Available on line www.jocpr.com

Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2010, 2(1): 211-227

ISSN No: 0975-7384

Production of Solid Lipid Nanoparticles-Drug Loading and Release Mechanism

Rabinarayan Parhi*, Padilama Suresh

GITAM Institute of Pharmacy, GITAM University, Visakhapatnam-530045, AP.

Abstract

Solid lipid nanoparticles (SLNs) introduced at the beginning of the 1990s represents an alternative carrier system to traditional colloidal carriers, such as emulsions, liposomes and polymeric micro and nanoparticles. A number of administration routes such as topical, oral, parenteral, nasal and pulmonary have been proposed for the delivery of SLNs. This paper reviews various production techniques for SLNs including their advantages and disadvantages, drug incorporation, loading capacity with the factors affecting drug incorporation and loading capacity and drug release, especially emphasizing on mechanism drug release.

Key Words: Colloidal carriers; Drug incorporation; loading capacity; Entrapment efficiency.

Introduction

In recent years, the development of new drug alone is not sufficient to provide the base for the progress in drug therapy and the *in-vitro* data obtained from various experiments are very often followed by disappointing results *in vivo* due to following reasons

a. Poor absorption, rapid metabolism and elimination lead to insufficient drug concentration at the specific site.
b. High fluctuation of the plasma levels due to unpredictable bioavailability after peroral drug administration
c. Poor drug solubility

A promising strategy to overcome these problems involves the development of the suitable drug carrier system.

The new drug delivery system for lipophilic drug was first started during 50s. The first safe fat emulsion (Intralipid) was developed for parenteral nutrition. The advantage of this carrier system is the reduction of pain during injection [1]. The major limitation is the instability of the physical state due to reduction of Zeta potential, which leads to agglomeration, drug expulsion and consequently breakage of the emulsion [2].

Liposomes are spherical vesicles composed of outer bilayer of amphipathic molecule such as phospholipids with an aqueous compartment inside. The amount of poorly water-soluble drug that is possible to incorporate is, however, limited due to the relatively small volume (0.7ml/mmol of lipid) of the hydrophobic region of the lipid bilayer in comparison to aqueous interior (2-3ml/mmol of lipid). In addition, other obstacles for the development of liposomal formulation are limited physical stability, drug leakage, and instability in the vascular system due to lipid exchange with lipoproteins and rapid removal from circulation following intravenous administration primarily by Kupffer cells of the liver and fixed macrophages of the spleen [3],[4].

Polymeric nanoparticles, which are generally made with suitable biodegradable polymers, such as polyalkylcyanoacrylate, polymethylmethacrylate have been shown to prolong the release of the incorporated drugs [5]. Advantage of polymer system is to provide chemical modifications, including the synthesis of block and co-polymers. Problem associated with it are lack of large scale production methods to yield a product of a quality acceptable for registration by the regulatory authorities, cost of the polymeric material, toxic solvent residues from the production process (e.g. solvent evaporation method), cytotoxicity of some polymeric particles (e.g. lactic acid release from polylactic acid particles), and chemical problems with the polymers (e.g. catalyst residues, molecular non-homogeneity) [6].

SLNs indicate lipids, which are used in the manufacturing of nanoparticles, are solid at room temperature and also at body temperature and with mean diameter approximately between 50 and 1000nm. Here SLNs combine advantages such as physical stability, protection of incorporated labile drugs, controlled release and excellent tolerability of other innovative carrier system (fat emulsion, liposomes and polymeric nanoparticles) while at the same time minimizing the associated problem. For high drug loading and entrapment efficiency, lipophilic drug with good compatibility with lipids, have often been selected to incorporate in to SLN. Many methods are developed to prepare SLNs, such as high pressure homogenization, microemulsion, solvent emulsion diffusion, solvent emulsification evaporation, high speed stirring and Ultrasonication. In addition to above traditional techniques, few novel techniques also used are supercritical fluid, membrane contactor, solvent injection and multiple emulsion technique.

II. Preparation techniques for solid lipid nanoparticles

Various methods have been developed for the preparation of SLNs which uses biocompatible lipids or lipid molecules with a history of safe use in medicine. The essential excipients of SLNs

are solid lipid as matrix material, emulsifier and water. The term lipid is used here in a broader sense and includes

a. Saturated monoacid triglycerides- tristearin, tripalmitin, trilaurin, trimyristin
b. Partial glycerides - glyceryl monostearate, glyceryl behenate, glyceryl palmitostearate
c. Fatty acids- stearic acid, behenic acid, palmitic acid, decanoic acid
d. Steroids- cholesterol and
e. Waxes - cetyl palmitate.

All classes of emulsifiers have been used but physiologically compatible emulsifiers such as phospholipids (Soyabean lecithin, egg lecithin, phosphatidyle choline), bile salts (Sodium cholate, Sodium taurocholate, sodium glycholate) and poloxamers (Poloxamer- 188, 182, 407) are preferred as stabilizers and in few cases co-emulsifiers (e.g. butanol) are used. A large number of drugs and solid lipids used for the preparation of SLNs by different techniques are given in the Table 1.

Table 1. Drugs and lipids used for preparation of solid lipid nanoparticles by different methods

Methods	Drugs	Lipids	Literature
1. High pressure homogenization technique;			
A-Hot homogenization	Porcine pancreatic lipase and colipase	Glyceryl tripalmitate (Dynasan® 116) Glyceryl trimyristin (Dynasan® 114)	[8]
	Diazepam	Cetyl palmitate	[9]
	Clotrimazole	Glyceryl tripalmitate (Dynasan® 116)	[10]
	Nitrenadipine	Glyceryl tripalmitate Cetyl palmitate Glyceryl monostearate	[11]
	Isotretinoin	Glyceryl palmitostearate (Precirol® ATO 5)	[12]
	Doxorubicine	Stearic acid	[23]
	Paclitaxel	Tripalmitin	[23]
	Vitamin-E	Cetyl palmitate	[26]
	All-trans retinoic acid	Tricaprin	[15]
	Retinol	Glyceryl behenate Tribehenate (Compritol 888 ATO)	[16]
	3'-azido-3'-deoxythymidine palmitate	Trilaurin	[17]
	Cyclosporine	Glycerol monostearate Imwitor®900	[20]
	Cyclosporine-A	Stearic acid	[21]
	oxybenzone	Cetyl palmitate	[73]
B-Cold homogenization	Vinorelbin-bitartrate	Glyceryl monostearate	[22]
2, Microemulsion technique;	Curcuminoides	Stearic acid Glyceryl monostearate	[29]
	Podophyllotoxin	Stearic acid	[34]
	Verapamil	Cacao butter	[53]

	Tea polyphenol	Glyceryl monostearate	[25]
	Cyclosporine A	Stearic acid	[30]
	Insulin	Stearic acid	[39]
3, Solvent emulsification-evaporation technique;			
	Oridonin	Stearic acid	[54]
	Insulin	Stearic acid	[39]
4, Solvent emulsification-diffusion technique;			
	Clobetasol propionate	Monostearin	[46]
	Gonadorelin	Monostearin	[43]
	Rifampicin, Isoniazid, Pyrazinamide	Stearic acid	[42]
5, Melt dispersion technique;	Oridonin	Stearic acid	[54]
6, Ultrasonication technique;	Indomethacin	Glyceryl behenate Tribehenate (Compritol 888 ATO)	[52]
	Vinpocetine	Glyceryl monostearate	[56]
	Triptolide	Tristearin glyceride	[51]
	Mifepristone	Glycerol monostearate	[55]
	Oridonin	Stearic acid Lecithin	[54]
7, Double emulsion technique;	Insulin	Tripalmitin	[59]
	Insulin	Palmitic and Stearic acid	[60]
	Ceramide	Tripalmitin	[61]
8, Membrane contactor technique;	Vitamin-E	Glyceryl behenate [Gelucire-44/14]	[62]
9, Supercritical fluid technology;	Indomethacin, Ketoprofen	Tristearin,Tripalmitin Glyceryl behenate [Gelucire-50/13]	[67]
	Bovine serum albumin	Trimyristin [Dynasan® 114] Glyceryl behenate [Gelucire® 50/02]	[69]

II.A. High pressure homogenization technique

In high pressure homogenization technique lipids are pushed with high pressure (100-200 bars) through a narrow gap of few micron ranges. So shear stress and cavitation (due to sudden decrease in pressure) are the forces which cause the disruption of particle to submicron range. Normally the lipid contents are in the range of 5-10%. At this concentration it does not cause any problem to homogenizer. SLN can be stabilized by wide range of surfactants or polymers and their mixture but the emulsion for parenteral nutrition is stabilized by lecithin only. In contrast to other preparation technique high pressure homogenization does not show scaling up problem [7]. Basically, there are two approaches for SLN production by high pressure homogenization, hot and cold homogenization techniques. For both the techniques depicted in Figure 1, the drug is dissolved or dispersed or solubilized in the lipid being melted at approximately 5-10ºc above the melting point.

1. Hot homogenization technique

For the hot homogenization technique the drug loaded melted lipid is dispersed under stirring by high shear device (e.g. Ultra Turrax) in the aqueous surfactant solution of identical temperature. The pre-emulsion obtained is homogenized by using a piston gap homogenizer (e.g. Macron LAB 40 or Macron LAB 60 or APV-2000) and the produced hot o/w nanoemulsion is cooled

down to room temperature. At room temperature the lipid recrystallizes and leads to formation of SLNs [8],[9],[10],[11],[12],[13],[14],[15],[16],[17]. In case of glycerides composed of short chain fatty acids (e.g. Dynasan-112) and glycerides with low melting point (too close to room temperature), it might be necessary to cool the nanoemulsion to lower temperature than room temperature to start recrystallization [18],[19].

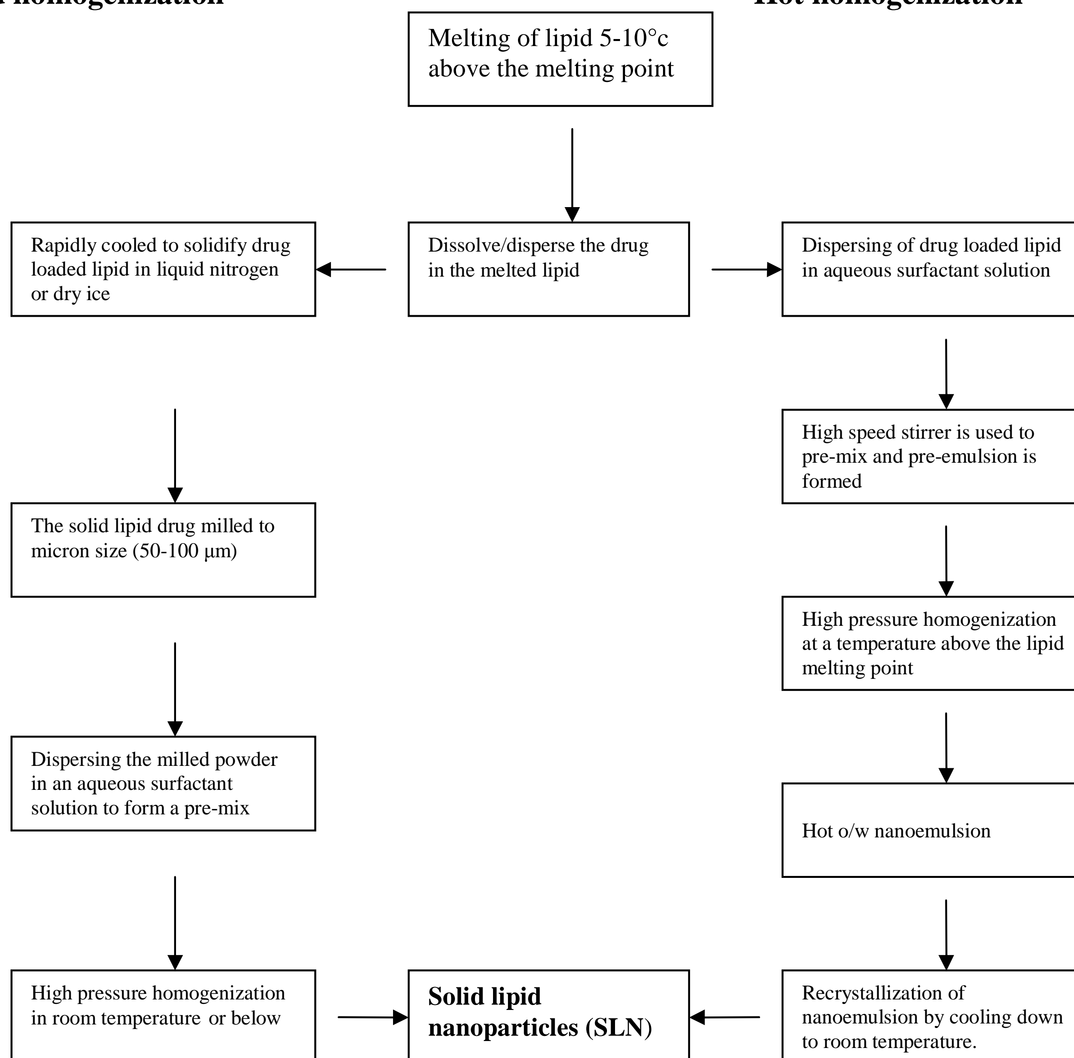

FIGURE 1. Schematic representation of SLN preparation by hot and cold homogenization

In general, high temperature results in lower particle size due to the decreased viscosity of the inner phase, but the disadvantage is that, at high temperature the rate of drug and carrier degradation is more and further due to small particle size and presence of emulsifier, lipid crystallization may be highly retarded and the sample remains as supercooled melt for several months [20]. A great disadvantage of hot homogenization technique is that, it is a poor technique for hydrophilic drug candidate because during heating the drug partitions into aqueous phase and

when cooled, most of drug particle remained at the outer layer of the solid lipid nanoparticles, which leads to burst release [21].

2. Cold homogenization technique

Cold homogenization is carried out with the solid lipid containing drug and therefore called as milling of a suspension. Cold homogenization has been developed to prevent:

- Temperature induced drug degradation
- Partitioning of hydrophilic drug from lipid phase to aqueous phase
- Complexity of the crystallization step of the nanoemulsion leading to several modifications and/or supercooled melts

The first step of preparation is same as hot homogenization which includes dispersion or dissolving or solubilisation of the drug in the melted lipid. Then the drug lipid mixture is rapidly cooled either by means of liquid nitrogen or dry ice. The drug containing solid lipid is milled by means of mortar or ball mill to micron size (50-100 micron) and these microparticles are dispersed in chilled emulsifier solution yielding a pre-suspension. Then this pre-suspension is subjected to high pressure homogenization at room or below room temperature, where the cavitation force is strong enough to break the microparticles to SLNs. This process avoids or minimizes the melting of lipid and therefore minimizing loss of hydrophilic drug to aqueous phase [22]. Another method to minimize the loss of hydrophilic drug to aqueous phase is to replace water with other media (e.g. oil or PEG 600) with low solubility for the drug. In comparison to hot homogenization, in cold homogenization particle size and polydispersity index are more. The cold homogenization only minimizes the thermal exposure of drug, but it does not avoid completely it due to melting of the lipid/drug mixture in the first step of preparation.

High pressure homogenization increases the temperature of the sample (e.g. 10-20ºC for each homogenization cycle) [23]. In most of the cases, 3-5 homogenization cycles at 500-1500 bar are sufficient to prepare SLN. Increasing the number of homogenization cycle or the homogenization pressure resulted in increase of particle size due to particle coalescence which resulted from high kinetic energy of particles [24].

II.B. Microemulsion technique

Microemulsions are clear, thermodynamically stable system composed of a lipophilic phase, surfactant and co-surfactant (in most cases) and water. The concept of microemulsion technique for the production of SLN was developed and optimized by Gasco and co-workers which has been adapted and/or modified by different laboratories [25].

Microemulsion needs to be produced at a temperature above the melting point of the lipids, so the lipid should have melting point above room temperature. At first solid lipids are taken (approximately 10%) and melted at a temperature 65-70°C. Separately, a mixture of surfactant of 15%, co-surfactant of 10% and water were heated to same temperature as the lipid and then added to melted lipid under mild stirring [26]. A transparent, thermodynamically stable system was formed which was then dispersed under stirring in excess cold water (2-3°C) in the typical ratio of microemulsion to cold water ranges from 1:10 to 1:50 using a specially developed thermostated syringe with gentle stirring [27],[28]. The composition of microemulsion

determines the dilution process. The SLN preparations were washed three times with distilled water and filtered using a membrane (e.g. Diaflo YM 100) having cut off 100,000 Dalton in order to remove any unwanted bigger lipid particles [29],[30]. The excess water was removed either by ultra-filtration or by lyophilisation in order to increase the particle concentration.

In the microemulsion, drugs are partitioned partly in the internal oil phase and partly at the interphase between internal and continuous phase, depending on their lipophilicity. When SLNs are formed by a quick quenching of the microemulsion, the presence of the droplet structure of the microemulsion does not allow the drug molecules to nucleate and form the crystal lattice, and consequently the drug molecules remain dispersed in the lipid matrix of the SLNs in an amorphous state [31].

The major parameters during scaling up are temperature gradient (the temperature difference between lipid melting and emulsion process), the pH value and the water content. High temperature gradients facilitate rapid lipid crystallization and prevent aggregation [32]. The removal of excess water from the prepared SLN dispersion is a difficult task with regard to the particle size and high concentrations of surfactants and co surfactants which are necessary for formulating purposes [33],[34].

II.C. Solvent emulsification-evaporation technique

In solvent emulsification-evaporation method, the lipophilic material and hydrophobic drug were dissolved in a water immiscible organic solvent (e.g. cyclohexane, dichloromethane, toluene, chloroform) and then that is emulsified in an aqueous phase using high speed homogenizer [35],[36],[37],38]. To improve the efficiency of fine emulsification, the coarse emulsion was immediately passed through the microfluidizer. Thereafter, the organic solvent was evaporated by mechanical stirring at room temperature and reduced pressure (e.g. rotary evaporator) leaving lipid precipitates of SLNs as indicated in Figure 2a. Here the mean particle size depends on the concentration of lipid in organic phase. Very small particle size could be obtained with low lipid load (5%) related to organic solvent. The great advantage of this technique is the avoidance of any thermal stress, which makes it suitable for the incorporation of highly thermolabile drugs. A clear disadvantage is the use of organic solvent which may interact with drug molecules and limited the solubility of the lipid in the organic solvent.

II.D. Solvent emulsification-diffusion technique

In solvent emulsification-diffusion technique, the solvent used (e.g. benzyl alcohol, butyl lactate, ethyl acetate, isopropyl acetate, methyl acetate) must be partially miscible with water and this technique can be carried out either in aqueous phase or in oil [39],[40],[41],[42],[43]. Initially, both the solvent and water were mutually saturated in order to ensure the initial thermodynamic equilibrium of both liquid [44]. When heating is required to solubilize the lipid, the saturation step was performed at that temperature. Then the lipid and drug were dissolved in water saturated solvent and this organic phase (internal phase) was emulsified with solvent saturated aqueous solution containing stabilizer (dispersed phase) using mechanical stirrer. After the formation of o/w emulsion, water (dilution medium) in typical ratio ranges from 1:5 to 1:10, were added to the system in order to allow solvent diffusion into the continuous phase, thus forming aggregation of the lipid in the nanoparticles which has been depicted in Figure 2b. Here the both the phase were maintain at same elevated temperature and the diffusion step was

performed either at room temperature or at the temperature under which the lipid was dissolved [45]. Throughout the process constant stirring was maintained. Finally, the diffused solvent was eliminated by vacuum distillation or lyophilization [46].

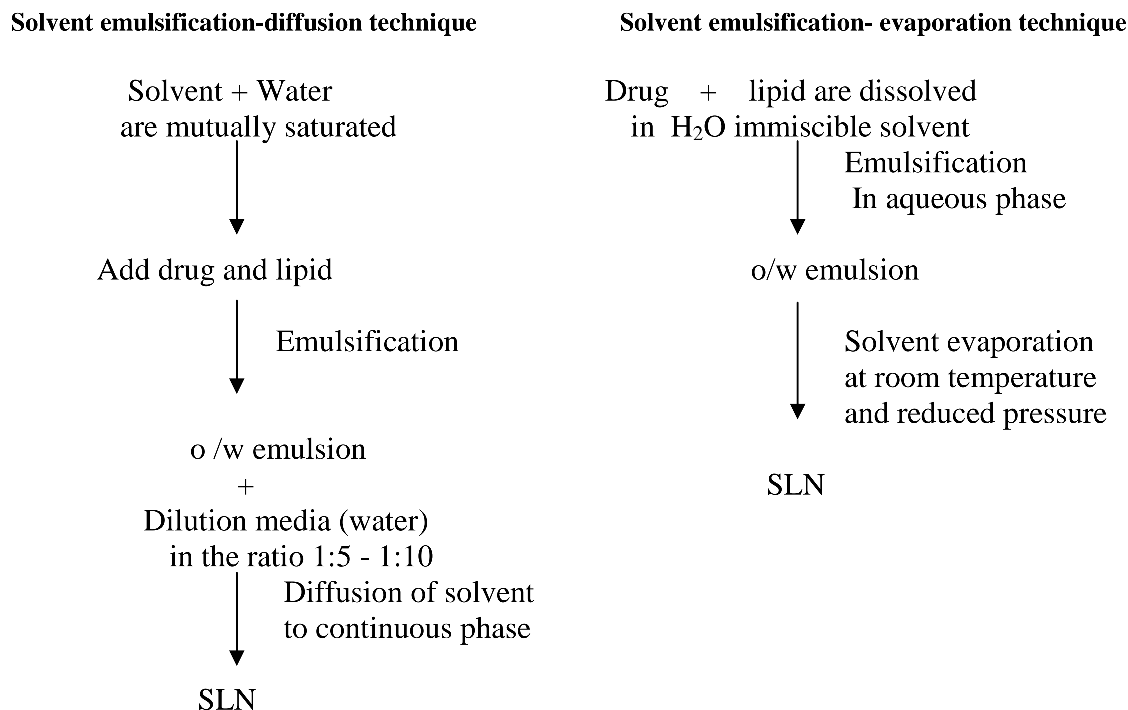

FIGURE 2. Schematic diagram of (a) Solvent emulsification-diffusion technique (b) Solvent emulsification-evaporation technique

This approach has clear advantages over the other methods, namely: (a) it is efficient and versatile. (b) easy implementation and scaling up (no need for high energy sources). (c) high reproducibility and narrow size distribution. (d) less physical stress (i.e., short exposure to high temperatures and to mechanical dispersion). (e) it is not necessary to dissolve the drug in the melted lipid. Drawbacks associated with this method are (a) the need to clean up and concentrate the SLN dispersion. (b) drug diffusion into aqueous phase occurred easily ,which leads to low entrapment of drugs in SLN [47].

II.E Melting dispersion method (Hot melt encapsulation method)

The melting dispersion method was as follows, in first step, drug and solid lipid were melted in an organic solvent regarded as oil phase and simultaneously water phase was also heated to same temperature as oil phase. Then in second step, the oil phase added in to a small volume of water phase and the resulting emulsion was stirred at higher rpm for few hrs. At last it was cooled down to room temperature to give SLNs. The last step was same as solvent emulsification-evaporation method except in melting dispersion method no organic solvent had to be evaporated. Reproducibility was less than that of solvent emulsification-evaporation method but more than ultrasonication method [48],[49].

II.F. High shear homogenization and/or Ultrasonication technique

This ultrasonication technique is a dispersing technique, which was initially used for the production of solid lipid nanodispersion [50]. Ultrasonication based on the mechanism of cavitation. In first step, the drug was added to previously melt solid lipid. In second step, the heated aqueous phase (heated to same temperature) was added to the melted lipid and emulsified by probe sonication or by using high speed stirrer or aqueous phase added to lipid phase drop by drop followed by magnetic stirring [51]. The obtained pre-emulsion was ultrasonicated using probe sonicator with water bath (at 0ºC). In order to prevent recrystallization during the process, the production temperature kept at least 5ºC above the lipid melting point [52]. The obtained nanoemulsion (o/w) was filtered through a 0.45μm membrane in order to remove impurities carried in during ultrasonication [53]. Then the obtained SLN is stored at 4ºC. To increase the stability of the formulation, was lyophilized by a lyophilizer to obtain freeze-dried powder and sometime mannitol (5%) was added into SLNs as cryoprotector [54].

Advantages of this technique are widespread and easy to handle. It is a simple, available and effective method to produce SLNs without organic solvents; but it also having the limitation that, it require an extra step of filtration of formed SLN emulsion in order to remove impurity materials (e. g. metal) produced during ultrasonication and is often compromised by the presence of microparticles [55],[56].

II.G. Double emulsion technique

In double emulsion technique the drug (mainly hydrophilic drugs) was dissolved in aqueous solution, and then was emulsified in melted lipid. This primary emulsion was stabilized by adding stabilizer (e.g. gelatin, poloxamer-407). Then this stabilized primary emulsion was dispersed in aqueous phase containing hydrophilic emulsifier (e.g. PVA). Thereafter, the double emulsion was stirred and was isolated by filtration [57],[58]. Double emulsion technique avoids the necessity to melt the lipid for the preparation of peptide-loaded lipid nanoparticles and the surface of the nanoparticles could be modified in order to sterically stabilize them by means of the incorporation of a lipid -PEG derivative. Sterical stabilization significantly improved the resistance of these colloidal systems in the gastrointestinal fluids [59]. This technique is mainly used to encapsulate hydrophilic drug (peptides). A major drawback of this technique is the formation of high percentage of microparticles. Sodium cromoglycate containing SLN was tried to be prepared by this technique but the produced colloidal system gave the average particle of micrometer range. Insulin loaded SLN was prepared by a novel reverse micelle-double emulsion technique, using sodium cholate-phosphatidylcholine based mixed micelle [60],[61].

II.H. Membrane contactor technique

It is a novel technique to prepare the SLN. In membrane contactor technique the liquid phase was pressed at a temperature above the melting point of the lipid through the membrane pores (kerasep ceramic membrane with an active ZrO_2 layer on an AlO_2-TiO_2 support) allowing the formation of small droplets as indicated in Figure 3. The aqueous phase was stirred continuously and circulates tangentially inside the membrane module, and sweeps away the droplets being formed at the pore outlets. SLNs were formed by the cooling of the preparation at the room temperature. Here both the phases were placed in the thermostated bath to maintain the required temperature and nitrogen was used to create the pressure for the liquid phase.

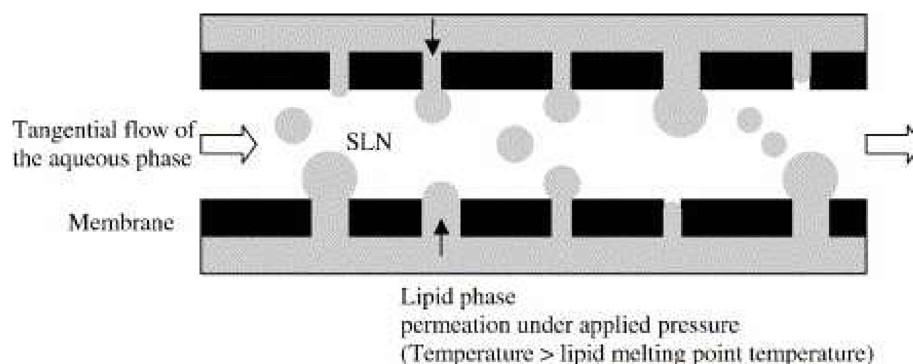

FIGURE 3. Schematic drawing of the membrane contactor for the SLN preparation [62].

The influence of various process parameters (aqueous phase cross flow velocity, the lipid phase pressure, aqueous and lipid phase temperature, lipid phase amount and membrane pore size) were studied [62]. The role of cross–flow velocity is to contribute to the detachment of the SLN formed at the pores outlet and to homogenize the SLN suspension. The aqueous cross flow velocity has little effect on the particle size as well as on lipid phase flux, because the lipid phase circulates from membrane outside to the membrane inside and is a case of classical ultrafiltration or microfiltration. The lipid phase flux is increased with the increase in lipid phase pressure within a range 3-6 bar but at the highest pressure the little decrease in size of particles was observed. It was seen that below the temperature of the lipid fusion point the smaller particles (70-80nm) were formed but the flux time increased where as above the melting point of the liquid the flux time decreased and the particle size was increased. The membrane contactor method is also 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) [63]. The advantages of this process of SLN preparation using a membrane contactor are shown to be its facility of use, the control of the SLN size by an appropriate choice of process parameters and its scaling up ability [64]. Vitamin-E loaded SLN was prepared by this technique [62].

II.I. Solvent injection technique

Solvent injection technique is a novel approach to prepare SLN, which has following advantages over other production methods like use of pharmacologically acceptable organic solvent, easy handling and fast production process without technically sophisticated equipment. It is based on lipid precipitation from the dissolved lipid in solution. In this technique, the solid lipid was dissolved in water-miscible solvent (e.g. ethanol, acetone, isopropanol) or a water-miscible solvent mixture. Then this lipid solvent mixture was injected through an injection needle in to stirred aqueous phase with or without surfactant. The resulted dispersion was then filtered with a filter paper in order to remove any excess lipid [65].

The presence of emulsifier within the aqueous phase helps to produce lipid droplets at the site of injection and stabilize SLN until solvent diffusion was complete by reducing the surface tension between water and solvent resulting in solvent [65].

II.J. Supercritical fluid technology

This is a novel technique which recently applied for the production of SLNs. A fluid is qualified as supercritical when its pressure and temperature exceed their respective critical value. Above

the critical temperature, it is not possible to liquefy a gas by increasing the pressure. The supercritical fluid has unique thermo-physical properties. As the pressure is raised, the density of the gas increases without significant increase in viscosity while the ability of the fluid to dissolve compounds also increases. A gas may have little to no ability to dissolve a compound under ambient condition can completely dissolve the compound under high pressure in supercritical range. Therefore, its solvation power is altered by careful control of changes in temperature and pressure. Many gases like, CO_2, ammonia, ethane, $CHClF_2$ and CH_2FCF_3 were tried, but CO_2 is the best option for SCF technique because, it is generally regarded as safe, easily accessible critical point [31.5ºC, 75.8 bar), does not causes the oxidation of drug material, leaves no traces behind after the process, is inexpensive, noninflammable, environmentally acceptable an easy to recycle or to dispose off. Most of the time solubility enhancers (e.g. ethanol) are used to increase the solubility of less soluble solvent (e.g. water) in the SCF phase or this technique generally use organic solvents (e.g. DMSO, DMFA) because they are fully miscible in SCF-CO_2. This technology comprises several processes for nanoparticles production such as rapid expansion of supercritical solution (RESS), particles from gas saturated solution(PGSS), gas/supercritical antisolvent (GAS/SAS), aerosol solvent extraction solvent (ASES), solution enhanced dispersion by supercritical fluid (SEDS),supercritical fluid extraction of emulsions (SFEE). Mainly SAS and PGSS were used for SLN preparation [66],[67],[68],[69],[70].

GAS/SAS

In this process SCF acts as antisolvent for processing solid that are insoluble in SCF. It exploits the ability of SCF to dissolve in organic solvent and reduce the solvation power of solid in solution, thus causing the solid to precipitate. At first, the near critical or supercritical fluid was introduced in a vessel containing an organic solvent in which the solid material to be crystallized was dissolved which causes the intimate mixing of the fluid and liquid resulting in liquid expansion and particle precipitation. A clear disadvantage of this technique is the lack of control on the particle formation. A modification of SAS technique was used to prepare lysozyme spherical nanoparticles, which combines both the atomization and anti-solvent process, by using water/ethanol solution [71].

PGSS

In this process, the SCF was dissolved in liquid substrate, or a solution of substrate in solvent, or a suspension of substrate in solvent followed by a rapid depressurization of this mixture through a nozzle causing the formation of SLN. The great advantage of this process is that it produces particles of great variety of substance that need not be soluble in SCF-CO_2. Limitations are, care must be taken for thermolabile solute and the final product may contain microparticles. Insulin nanoparticles are produced by this process, in which the solvent used, was DMSO and the lipid mixture (tristearin, phosphatidylcholine, dioctylsulfosuccinate) were atomized to produce insulin SLN (<500nm) [72].

III. DRUG LOADING CAPACITY AND INCORPORATION

A novel carrier system should allow a high loading capacity for the incorporated drugs and provide long term incorporation. The loading capacity is generally expressed in percent related to lipid phase (lipid + drug) [73].

$$\text{Loading efficiency (\%)} = \frac{\text{Total weight of drugs - weight of free drugs}}{\text{Total weight of drugs}} \times 100$$

Here, total weight of free drugs is measured by using titration method or spectroscopic method from the supernatant liquid after filtering the nanosuspension/nanoemulsion through membrane filter.

Factors influencing the loading capacity and incorporation of drug in lipid are:

1. Drug solubility in the melted lipid.
2. Chemical and physical structure of solid lipid matrix.
3. Polymorphic of lipid material.
4. Gelatin phenomena.
5. The existence of supercooled melts.

To achieve high loading capacity, it is necessary that the drug should have sufficiently high solubility (i.e. should be higher than required, because it decreases when cooling down the melt) in the lipid melt. To enhance the drug solubility in the lipid melt, sometime solubilizers are used. In addition, the presence of mono-and diglycerides in the lipid matrix enhance the drug solubilization.

The drug can be incorporated between fatty acid chains, between lipid layers or in imperfections [74]. According to Western et al. the chemical nature of lipid shows great importance in loading and incorporation of drugs, because the lipid with perfect lattice (e.g. monoacid triglycerides) leads to drug expulsion [75]. Complex glycerides mixture of mono-, di- and triglycerides and hard fats of different chain length form less perfect crystals with many imperfections which provide space to accommodate the drugs. For the controlled optimization of the drug incorporation and drug loading capacity, characterization of physical state of the lipid particles is done by NMR and X-ray. In addition, a new technique ESR is very essential.

Polymorphic forms also influence the drug incorporation. Lipid nanoparticles recrystallize least in α-form where as the bulk lipid recrystallizes preferentially in β-modification and transferring rapidly into β-form (most stable form). The formation of more stable form leads to more perfect lattice, the number of imperfection decreases, which promotes drug expulsion. To optimize SLN carrier system in controlled way, it is necessary to create certain fraction of α-form and is to be preserved during storage. By doing this SLN carrier system transform in to an intelligent drug delivery system by having a built-in trigger mechanism to initiate transformation from α-form to β-forms and consequently controlled drug release. The to β transformation can be retarded by surfactants (e.g. poloxamer) or nitrogen environment, acts by inhibiting lipid hydrolysis [76].

Gelatin phenomena, which in most of the cases is an irreversible process, deals with the transformation of low-viscosity SLN dispersion into a viscous gel which may occur very rapidly and unpredictably. In this process increase in particle surface takes place due to the formation of platelets in β-modification, so that the surfactant molecules no longer provide coverage of the new surfaces and therefore, particle aggregation is seen [76].

Supercooled melts is a phenomenon in which the lipid crystallization may not occur although the sample is stored at a temperature below the melting point of lipid. It is a size dependent crystallization process i.e. a critical number of crystallization nuclei are required to start the crystallization process. But in this phenomenon critical number of nuclei is less likely to be formed. So the tendency of the formation of supercooled melts increases with decreased particle size [77].

IV. DRUG RELEASE FROM SLN

Depending upon the method of preparation, drug solubility and drug/ lipid ratio, the drug is located mainly in the core of the particles, in the shell or molecularly dispersed throughout the matrix.

There are mainly three drug incorporation models which describe the incorporation of drug into SLN.

1. Homogenous matrix model.
2. Drug enriched shell, core shell model.
3. Drug enriched core, core shell model.

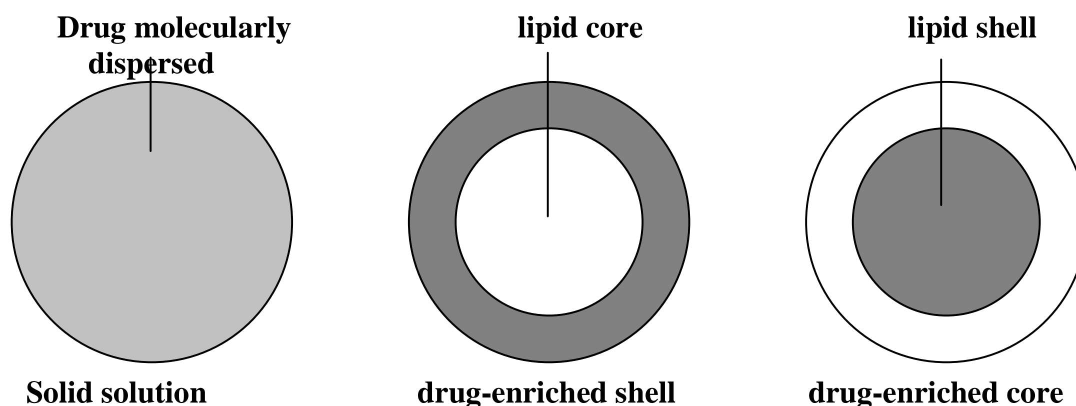

FIGURE 4. Models of incorporation of active compounds into SLN: (a) Homogeneous matrix, (b) Drug enriched shell with lipid core, (c) Drug enriched core with lipid shell.

The above three model are the function of formulation combination (solid lipid, active ingredients, surfactants and sometime co-surfactant) and of the production techniques (hot vs. cold homogenization)

Homogenous matrix model or solid solution model with drug being present in amorphous clusters or molecularly dispersed is mainly obtained when incorporating highly lipophilic drugs into SLN with using hot homogenization technique or applying cold homogenization method or by avoiding potentially drug solubilizing surfactants. In the cold homogenization technique the drug (in molecularly dispersed form) is dispersed in bulk of melted lipid, then the mechanical force of high pressure homogenization leads to the break down of molecular form to nanoparticles and giving rise to homogenous matrix model as shown in Figure 4a. The same will happen when highly lipophilic drug nanoparticles is formed by hot homogenization or without

using the surfactant, is being cooled, crystallizes and no phase separation occur during cooling process. Etomidate SLN represents the homogenization matrix model [78].

The drug enriched shell with core shell model will be obtained when performing the production as shown in Figure 5. During the production, the drug partitioned to water phase. Upon cooling, the lipid precipitates first, forming a practically drug free lipid core due to phase separation. At the same time, the drug re-partitions into the remaining liquid-lipid phase and drug concentration in the outer shell increasing gradually [79]. Finally drug enriched shell crystallizes as depicted in 4b. The amount of drug partitioning to the aqueous phase will increases with the increase of solubility of drug in the aqueous phase. Mainly two factors, increasing temperature of the aqueous phase and increasing surfactant concentration, are increasing the saturation solubility of drug in water phase. Tetracaine SLN were prepared by hot HPH shows drug enriched shell model [80]. In this model burst release is highly likely because most of drugs are present in the outer shell. Other factors contributing to first release are short diffusion distance for the drug and large surface area due to small molecular size.

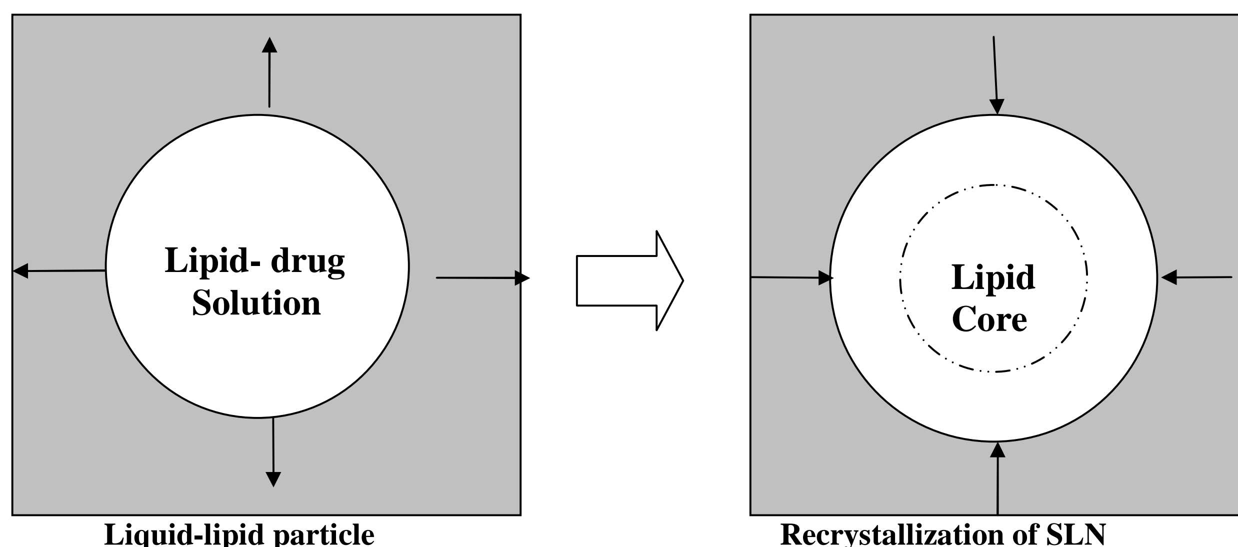

FIGURE 5. **Partitioning effects on drug during the production of SLN by the hot homogenization technique. (a) Partitioning of drug from the lipid phase to water phase at increased temperature, (b) Re-partitioning of the drug to the lipid phase during cooling** (Modified from Ref. 80).

A drug enriched core obtained when dissolving a drug (e.g. prednisolone) in the lipid melts at or close to its saturation solubility. In this model, cooling of the formed nanoemulsion will lead to supersaturation of drug in melted lipid and it further leads drug precipitation prior to lipid precipitation. Further cooling will lead to precipitation of lipid surrounding the drug enriched core as a membrane as indicated in Figure 4c. Due to increased diffusional distance and hindering effect of surrounding solid lipid shell, the carrier system shows sustained release profile [80].

Conclusion

The important and clear advantages of SLN include the composition (solid lipid similar to physiological lipid), large scale production is possible because it can rapidly and effectively produced, avoidance of organic solvent (except solvent emulsification-diffusion and solvent emulsification-evaporation). Disadvantages include low load for a number of drugs, complexity of the physical state of the lipid (transformation between different modifications, possibility of supercooled melt) which cause stability problem during drug storage and administration (gelatin, increase in particle size and drug expulsion), high water content of SLN dispersion. To avoid these disadvantages, alternative carrier systems like nanostructured lipid carrier (NLC) and lipid drug carrier (LDC) were developed. Further works on this carrier system are carried out by various scientists. The entrapment efficiency and drug release depend not only on lipid mixture employed or type of drug (lipophilic or hydrophilic drug) but mostly on the production technique used.

References

[1] RH Muller, K Menhert., *Eur J Pharm Biopharm.* **1995;** 41:62-9.
[2] L Collins-Gold, N Feichtinger, T Warnheim. *Mod Drug Discov*. **2000**;3:44– 8.
[3] NK Jain. Advances in controlled and novel drug delivery. 1st ed. New Delhi: CBS publishers and distributers; **2001.**
[4] TM Allen, A Chonn. *Federation of Eur Biochem Societies*. **1987**; 223:42-6.
[5] P Blasi, S Giovagnoli, *Adv Drug Deliv Rev*. **2007**;59:454-77.
[6] AA Date, MD Joshi, VB Patravale. *Adv Drug Deliv Rev*. **2007**;59:505-21.
[7] A Lippacher, RH Muller, K Mader. *Int J Pharm.* **2000**;196:227–30.
[8] C Olbrich, O Kayser, RH Muller. *Int J Pharm.* **2002;**237:119–28.
[9] M Sznitowska, M Gajewska, S Janicki, A Radwanska, G Lukowski. *Eur J Pharm Biopharm*. **2001**;52:159-63.
[10] EB Souto, SA Wissing, CM Barbosa, RH Muller. *Int J Pharm*. **2004**;278:71–7.
[11] VV Kumar, D Chandrasekar, S Ramakrishna, V Kishan, YM Rao, PV Diwan. *Int J Pharm*. **2007**; 335:167–75.
[12] J Liu, W Hu, H Chen, Q Ni, H Xu, X Yang. *Int J Pharm*. **2007**; 328:191-5.
[13] AA Attama, CC Muller-Goymann. *Int J Pharm*. **2007;**334:179–89.
[14] JP Jee, SJ Lim, JS Park, CK Kim. *Eur J Pharm Biopharm*. **2006;**63:134–9.
[15] SJ Lim, CK Kim. *Int J Pharm*. **2002**;243:135– 46.
[16] V Jenning, AF Thunemann, SH Gohla. *Int J Pharm***. 2000**;199:167–77.
[17] H Heiati, R Tawashi, NC Phillips. *Int J Pharm*. **1998;**174:71–80.
[18] V Jenning, M Schafer-Korting, S Gohla. *J Control Release*. **2000**;66:115–26.
[19] RH Muller, K Mader, S Gohla. *Eur J Pharm Biopharm***. 2000**;50:161-76.
[20] RH Muller, S Runge, V Ravelli. W Mehnert. AF Thunemann. EB Souto. *Int J Pharm.* **2006**;317:82–9.
[21] Q Zhang, G Yie, Y Li, Q Yang, T Nagai. *Int J Pharm*. 2000;200:153–59.
[22] J You, F Wan, FD Cui, Y Sun, Y Du, FQ Hu. *Int J Pharm*. **2007**;343:270–76.
[23] A Miglietta, R Cavalli, C Bocca, L Gabriel, MR Gasco. *Int J Pharm*. **2000**;210:61–7.
[24] B Siekmann, K Westesen. *J Pharm Pharmacol*. **1994**;3:194–7.

[25] QH Ma, Q Xia, YY Lu, XZ Hao, N Gu, XF Lin, D Luo. *Solid State Phenomena*. **2007**;121-123: 705-8.
[26] A Dingler, RP Blum, H Niehus, RH Mueller, S Gohla. *J. Microencapsul*. **1999**;16:751-67.
[27] E Marengo, R Cavalli, O Caputo, L Rodriguez, MR Gasco. *Int J Pharm.* **2000;**205:3–13.
[28] AV Heydenreich, R Westmeier, N Pedersen, HS Poulsen, HG Kristensen. *Int J Pharm*. **2003;**254:83–7.
[29] W Tiyaboonchai, W Tungpradit, P Plianbangchang. *Int J Pharm*. **2007**;337:299–06.
[30] E Ugazio, R Cavalli, MR Gasco. *Int J Pharm*. **2002**;241:341–4.
[31] R Cavalli, E Peira, O Caputo, MR Gasco. *Int J Pharm*. **1999**;182:59–69.
[32] R Cavalli, E Marengo, L Rodriguez, MR Gasco. *Eur J Pharm Biopharm*. **1996**;43:110–5.
[33] SA Wissing, O Kayser, RH Muller. *Adv Drug Deliv Rev*. **2004**;56:1257–72.
[34] FM Xie, K Zeng, GF Li, ZF Lin, LD Sun. Di Yi Jun Yi Da Xue Xue Bao**. 2005**;25:99-01.
[35] C Jin, L Bai, H Wu, F Tian, G Guo. *Biomaterials*. **2007**;28:3724–30.
[36] E Lemos-Senna, D Wouessidjewe, S Lesieur, D Duchene. *Int J Pharm*. **1998;**170:119–28.
[37] P Shahgaldiana, L Quattrocchia, J Gualberta, AW Colemana, P Goreloff. *Eur J Pharm Biopharm*. **2003**;55:107–13.
[38] CP Tan, M Nakajima. *Food Chemistry*. **2005**;92:661–71.
[39] N Zhang, Q Ping, G Huang, W Xu, Y Cheng, X Han. *Int J Pharm*. **2006**;327:153-59.
[40] M Trotta, D Chirio, R Cavalli, E Peira. *Pharm Res***. 2004**;21:1445-9.
[41] Y Kawashima, H Yamamoto, H Takeuchi, T Hino, T Niwa. *Eur J Pharm Biopharm*. **1998;**45:41–8.
[42] R Pandey, S Sharma, GK Khuller. *Tuberculosis.* **2005;**85:415–20.
[43] FQ Hu, Y Hong, H Yuan. *Int J Pharm.* **2004**;273:29–35.
[44] M Trotta, F Debernardi, O Caputo. *Int J Pharm***. 2003**;257:153–60.
[45] M Trotta, R Cavalli, ME Carlotti, L Battaglia, F Debernardi. *Int J Pharm*. **2005;**288:281–8.
[46] FQ Hu, H Yuan, HH Zhang, M Fang. *Int J Pharm*. **2002**;239:121–8.
[47] D Quintanar-Guerrero, D Tamayo-Esquivel, A Ganem-Quintanar, E Allemann, E Doelker. *Eur J Pharm Sci*. **2005**;26:211–8.
[48] H Reithmeier, J Herrmann, A Gopferich. *J Control Release*. **2001;**73:339–50.
[49] H Reithmeier, J Herrmann, A Gopferich. *Int J Pharm*. **2001**;218:133–43.
[50] W Mehnert, K Mader. *Adv Drug Deliv Rev*. **2001;**47:165–96.
[51] Z Mei, X Li, Q Wu, S Hu, X Yang. *Pharm Res.* **2005**;51:345–51.
[52] F Castelli, C Puglia, MG Sarpietro, L Rizza, F Bonina. *Int J Pharm*. **2005;**304:231–8.
[53] BD Kim, K Na, HK Choi. *Eur J Pharm Sci*. **2005**;24:199–05.
[54] D Zhang, T Tan, L Gao. *Nanotechnology.* **2006**;17:5821–28.
[55] DZ Hou, CS Xie, KJ Huang, CH Zhu. *Biomaterials.* **2003**;24:1781–5.
[56] YF Luo, DW Chen, LX Ren, XL Zhao, J Qin. *J Control Release*. **2006**;114:53–9.
[57] R Cortesia, E Espositoa, G Lucab, C Nastruzzib. *Biomaterials.* **2002;**23:2283–94.
[58] KS Jaganathan, YUB Rao, P Singh, D Prabakaran, S Gupta, A Jain, SP Vyas. *Int J Pharm*. **2005;**294:23–32.
[59] M Garcıa-Fuentes, D Torres, MJ Alonso. *Biointerfaces.* **2002**;27:159-68.
[60] J Liu, T Gong, C Wang, Z Zhong, Z Zhang. *Int J Pharm.* **2007;**340:153-62.
[61] S Hatziantoniou, G Deli, Y Nikas, C Demetzos, GT Papaioannou. *Micron.* **2007**;38:819-23.
[62] C Charcosset, A El-Harati, H Fessi. *J Control Release.* **2005;**108:112–20.
[63] AA El-Harati, C Charcosset, H Fessi. *Pharmaceutical Development and Technology*. **2006;**11:153-7.

[64] C Charcosset, AA El-Harati, H Fessi. *Desalination*. **2006**;200:570–1.
[65] MA Schubert, CC Muller-Goymann. *Eur J Pharm Biopharm*. **2003**;55:125–31.
[66] N Jovanovic, A Bouchard, GW Hofland, GJ Witkamp, DJA Crommelin, W Jiskoot. *Pharm Res.* **2004**;21:1955-69.
[67] P Chattopadhyay, BY Shekunov, D Yim, D Cipolla, B Boyd, S Farr. *Adv Drug Deliv Rev*. **2007;**59:444–53.
[68] WZ He, QL Suo, ZH Jiang, A Shan, HL Hong. *J Supercritical Fluids*. **2004;**31:101–10.
[69] IRD Santos, J Richard, B Pech, C Thies, JP Benoit. *Int J Pharm*. **2002**;242:69–78.
[70] J Jung, M Perrut.. *J Supercritical Fluids*. **2001**;20:179–219.
[71] A Bouchard, N Jovanovic, W Jiskoot, E Mendes, GJ Witkamp, DJA Crommelin, GW Hofland*J Supercritical Fluids***. 2007**;40:293–307.
[72] AJ Almeida, E Souto. *Adv Drug Deliv Rev*. **2007**;59:478-90.
[73] SA Wissing, RH Muller. *J Control Release*. **2002**;81:225-33.
[74] SA Wissing, O Kayser, RH Muller. *Adv Drug Deliv Rev.* **2004**;56:1257–72.
[75] K Westesen, H Bunjes, MHJ Koch. *J Control Release*. **1997**;48:223-36.
[76] B Siekmann, K Westesen. *Biointerfaces.* **1994**;3:159–75.
[77] K Westesen, B Siekmann, KHJ Koch. *Int J Pharm*. **1993**;93:189-99.
[78] A Muhlen, C Schwarz, W Mehnert. *Eur J Pharm Biopharm*. **1998;**45:149-55
[79] RH Muller, M Radtke, SA Wissing. *Adv Drug Deliv Rev***. 2002**;54:131-55.
[80] A Muhlen, W Mehnert. *Pharmazie*. **1998**;53:552-5.