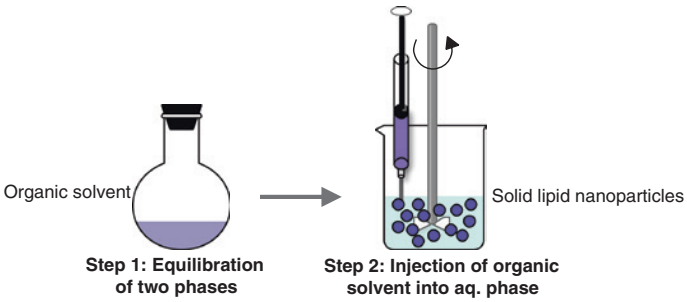

Fig. 3.8 Schematic steps involved in the solvent injection technique

into an aqueous phase containing the surfactant. Lipid nanoparticles precipitate upon solvent distribution into the continuous aqueous phase. A schematic showing the mechanistic steps in solvent injection is depicted in Fig. 3.8.

The particle sizes of the lipid nanoparticles are determined by the velocity of the diffusion of the solvent across the lipid-solvent interface into the aqueous phase. Lipid nanoparticles have been precipitated with polar solvents such as ethanol, acetone, isopropanol and methanol that distribute rapidly into the aqueous phase. Typical diameters of the nanoparticles produced were in the range of 100–200 nm (Dubes et al. 2003; Hu et al. 2002, 2004; Schubert and Müller-Goymann 2003).

Wang et al. (2010) introduced a modified solvent injection method called "solvent injection lyophilization". Lipid nanoparticles were prepared according to the steps discussed earlier for the solvent injection technique except that the organic phase (t-butyl alcohol, in this case) was injected into a stirred aqueous solution containing lyoprotectants to form lipid nanoparticles dispersed in a t-butyl alcohol/water co-solvent system. Subsequent lyophilization of the co-solvent system yielded a dry lipid nanoparticle product which, upon rehydration, formed an aqueous lipid nanoparticle dispersion.

3.2.8 High Shear Homogenization and/or Ultrasound

High shear homogenization and ultrasonication are dispersing techniques. SLN dispersions can be obtained by dispersing a molten lipid in an aqueous phase and then stabilizing with surfactants. Speiser (1986) described the use of high sheer homogenization followed by ultrasonication to prepare lipid nanopellets as an oral drug carrier. The lipid nanopellets obtained had an average particle diameter of 80–800 nm and were suitable for peroral administration.

Lipid nanoparticle dispersions are obtained by dispersing the melted lipid in the warm aqueous phase containing surfactants by high sheer homogenization followed by ultrasonication. Figure 3.9 describes a schematic protocol of manufacturing lipid nanoparticle dispersions by the high sheer homogenization and

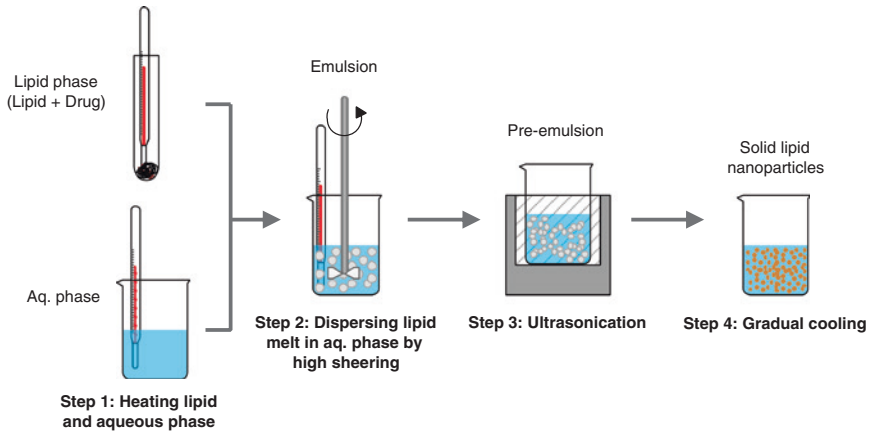

Fig. 3.9 Schematic steps involved in the high shear homogenization and ultrasonication technique

ultrasound technique. This method primarily involves heating of a solid lipid to approximately 5–10 °C above its melting point. The lipid melt is dispersed in an aqueous surfactant solution at the same temperature under high speed stirring to form an emulsion. Subsequent sonication reduces the droplet size of the emulsion. Gradual cooling of the warm emulsion below the crystallization temperature of the lipid yields a lipid nanoparticle dispersion. Concentrated lipid nanoparticle dispersions can be obtained by ultracentrifugation.

3.2.9 Membrane Contactor Method

Membrane contactors have been increasingly used in recent times (Drioli et al. 2003, 2011; Sirkar et al. 1999). Membrane emulsification is a well-established method of preparation of emulsions (Charcosset et al. 2004; Joscelyne and Trägårdh 2000). Membrane contactors have been applied in the manufacture of precipitates including barium sulphate and calcium carbonate (Chen et al. 2004; Jia et al. 2003).

For the preparation of lipid nanoparticles, the lipid melt is initially pressed through the pores of a membrane contactor. The pores in the membrane act as parallel capillaries for introduction of the lipid phase. The passage of the lipid melt allows formation of small droplets into the aqueous phase that flow tangentially to the membrane surface. The aqueous flow carries the droplets formed at the pore outlets to give the lipid nanoparticles. A schematic representation of this method is shown in Fig. 3.10.

The mean particle diameters increase with increasing amount of lipid and the increased lipid content also deteriorates membrane performance, lowering the

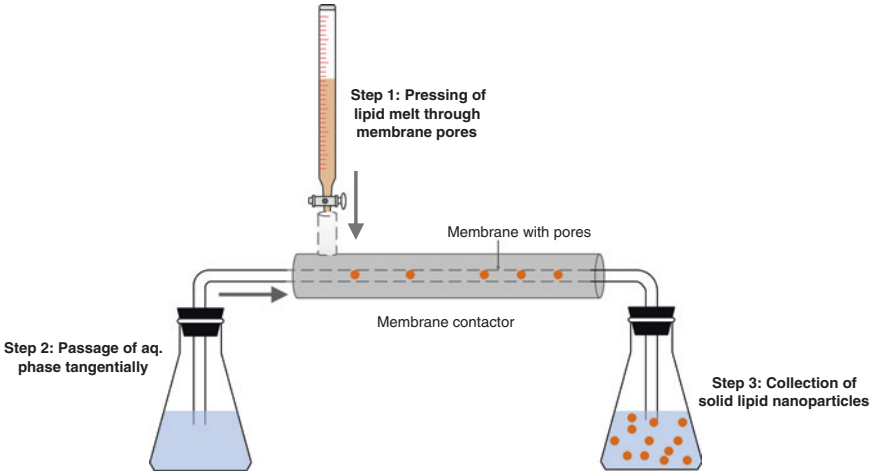

Fig. 3.10 Schematic steps involved in the membrane contactor technique

lipid flux (Charcosset et al. 2005). The dispersed lipid flux is calculated as the volume of the lipid phase divided by the reaction time and membrane surface area. The cross flow velocity and temperature of the aqueous phase also influence the size of the nanoparticles produced and the lipid flux (Charcosset and Fessi 2005). The choice of emulsifiers and their concentration affects the lipid flux and the size of nanoparticles (El-Harati et al. 2006).

3.2.10 Supercritical Fluid Extraction of Emulsions

Supercritical fluid (SCF) technology has been used in the production of microparticles and drugs (Yasuji et al. 2008). SCF technology is based on the principle of precipitation of drug or microparticles using a compressed anti-solvent such as supercritical carbon dioxide. The solutes are dissolved in a solvent. The supercritical fluid chosen here has complete or partial miscibility with the solvent, but acts as the anti-solvent to the solutes. The micron-sized solute particles precipitate upon spraying the solution into flowing SCF (Byrappa et al. 2008).

The process of preparing lipid nanoparticles from emulsions using SCF technology is referred to as "supercritical extraction of emulsions" (SFEE) (Chattopadhyay et al. 2006, 2007). Figure 3.11 is a schematic depiction of the preparation of lipid nanoparticles by the SFEE process. The organic solution is prepared by solubilizing the lipid material and the drug in an organic solvent such as chloroform with the addition of a suitable surfactant. The organic solution is dispersed into an aqueous solution (which may contain a co-surfactant) and the mixture is subsequently passed through a high pressure homogenizer to form an o/w emulsion. The o/w emulsion is introduced from one end of the extraction

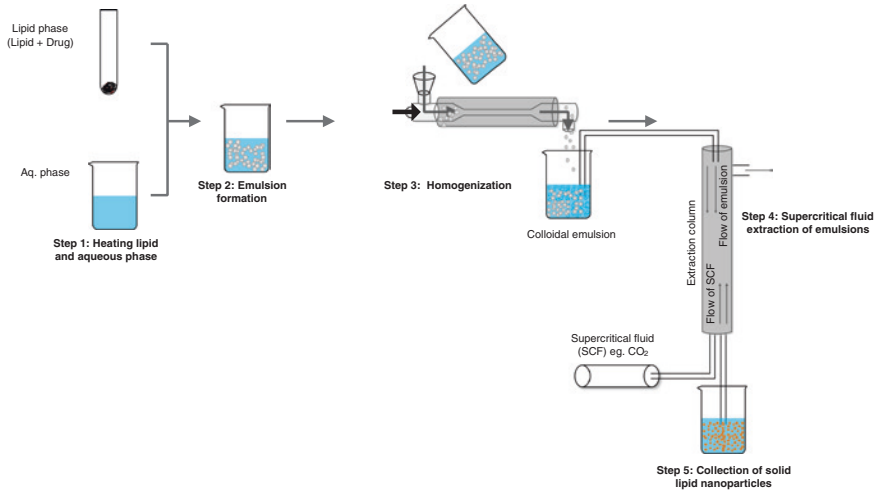

Fig. 3.11 Schematic steps involved in preparation of lipid nanoparticles by the supercritical fluid extraction of emulsions technique

column (usually the top) at a constant flow rate and the supercritical fluid (maintained at constant temperature and pressure) is introduced counter-currently at a constant flow rate. Lipid nanoparticle dispersions are formulated by continuous extraction of solvent from the o/w emulsions.

3.2.11 Coacervation Technique

The coacervation technique has been most commonly employed in the production of polymeric nanoparticles (Maculotti et al. 2009; Silva et al. 2008). Recently, it has been used in the preparation of fatty acid lipid nanoparticle dispersions (Battaglia et al. 2010, 2011). Lipid nanoparticles are produced by acidification of a micellar solution of fatty acid alkaline salts (Bianco et al. 2010; Chirio et al. 2011; Gallarate et al. 2010).

Prior to preparation of lipid nanoparticles, a stock solution of polymeric stabilizer is prepared by heating in hot water. A sodium salt of the fatty acid is homogenously dispersed in the polymeric stabilizer stock solution and the solution is heated above the Krafft point of the sodium salt of the fatty acid, under constant stirring, to obtain a “clear” solution. The drug (solubilized in ethanol) is later added to the clear solution, with constant stirring, until a single phase is obtained. Gradual addition of coacervating solution (or on acidifying the solution) to this mixture yields a suspension. Further cooling of the suspension in a water bath, under constant agitation, yields drug-loaded nanoparticles which are well dispersed. Figure 3.12 gives a schematic depiction of the coacervation technique used in the preparation of lipid nanoparticles.

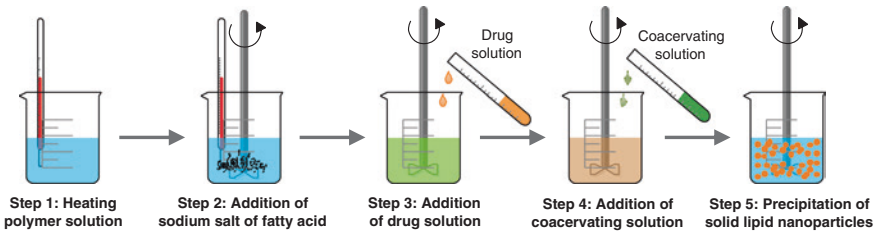

Fig. 3.12 Schematic steps involved in the coacervation technique

3.2.12 Phase Inversion Temperature Technique

Emulsification by the phase inversion temperature (PIT) method was first reported in 1968 by Shinoda and Saito (1968). Transformation of an o/w type to a w/o type of emulsion is termed "phase inversion", can be induced by changing the temperature, and the temperature at which the inversion occurs is referred to as the PIT. Rapid cooling of an emulsion prepared at the PIT produces a fine, stable emulsion. This procedure was used in the preparation of stable lipid nanocapsules in solvent-free conditions by Heurtault et al. (2002). Lipid nanocapsules are an intermediate between lipid and polymeric nanoparticles.

Heurtault et al. (2002) developed a novel solvent-free technique for the formulation of lipid nanocapsules that was based on the phase inversion of an emulsion. In this PIT technique, the formulation ingredients (i.e. lipid, surfactant, drug and water) are thoroughly mixed under constant magnetic agitation. The mixture is then subjected to three cycles of heating and cooling (from room temperature to 85 °C (the PIT), to 60 °C to 85 °C to 60 °C to 85 °C to room temperature) applied at a constant rate of 4 °C/min. In the final step, the emulsion is diluted under cooling conditions. Figure 3.13 gives a schematic representation of steps involved in phase inversion temperature method.

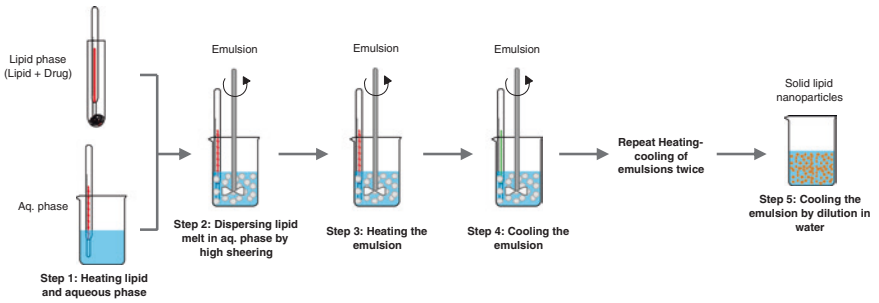

Fig. 3.13 Schematic steps involved in the phase inversion temperature method

3.3 Conclusions

Several production techniques are available and have been discussed in this chapter. The particle characteristics of the lipid nanoparticles is expected to be greatly influenced by the choice of method employed in its production (see next chapter). The choice of method also depends on the composition of the lipid nanoparticle formulation. Table 3.1 outlines the various techniques discussed here with the possible mechanism involved in particle formation. Each of the methods developed to date have their own advantages and disadvantages (also discussed in Table 3.1). A novel method developed by the authors is a rapid technique with many advantages, and few disadvantages, but may face limitations in scalability and is not yet fully tested. Based on literature, it can thus be concluded that no method is perfect, and choice of method will depend on availability of equipment, composition of the lipid formulation and desired property outcomes.

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