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Solid Lipid Nanoparticles – A Promising Drug Delivery System

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Introduction

The lipids are a large group of organic compounds that has a fundamental role in life on Earth. Whether they act as the energy storage in our bodies or as the building blocks of the cell membranes they play a key role in different physiological and biochemical processes. Due to their intrinsic properties lipids can dissolve water-insoluble substances, pass through biological membranes and undergo digestion by enzymes. In the pharmaceutical industry lipids are used mainly as vehicles in different formulations intended for all routes of administration – as emulsions, ointments, pellets, suppositories etc. The emulsions (type “oil-in-water”) are one of the most important systems because they enable the administration of fat-soluble active pharmaceutical ingredients (APIs) throughout various routes. For example, the *i.v.* administration of lipids and lipid soluble substances wouldn't be possible if the first parenteral nanoemulsions weren't invented in the 1950s. However, the small size of the lipid droplets in the nanoemulsions results in very high surface area and rapid release of the drug. In addition, the liquid nature of the droplets favors oxidation and specific instability processes (i.e. Ostwald ripening).

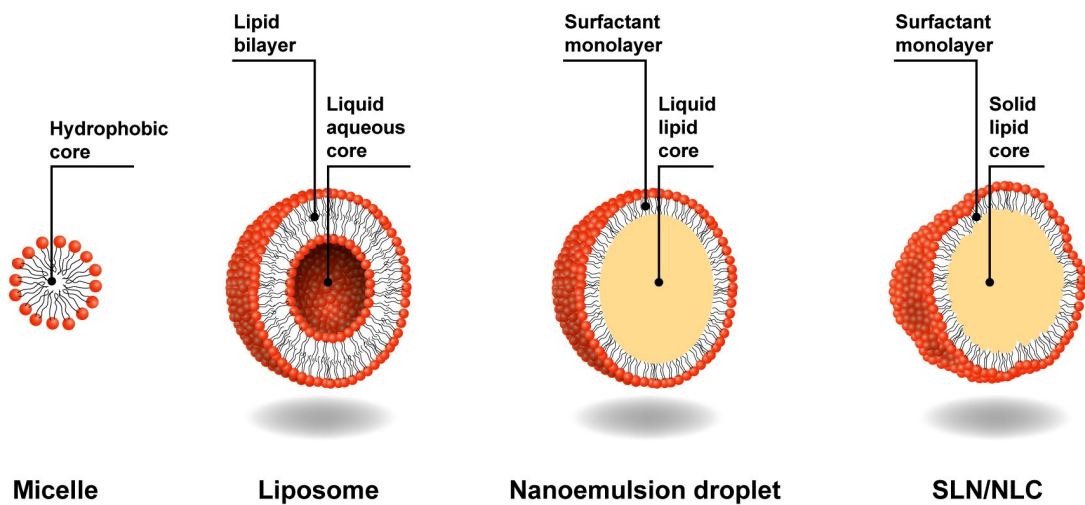


FIGURE 8.1 Comparison between micelles, liposomes, nanoemulsions and solid lipid nanoparticles. (Micelles with hydrophobic core which is formed by the tails of the surfactant molecules. Liposomes with aqueous core surrounded by a double phospholipid layer. Nanoemulsions droplets with hydrophobic liquid core composed of the oil that is dispersed in the water and stabilized by a surfactant monolayer. SLN and NLC with hydrophobic core of solidified lipid; often the solidification/crystallization of the lipid results in non-spherical shape of the particles).

The demand to broaden the area of application of oil (lipid) in water dispersions and their continuous optimization resulted in the development of the first solid lipid microparticles in the 1980s by Speiser and coworkers [1]. In the following years, extensive work and experiments with solid lipids resulted in the invention of lipid based solid particles in the submicron range by the groups of Westesen, Müller and Gasco [2 - 4]. This system called Solid Lipid Nanoparticles (SLN) is defined as drug carrier in the submicron size range made of biocompatible and biodegradable lipids solid at room and body temperature.

Some authors describe SLN as nanoemulsion in which the liquid lipid core of the droplets is substituted by a solid one [5]. Such “substitution”, however, is related to the formation of a distinctly different system that exhibits its own specific properties and such a comparison of SLN to nanoemulsions is inappropriate.

In the following years SLN proved as a blockbuster in nanotechnology because many believed that SLN “offer some of the advantages of polymeric nanoparticles, fat emulsions and liposomes along with the possibility to successfully resolve problems related to drug physical and chemical stability, drug delivery and absorption” [6]. Logical sequel was the development of Nanostructured Lipid Carriers (NLC) which, still being solid at room and body temperature, consist of solid and liquid lipids. NLC have met the expectations and managed to resolve some of the problems related to crystallinity and poor drug loading [7].

The goal of this chapter is to summarize the most important information about Solid Lipid Nanoparticles for the past two decades. It includes examples of the most frequently used excipients and explanations of the production techniques. Moreover, we have given a short overview of the analytical techniques that are considered the most important in the process of characterization of SLN. Finally, we have discussed the current and future applications of SLN from our point of view. We believe that this chapter will be useful to students and researches who want to learn more about this type of nanoparticles. In addition, the chapter contains many tables and citations which will be helpful to anyone who wants to know what research has been done so far in this area.

Composition

SLN and NLC are composed of lipids and of stabilizers – in most cases surfactants, co-surfactants and coating materials. Antioxidants, electrolytes, preservatives, viscosity enhancing agents, adhesives, absorption enhancers and other excipients also find application. Most of the formulation ingredients are safe and under the Generally Recognized as Safe (GRAS) status issued by the Food and Drug Administration (FDA).

Lipids

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Lipids can be defined as fatty or waxy organic compounds. Generally, they are soluble in nonpolar and insoluble in polar solvents. Their typical constituents are free fatty acids, free fatty alcohols, glycerol esters of fatty acids and waxes. More complex structures as phospholipids, glycolipids and sphingophospholipids are also referred to this group. Lipids “build” the core (the lipid matrix) of SLN/ NLC. The lipid matrix itself determines the particles’ pharmaceutical properties as it is the structure that stores, transports and releases the drug.

Often the combination of two and more lipids is favorable because it enables adjustments in the physical state (melting point, crystallinity and polymorphism), drug loading and drug release kinetics of the particles to be made. Examples of different groups of lipids used in the preparation of SLN and NLC can be found in *Table 8.1*.

Surface active compounds (SACs)

When one of two immiscible phases (e.g., lipid and water) is dispersed into the another an interfacial boundary is formed. The surface energy at this boundary is expressed as Gibbs free energy (G). At constant temperature and pressure G depends on the surface area (A) and the interfacial tension (γ) (Eq.1) [62]:

$$\Delta p = P_{inside} - P_{outside} = \frac{2\gamma}{r} \quad (\text{Eq. 2})$$

Where P_{inside} is the pressure inside the droplet, $P_{outside}$ is the pressure outside the droplet, γ is the surface tension and r is the radius of the spherical droplet. The lower the value of the Laplace pressure is the less energy to break the droplets is needed. However, as the droplets become smaller their radius decreases which results in proportional increase in the pressure (e.g. tenfold reduction in droplets diameter increases the Laplace pressure 10 times). Therefore, more energy will be required to produce smaller and smaller particles. increases the Laplace pressure 10 times). Still, smaller droplet formation at constant amount of energy applied on the system can be facilitated by lowering the surface tension. Surface active compounds, due to their amphiphilic structure, exhibit a tendency to accumulate at phase boundary and form monomolecular layer around the droplets/particles. This normally results in system stabilization by lowering the surface tension, decrease in the Gibbsfree energy and the Laplace pressure.

TABLE 8.1 Commonly used lipids in the preparation of SLN and NLC.

Group	Reference
Triglycerides	
Glyceryl tristearate	[8-13,38]
Glyceryl tripalmitate	[8,9,13,15,17,38]
Glyceryl trimyristate	[8,9,12,13,16,38]
Medium chain triglycerides	[18,20,48,49]
Glyceryl trioleate	[21]
Monoglycerides	
Glyceryl monostearate	[12,13,23,24–26,39,89]
Mixtures	
Glyceryl behenate	[12,16,18,19,26,27]
Glyceryl palmitostearate	[22,29,45]
Witepsol grades	[28,30,31,88]
Free fatty acids	
Behenic acid	[33,34]
Stearic acid	[13,24,27,33,34,37,39,41]
Palmitic acid	[33-36]
Myristic acid	[33,34]
Oleic acid	[22,37-40]
Free fatty alcohols	
Stearyl alcohol	[42,43,47]
Cetyl alcohol	[38,44-47]
Myristyl alcohol	[47]
Lauryl alcohol	[47]
Waxes	
Cetyl palmitate	[41,42,48,49,75]
Beeswax	[50-52]
Carnauba wax	[52,53]
Others	
Castor oil	[14]
Hydrogenated castor oil	[54–56,85]
Hydrogenated palm oil	[57,58]
Cacao butter	[59]
Goat fat	[60]
Anhydrous milk fat	[61]

However, surfactants properties are predetermined by their chemical structure and they lower the surface tension only to a certain limit. Further decrease can be achieved with the incorporation of co-surfactants [65].

The choice of SAC(s) is essential to the final properties of the formulation. Surfactants from almost all groups that find application in the formulation of SLN and NLC are presented in *Table 8.2*. Elevation of the temperature also decreases the surface tension and can be used to produce hot-emulsions with low energy input [64].

Beside the main components constituting the SLN/NLC (i.e., the lipids and surfactants) a variety of different additives can find place in each particular formulation. Good illustrations of such examples are the following: cryoprotectants in freeze dried formulations – d-sorbitol, d-glucose, d-fructose [28]; polysaccharide coating materials – chitosan [30,34]; protein coating materials – silk fibroin [72]; alternative polymeric emulsifiers – polyvinyl alcohol (PVA) [35] and poly(lactic-co-glycolic acids) (PLGA) [85]; tonicity adjusting agents – electrolytes and glycerol [22]; preservatives – parabens [69], thiomersal [58, 66], imidazolidinyl urea, chloromethylisothiazolinone and methylisothiazolinone [75]. Other relevant examples are the combinations of SLN and NLC with different viscosity enhancing excipients to produce carbopol [26] and dextran [70] hydrogels.

A bright demonstration of the endless and diverse applications of the excipients is the innovative approach to obtain polyvinylpyrrolidone/tristearine microparticles with electrospraying which upon dissolution form SLN [11].

TABLE 8.2 Commonly used surfactants and co-surfactants in SLN and NLC formulations.

Surfactant	Reference
Nonionic	
Polyoxyethylene sorbitan fatty esters	
Polysorbate 20	[23,57,78]
Polysorbate 60	[17,43,44]
Polysorbate 80	[12,13,15,17,18,24,26,57]
Polysorbate 85	[57]
Polyoxyethylene alkyl/aryl ethers	
Polyoxyethylene(20)cetyl ether	[75,76]
Polyoxyethylene(20) isoheca decyl ether	[75,76]
Polyoxyethylene(20)oleyl ether	[75,76]
Polyoxyethylene(20)stearyl ether	[43,76–79]
Tyloxapol	[80,81,86]
Ethoxylated castor oils	
PEG-35 castor oil	[131]
PEG-40 hydrogenated castor oil	[83,84]
Poloxamers	
Poloxamer 188	[14,16,18,19,33,69,78]
Poloxamer 407	[30,87,90]
Others	
Polyoxyethylene- glycerine monostearate	[68]
Macrogol(15) hydroxystearate	[14,16]
PEG caprylic/capric triglycerides	[29]
Polyglyceryl-3 methylglucose distearate	[46]
Polyglyceryl-6 distearate	[48]
Anionic	
Sodium dehydrocholate	[24]
Sodium taurocholate	[27,59,67]
Sodium glycocholate	[66,68,86]
Sodium cholate	[69,70]
Sodium lauryl sulphate	[71,72]
Cationic	
Cetrimonium bromide	[42-44,73]
DOTAP	[38,82]
DOTMA	[73]
Chlorhexidine salts	[74]
Dimethyldiocta- decylammonium bromide	[37]
Amphoteric	
L- α -phosphatidyl- choline	[21,59]
Soya lecithin	[10,14,24,29,39,66,89]
Egg lecithin	[52,88]
Co-surfactants	
1-Butanol	[13,37,59]
Low molecular weight PEG	[15,89]
Diethylene glycol monoethyl ether	[23]
Propylene glycol	[26]
Ethanol	[27]
Sorbitan monostearate	[71]

Preparation

The techniques for SLN and NLC preparation can be grouped into three main branches – high-energy approaches, low-energy approaches and methods employing organic solvents.

High-energy approaches

High pressure homogenization

High pressure homogenization is one of the first techniques used in the preparation of SLN [3]. Nowadays, it is well-established in the production of nanoemulsions for parenteral foods, homogenization of milk, ice cream and others. In this technique a liquid is pushed at high pressure through a narrow gap. Both the high pressure (in the range of 100-2000 bar) and the small size of the gap (in the range of few microns) cause a very high acceleration and pressure drop. As a result a very high shear stress and cavitation forces disrupt particles/drops in the liquid. An increase in the temperature during the process (usually around 10 °C for every 500 bar) is also possible due to the high acceleration and friction. The method is easy accessible and scalable.

Two approaches to produce SLN and NLC are utilized via this technique – homogenization of hot pre-emulsion and homogenization of cold pre-suspension (*Figure 8.2* and *Figure 8.3*).

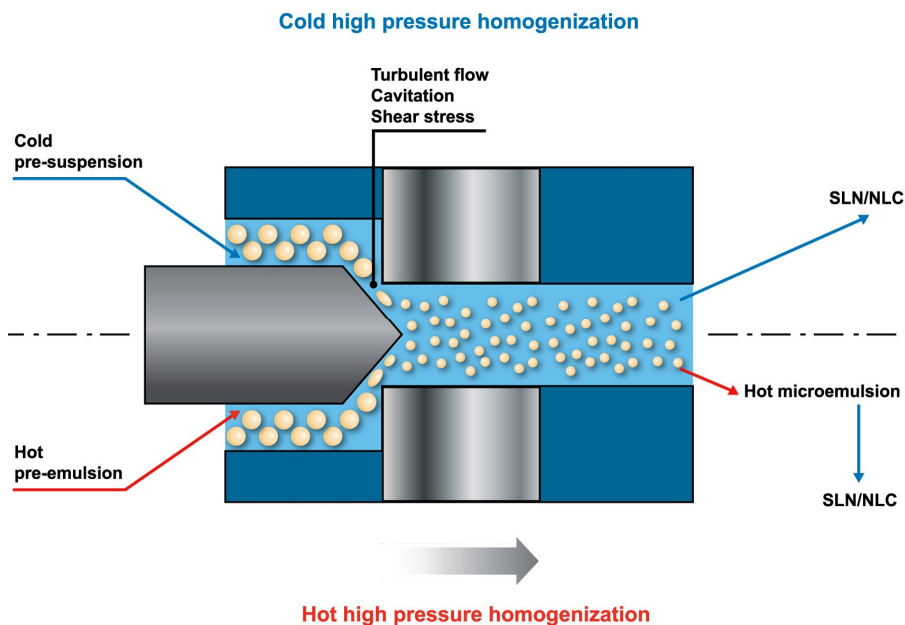


FIGURE 8.2 Mechanism of high pressure homogenization of cold pre-suspension and hot pre-emulsion respectively in the "cold" and "hot" technique.

In the “hot” method the procedure is carried at temperatures above the melting temperature of the lipid. A pre-emulsion is formed usually with the help of high shear mixers. This emulsion is passed through a narrow orifice – valve or nozzle. Usually several cycles are applied to achieve submicron size with low polydispersity. The product obtained after the homogenization is a hot microemulsion. The latter should be cooled fast so that the liquid lipid droplets can solidify and form the intrinsic structure of the SLN and NLC.

The first stage of the cold homogenization approach is the formation of a hot lipid melt mixture of the substances that form the lipid matrix and the APIs. Then the melt is cooled down to a solid state and grinded in powder mill to obtain particles in the size range of 50 to 100 micrometers. The obtained lipid powder is dispersed in aqueous solution of surfactant to form a pre-suspension. This pre-suspension is then passed through a homogenizer. Usually, more cycles and higher pressure (compared to the hot approach) are required because the particles are more rigid and harder to break. The cold approach is desirable in formulations with drugs that are not stable at high temperatures or can distribute in the aqueous phase during the preparation.

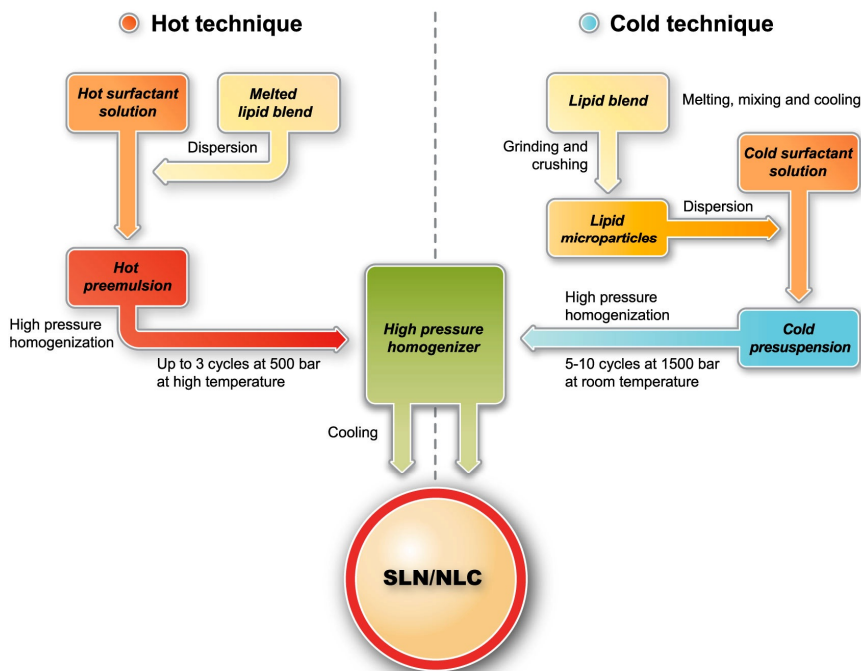


FIGURE 8.3 "Hot" and "Cold" high pressure homogenization technique in the production of SLN/NLC.

High shear homogenization

This is a simple and widely used technique in the production of aqueous dispersions. It is usually processed in chamber with a rotor-stator homogenizer. The procedure starts with placing the lipid ingredients and the water phase into the homogenizer followed by applying high shear mixing (5 000 – 25 000 rpm) at temperatures above the melting temperatures of the lipids (*Figure 8.4.*). After homogenization, the formed hot microemulsion is cooled to form SLN and NLC. Although it is very simple procedure to perform the properties of the final product are usually poor and particles

in the micrometre range are detected. Higher shearing rates can result in smaller droplets only until a certain critical value due to processes of coalescence. Although higher shearing rates cannot decrease particle size beyond that certain value they can reduce the polydispersity [91]. The poor properties of the final product are the cause why this technique is preferably used as a pre-homogenization step in high pressure homogenization and ultrasonication.

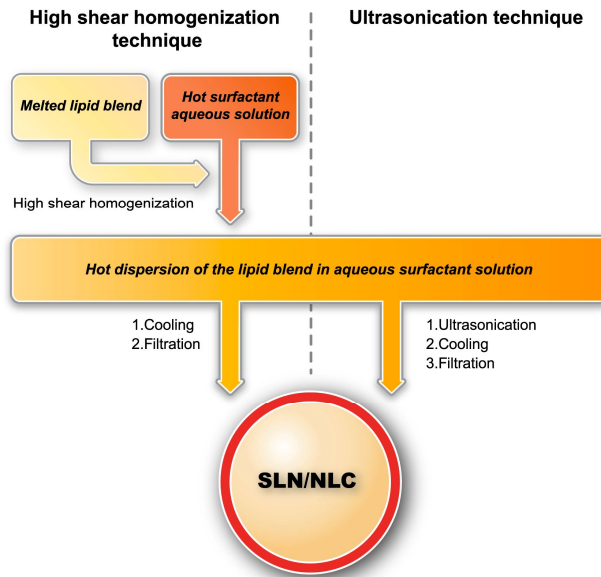


FIGURE 8.4 High shear homogenization and ultrasonication techniques in the production of SLN/NLC.

Ultrasonication

Ultrasonication is based on the cavitation in aqueous dispersions caused by powerful ultrasound with wave frequency usually around and above 20kHz. In the production of SLN and NLC a mixture of pre-emulsion from melted lipid and hot surfactant solution is first prepared (*Figure 8.4.*). Then the ultrasound is applied with a sonotrode that is in contact with the liquid. The cavitation causes disintegration of the lipid phase into smaller droplets. The obtained hot microemulsion is then cooled to form the solid particles (*Figure 8.3.*). Advantages of this technique, without doubt, are the possibility for scale up with flow cells, the low number of wetted and moving elements (easier cleaning) and the possibility to control the process by controlling the sound wave amplitude. Main drawback, however, is the risk of metal contamination, which increases with longer sonication times.

Electro-spray technique

In this relatively new technique an electrodynamic atomization is used to produce SLN directly in powder form [92]. The obtained particles by this method have narrow distribution and size below one micrometre. However the method is still under investigation for its applicability in the production of larger quantities of dispersions.

Low-energy approaches

Microemulsion method

Microemulsion formation is used as a stage in the production of SLN and NLC since the early 90s [4]. In this method the microemulsion is spontaneously formed due to the high surfactants/lipid ratio. The proportions of the excipients are essential and in most cases pseudo ternary diagrams are used to study and describe the areas of microemulsion formation. This method is simple and is performed by several common steps. Initially the lipids are melted and mixed with hot surfactant solution. Gentle stirring is applied until the microemulsion is formed. In the second stage the hot microemulsion is dispersed in high volume of cold water (2-3 °C) under moderate stirring. This causes the liquid droplets to solidify. SLN or NLC obtained by this technique are spherical in shape and have narrow size distribution. However, the method suffers from several drawbacks - the final dispersion is very diluted (ranging between 1:25 up to 1:50 with respect to the hot emulsion). This may require further concentration by ultrafiltration, lyophilization or other method. The high concentration of surfactants/co-surfactants used is another major disadvantage of this technique.

Membrane contactor

Membrane contactor technique is a method which allows a gaseous and liquid phase to come in direct contact without dispersing one of the phases into the other and is used for purposes which require mass transfer between them. The two phases are separated by a hydrophobic membrane with typical pore size of 0,05 microns. This membrane doesn't allow water to pass because of the high breakthrough pressure needed. In the production of SLN and NLC this technique is modified and the gaseous phase is replaced with a melted lipid blend [93]. This blend is forced to pass through the membrane. Small droplets are formed spontaneously (*Figure 8.5.*). On the other side of the membrane a hot surfactant solution is circulating and swapping away the droplets. The liquid lipid droplets are enveloped and stabilized by the surfactant molecules. Then, after cooling down the dispersion the droplets transform into solid particles. The method is scalable and the particle size can be tuned by using membranes with different pore size.

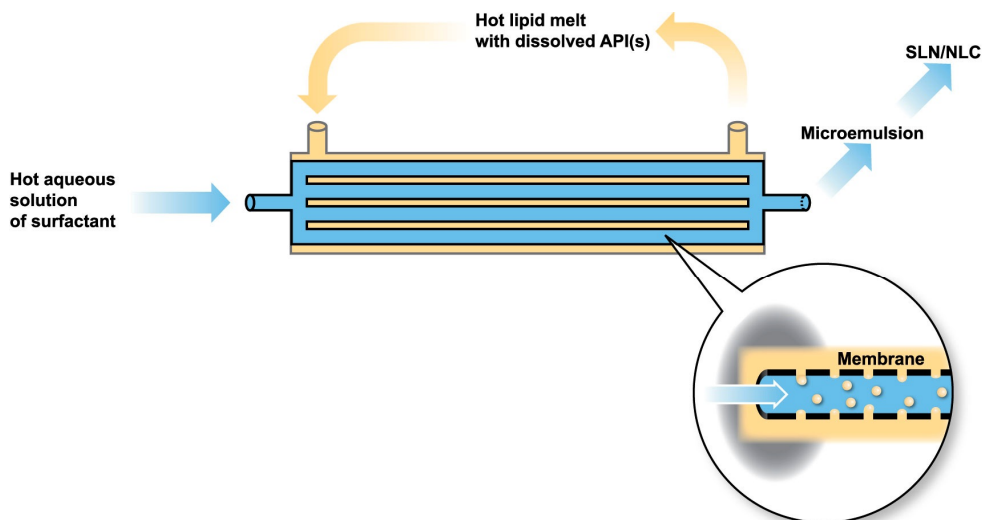


FIGURE 8.5 Membrane contractor technique in preparation of SLN/NLC.

Phase inversion temperature (PIT) method

Phase inversion of O/W to W/O emulsions and *vice versa* induced by temperature change is a long known method to produce microemulsions stabilized with non-ionic surfactants [94]. The technique is based on the change in the properties of polyoxyethylated surfactants at different temperatures. The hydrophilic-lipophilic balance (HLB) value of surfactants defined by Griffin is valid at 25°C. At this temperature the hydrophilic parts of the SAC molecules are hydrated to a certain extent. An increase in the temperature causes dehydration of the ethoxy groups. As a result, the lipophilicity of the molecules of the SAC rises with corresponding decrease in HLB value.

At a certain point the affinity of the SAC to the aqueous and lipid phase is equal - this temperature is defined as the phase inversion temperature. This particulate state is characterized by very low surface tension and presence of complex structures in the system. If the temperature is further increased the SAC's affinity to the lipid phase becomes higher enough to stabilize emulsions of W/O type (*Figure 8.6*).

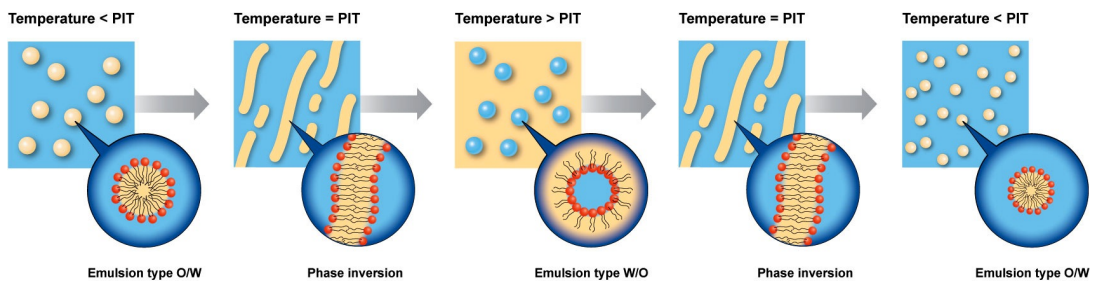


FIGURE 8.6 Phase inversions of hot emulsion with variations of the temperature above and below PIT.

If cooled down the system goes through the reverse process. Due to the specific properties of the system around the PIT very small particles are spontaneously formed just below that temperature. If rapid cooling is applied at this point stable particles with desirable size and polydispersity can be obtained [95]. In the last decade different groups report the feasibility of the method in the production of lipid nanocapsules, SLN and NLC [75, 76, 95, 96]. Several modifications to improve the final product are also developed e.g., cycling around the PIT to achieve better distribution of SAC at the phase interface and therefore smaller size and lower polydispersity [97].

Coacervation method

The coacervation method is relatively new, low-energy and solvent free technique. It is based on precipitation of free fatty acids from their sodium salt micelles in presence of surfactants [33]. Particles obtained by this technique are usually spherical in the range from 250 to 500 nm. However, the feasibility of the method is still under investigation.

Double emulsion method

The double emulsion technique in the preparation of SLN and NLC is suitable for hydrophilic APIs and peptides [98]. In this method an aqueous solution of the drug is emulsified in melted lipid blend to form primary W/O emulsion stabilized with suitable excipients (*Figure 8.7*). The primary

W/O emulsion is dispersed in aqueous solution of hydrophilic emulsifier to form a double W/O/W emulsion. Then the double emulsion is stirred and isolated by filtration. Relatively large particles are obtained with this technique but beside the incorporation of hydrophilic molecules it offers the possibility of surface modification, e.g. with PEGs.

Approaches with organic solvents

Emulsification-solvent evaporation

This technique is based on precipitation of the lipids from O/W emulsions [99, 100]. The lipid material and drug are dissolved in organic water-immiscible solvent (e.g., chloroform). The organic solution is emulsified in an aqueous phase with the help of suitable surfactants to form O/W emulsion. Then the organic solvent from this emulsion is evaporated at low pressure. This causes the lipid and API(s) to precipitate in the form of SLN or NLC (*Figure 8.8.* and *Figure 8.9.*). The particles obtained by this method have optimal properties – small size (25-100 nm) and narrow size distribution. Main disadvantages are the limitation of lipid concentration in the organic solvent to form desirable particles and the use of organic solvents *per se*. The method is appropriate especially in the encapsulation of thermo sensitive APIs because of the absence of any thermal stress. It is notable that identical formulations don't achieve the same results when they are processed by other technique.

Emulsification solvent diffusion

This method is based on the emulsification of partially water miscible solvent (e.g. butyl lactate, benzyl alcohol) solution of a solid lipid in an aqueous solution of suitable surfactant [101]. The aqueous phase of the obtained emulsion is saturated with the organic solvent and the excess of the solvent is in the form of emulsion droplets. A dilution with fresh water causes the organic solvent, driven by the concentration gradient, to diffuse from the droplets to the water. As a result the solubility of the lipids decreases until they precipitate (*Figure 8.9.*). SLN and NLC below 100 nm with narrow size distribution can be produced by this technique. The solubility of the water miscible solvent in water and the solubility of the lipid in the organic solvent are crucial to the final results. However, the type and the concentration of the lipid, surfactant and organic solvent would require a substantial optimization work. Noteworthy advantage is the low processing temperature.

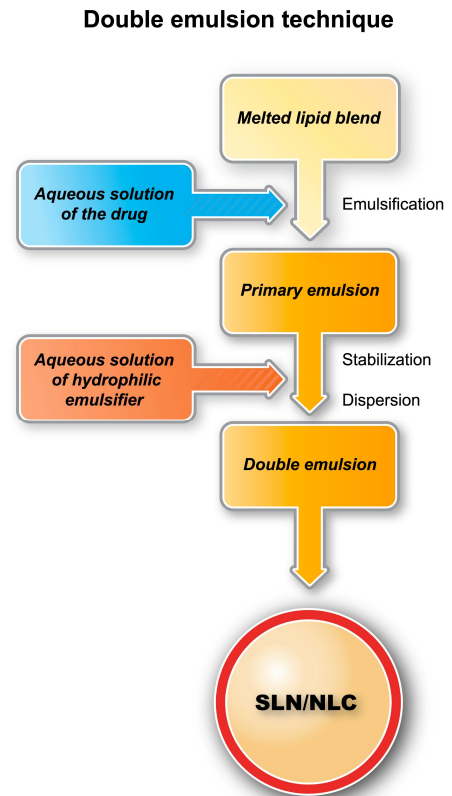


FIGURE 8.7 Double emulsion technique in preparation of SLN/NLC.

Solvent injection

The method is similar to the emulsification solvent diffusion method but the organic solvents used are selected from the group of the very miscible with water solvents (DMSO, ethanol) thus eliminating the chance for emulsion to be formed [102]. Firstly, the lipid(s) and API(s) are dissolved in the organic solvent. Then the organic solution is injected in aqueous solution of surfactant under stirring. This causes a rapid migration of the organic solvent in the water and precipitation of the lipids (*Figure 8.9.*). The obtained particle size depends strongly on the velocity of extraction respectively on the lipophilicity of the solvent. The more hydrophilic the solvent the smaller the particles but the less its capacity to dissolve lipids. The method offers advantages such as low processing temperatures and low shear stress.

Supercritical fluid (SCF) technique

A SCF is defined as a substance above its critical temperature (T_c) and critical pressure (P_c). The critical point represents the highest temperature and pressure at which the substance can exist as a vapour and liquid in equilibrium. The supercritical fluid has unique thermo-physical properties which can be finely tuned by small changes in the pressure. As the pressure raises the density and the ability of the fluid to dissolve compounds increases while the viscosity remains relevantly constant. Accordingly under high pressure and appropriate temperature in the supercritical range the fluid can act as an alternative to organic solvents and dissolve different APIs and lipids [103]. SCF like carbon dioxide are safe, cheap, non-irritable, relatively inert and has a low critical point. However, the method often yields particles in the micrometre range and is often combined with other homogenization technique like ultrasound [104].

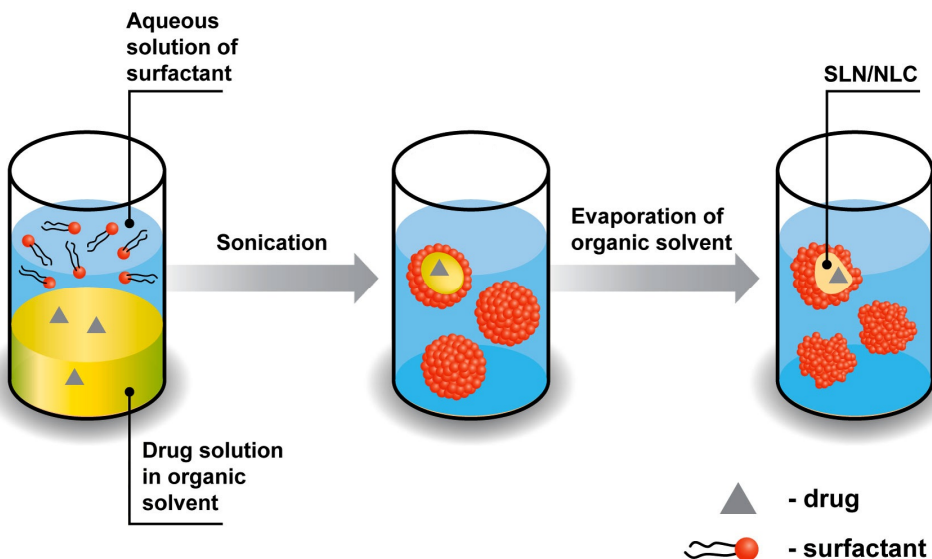


FIGURE 8.8 Emulsification solvent evaporation technique in the preparation of SLN/NLC.

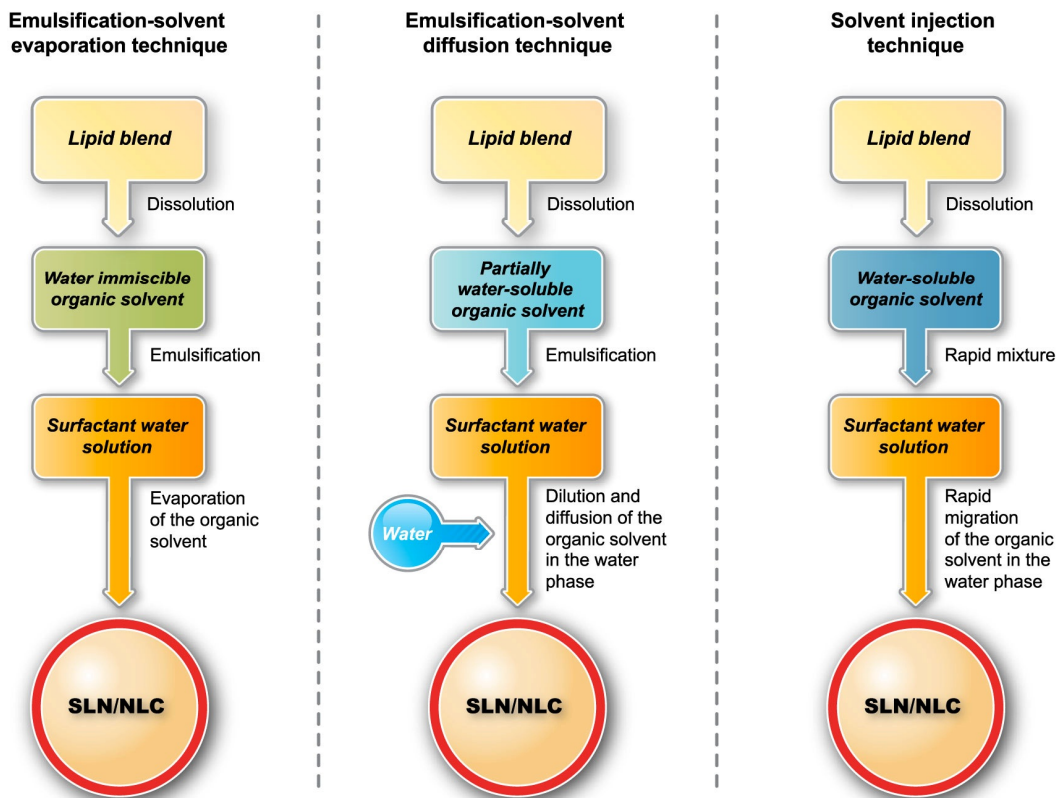


FIGURE 8.9 Organic-solvent based methods for preparation of SLN/NLC.

Characteristics and characterization

Size and shape

Size, size distribution (polydispersity) and shape of the particles are important to the physicochemical and biopharmaceutical properties of the SLN and NLC formulations. The accurate analysis of these parameters is crucial to the complete understanding of the formulations. Still, particle characterization in the nanometer range can be a serious challenge due to the presence of concomitant colloidal structures (e.g., liposomes, micelles, nanocrystals etc.) that are often formed alongside with the SLN and NLC [105]. These structures may disappear after dilution or drying of the system [106]. Therefore, it is highly recommended that the analysis should be performed on samples with minimal additional treatment.

Two of the widely used techniques to measure particle size and particle size distribution in aqueous suspensions are *Laser Diffraction (LD)* and *Dynamic Light Scattering (DLS)*. LD is based on the phenomenon that when a laser beam is passed through a sample with dispersed particles the larger ones scatter light at small angles while the smaller ones scatter light at large angles [107]. The data collected by LD is used to calculate the equivalent sphere diameter of particles according

to the Mie scattering solution (also known as the “Mie theory”). The method is effective in measuring particles in the range from dozens of nanometers to thousands of micrometers depending on the laser source. Blue lasers are used in the analysis of smaller particles while red lasers are more suitable for larger particles. However the method lacks accuracy for particles in colloidal suspensions with diameter significantly smaller than the wavelength of the laser. DLS, also known as *Photon Correlation Spectroscopy* (PCS) or *Quasi-elastic Light Scattering* (QLS) is based on time-dependent fluctuations in the scattering intensity caused by small particles in suspension when a laser beam is applied to the sample [108]. These fluctuations are result of the Brownian motion of the dispersed particles. Smaller particles have higher velocity of Brownian motion while larger particles have less velocity. Using the Stokes-Einstein equation and the data from these fluctuations the hydrodynamic diameter of the particles can be calculated. The hydrodynamic diameter is that of a sphere with the same translational diffusion coefficient as the particle being measured. The translation diffusion coefficient may depend on additional surface structures attached to the particles and ions in the medium. Therefore, the size measured with this technique is usually larger than the one obtained by microscopic techniques. DLS is very sensitive and can detect particles below 1 nm. However, there are some limitations in the micron range (particles above 6-10 microns are not suitable for analysis by this technique). The size distribution can also be calculated using DLS or LD. Size distribution can be presented either as graphic or as the polydispersity index (PDI). PDI values range from zero to one. Monodisperse samples have PDI equal to zero and very polydisperse samples have PDI close to one. The priorities and drawbacks of LD and DLS suggest that the two techniques may supplement each other. In that manner more accurate information for colloidal systems with several particle populations can be achieved. Both methods don't measure particle size directly, instead they use light diffraction and scattering to compute diameter, assuming that the particles are spherical. Therefore, for example, particles with irregular shape that is quite different from spherical may result in misleading data. Particle concentration in the sample is also critical. Analysis of concentrated dispersions may result in lower average hydrodynamic diameter and higher polydispersity. The Information obtained from LD and DLS is important but not sufficient for characterization of SLN and NLC dispersions [105]. The particle size data should be confirmed with another suitable method. Microscopic techniques are very helpful at this point. Even the simple *light microscopy* can provide information about the presence of artefacts and particles in the micrometre range. However, to visualize particle in the nanometre range more powerful methods are need.

Both *Scanning Electron Microscopy* (SEM) and *Transmission Electron Microscopy* (TEM) find application in SLN/NLC analysis [109]. These techniques employ electrons instead of light to visualize particles in the nanometre range. SEM detects the scattered electrons from the surface of the particles and visualizes the surface. TEM detects transmitted electrons and allows the researcher to “look beyond” the surface. Both techniques suffer from disadvantages related to the processing conditions (e.g., vacuum, heating) and sample preparation which may include dehydration, staining, conductive coating etc. Sample preparation and analysis itself can trigger changes within the nanoparticles thus giving invalid results [110]. Here *Atomic Force Microscopy* (AFM) offers some advantages compared to TEM and SEM. AFM uses calculations based on the force that acts between the surface of the particles in the sample and probing tip. The method provides adequate resolution with simplicity of sample preparation and fast image capturing. In AFM no vacuum is needed and the sample does not need to be conductive. However the measured particles should be immobilized before visualization. This is easily achieved for larger particles due to their sedimentation. For smaller particles that undergo Brownian motion immobilization is a

serious issue which is resolved with removal of the aqueous media. Still, dehydration can cause changes associated with cluster formation, shrinkage, crystallization of the lipid etc. [111]. A strong advantage of AFM is that it can give important details which other microscopic techniques are unable to provide i.e. information about soft surfactant layering around the particles [112]. Often the information obtained by the latter microscopic methods and LD and DLS can differ significantly [113]. As we have stated earlier this can be result of water removal and dehydration of the particles. Contemporary method that can visualize particles in their native state is the *Cryo-Electron Microscopy* (Cryo-EM). Cryo-EM allows observations in samples in their native environment at cryogenic temperatures. The sample is frozen according to specific procedure and exposed to a very low electron dose. The electron dose affects image resolution and usually a compromise is settled. The 2D image shows the SLN and NLC particles in their hydrated state [114].

Zeta potential

Zeta potential gives information about the magnitude of the electrostatic repulsion or attraction between particles in the aqueous suspension of SLN and NLC. Zeta potential can serve as an important parameter in the predictions for long term stability of the formulations [115]. High values of zeta potential (e.g., more than +30 mV or less than -30 mV) can stabilize the colloidal suspension by electric repulsion [115] (*Figure 8.10.*). Electric repulsion generally results in less contact between the particles and less aggregation. Particles charge can also influence their interaction with tissues and cells. Both positive and negative values of zeta potential can be induced with the help of ionic surfactants and charge inducers [66-68, 73, 74, 116, 117]. Although zeta values around 0 mV cannot stabilize the suspension by the means of electric repulsion, lack of particles charge doesn't necessarily result in particle aggregation. For example colloidal systems that contain steric stabilizers can express good long term stability even in cases when zeta potential is as low as around 0 mV [115].

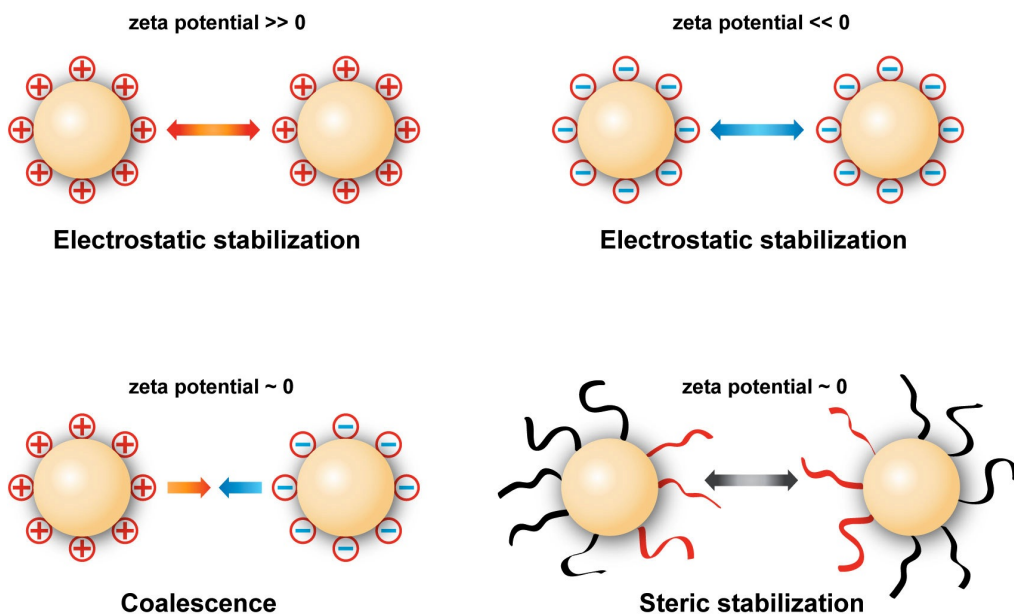


FIGURE 8.10 Influence of zeta potential on the repulsion/coalescence of particles.

Electrophoretic Light Scattering (ELS) or other appropriate methods are usually used to determine particle velocity in electric field. This information allows the zeta potential to be calculated. Modern instruments typically combine the analytical methods for particle size measurement by DLS and zeta-potential by ELS [118].

Melting point and crystallinity

The melting point of the lipid depends on its chemical nature (e.g., double bonds, free hydroxyl groups, chain length). Saturated fats have a uniform shape that allows them to pack together in a crystal lattice. Unsaturated fats have double bonds that introduce kinks into the hydrocarbon chain making crystal formation more difficult which results in lower melting temperatures. The melting point of the fatty acids also increases proportional to the chain length and triglycerides of the constituent fatty acids express a similar trend. In *Table 8.3* are shown the melting points of some of the most frequently used fatty acids and triglycerides. The melting point (or in some cases the melting interval) does not depend only on the chemical nature of molecules but it is also determined by the degree of crystallinity or crystal modification of the substance. For example triglycerides have three main crystal modifications, namely α (alpha), β' (betaprime), β (beta). The α -modification is in hexagonal subcell loosely packed in random order in which acyl groups are oriented at 90° to the plane of the glyceryl group and are assumed to be oscillating with a high degree of molecular freedom. The β' orthorhombic subcell is closely packed whereas acyl groups are tilted $68\text{--}70^\circ$ from plane of the glyceryl group. The β subcell is triclinic with highly ordered and most closely packed molecules with acyl groups tilted about 59° from the plane of the glyceryl groups.

TABLE 8.3 Melting points of commonly used saturated and unsaturated fatty acids and triglycerides in SLN/NLC.

Saturated fatty acids			
Name	Number of C atoms	Double bonds	Melting point
Lauric acid	12	-	45 °C
Myristic acid	14	-	55 °C
Palmitic acid	16	-	63 °C
Stearic acid	18	-	69 °C
Arachidic acid	20	-	76 °C
Unsaturated fatty acids			
Palmitoleic acid	16	1	0 °C
Oleic acid	18	1	13 °C
Linoleic acid	18	2	-5 °C
Linolenic acid	18	3	-11 °C
Arachidonic acid	20	4	-49 °C
Triglycerides			
Tricaprylin	8*	-	9 °C
Tricaprin	10*	-	33 °C
Trilaurin	12*	-	46 °C
Trimyristin	14*	-	56 °C
Tripalmitin	16*	-	66 °C
Tristearin	18*	-	68 °C

* Number of C atoms in the fatty acid

Beside these three main modifications sub-modifications exist. Cooling down a SLN/NLC formulation below the melting point of the lipids not always results in crystalline structure [119]. Such a state of the particles is defined as a supercooled melt [120]. The supercooled melts are considered similar to the emulsions prepared with liquid at room temperature lipids. The

phenomenon of supercooled melts can be explained with the lower chance of a sufficient number of crystallization nuclei to form in the small volume of the particles in the colloidal dispersion. Polymorphism and supercooled melts are considered major problems in the production of SLN and NLC; they seriously affect their long term stability and final characteristics which are later discussed in the *Stability and stabilization* part. Therefore the characterization of the state of the particles and degree of crystallinity is necessary to fully understand the formulations.

Differential scanning calorimetry (DSC) is a widely used technique that measures differences in the amount of heat required to increase the temperature of a sample compared to a reference [121]. Differences in heat flow may be positive or negative and are presented as function of the temperature. At phase transition there are differences in the sample compared to the reference. Owing to the different melting points and melting enthalpies of different lipid modifications DSC can give information about the sample structure and interactions between the components. It is highly recommended to confirm the results from DSC by another technique, especially in the presence of high melting APIs, which can dissolve in the melted lipid blend but tend to crystallize in the solid lipids. This may lead to the false interpretation that the API is in dissolved state in the solid core of the particles.

Powder X-ray Diffraction (PXRD) is a technique in which the sample is illuminated with X-rays of fixed wave-length and the intensity of the reflected radiation is recorded. This data is used to calculate the inter-atomic spacing. PXD is commonly used technique by scientists to analyze the crystal structure of the SLN and NLC [122]. However, it is performed on powders and the removal of the water from the colloidal suspension is required. Different polymorph modifications can be formed after dehydration of the sample. Thus PXD is not suitable to analyze crystallinity of particles which are originally formulated as colloidal suspension during storage. Nonetheless PXD is useful in the characterization of lyophilized and spray dried SLN/NLC.

X-Ray scattering is another X-Ray technique that finds application in lipid lattice structure examination of the particles. The X-Ray beam passed through the sample is obtained by a synchrotron. A main advantage is the possibility to perform the experiment on colloidal suspensions in their native state. Both *small angle X-ray scattering (SAXS)* and *wide angle X-ray scattering (WAXS)* find application in the characterization of SLN and NLC [123-125].

Alongside the above described methods, *Nuclear magnetic resonance (NMR)* can be used to detect fast and easy supercooled melts [126]. NMR measures the mobility of the molecules and the different relaxation time indicated by the width of ^1H NMR line. Low molecule mobility results in broader ^1H NMR lines. NMR also allows characterization of liquid nanocompartments in NLC and examinations on drug mobility [127].

Other valuable methods used to determine the physical state of SLN/NLC are *Electron Spin Resonance (ESR)*, *Infrared-*, *Raman-* and *External Reflection Spectroscopy* [128-131].

Entrapment efficiency (EE) and drug loading capacity (DL)

The amount of the drug incorporated in the particles depends on the capacity of the lipid to dissolve/disperse it. Two parameters expressing the efficiency of drug loading are most widely used. *Entrapment efficiency* is the amount of the drug incorporated in the particles divided by its overall amount in the formulation. EE is expressed in % (Eq.3):

$$EE\% = \frac{\text{Amount of drug in the particles}}{\text{Amount of drug added to the formulation}} * 100 \quad (\text{Eq.3})$$

Entrapment efficiency is influenced by the characteristics of both the lipid and the API. Lipophilic APIs distribute preferably in the lipid particles and have inherently higher entrapment efficiency while the more hydrophilic drugs tend to distribute in the aqueous media. *Drug loading capacity* (DL) is another parameter that expresses the amount of drug in the particles divided by the weight of total carrier system (all ingredients taken together). DL is also expressed in % (Eq.4):

$$DL\% = \frac{\text{Amount of drug in the particles}}{\text{Amount of drug + excipients added to the formulation}} * 100 \quad (\text{Eq. 4})$$

Challenges with the determination of these parameters are related to the correctness of drug analysis. A combination of suitable separation and analytical method should be used. Usually dialysis, ultracentrifugation, gel filtration or membrane filtrations are utilized. Drug concentration can be measured either in the separated aqueous media or in the particles.

Drug localization and drug release

APIs can be localized either in the SLN/NLC or in co-existing structures - micelles, liposomes, drug nanocrystals. The drug state and kinetics in the co-existing structures can be different from these of the NLC/SLN. It is necessary to determine correctly the presence/absence of co-existing structures before examining drug localization and release. Even when the drug is entrapped in the SLN/NLC it is not always homogeneously dissolved in the lipid matrix [132] and can be localized in different regions of the particles (*Figure 8.11*).

Localization can affect drug release and biological properties of the product. In some cases drug release kinetics of the drug from the SLN or NLC can be altered by varying the production method and/or its parameters. In other cases a burst release is observed independently of the production technique [133,134]. However, controlled release is achievable and reported for some formulations [135]. The type of in-vitro dissolution technique itself may also influence the drug release kinetics of SLN/NLC. Careful evaluation of the release method should be performed and the components of the dissolution media and conditions should be optimal for the specific formulation. For example, one of the most preferred methods is with a dialysis bag. The bag, however, can sometimes retard the diffusion and interact with drug molecules. Dissolutions studies on SLN/NLC cannot always correctly predict the release behavior *in vivo* because of possible enzyme degradation and interaction with cell organelles and lipid membranes in the body [136].

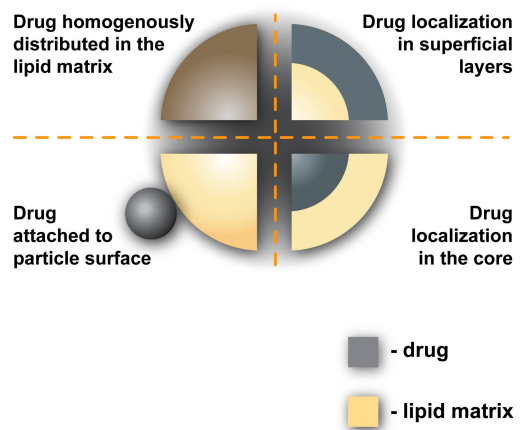


FIGURE 8.11 Possible drug localization in SLN/NLC.

Stability and stabilization

Chemical stability

The chemical stability of the lipids in formulations is often set aside by researchers. However it is important for the feasibility of the final product. The mechanism of lipid oxidation in water dispersion differs from bulk lipids due to the aqueous phase that may contain prooxidants and antioxidants [138].

Major prooxidants in the aqueous phase are the transition metals [139]. In emulsions the transition metals promote oxidation by decomposing lipid hydroperoxides located at the droplet surface into free radicals [140]. Free fatty acids are also promoters of the lipid oxidation in water dispersions [141]. The rigid structure of the SLN and NLC is supposed to protect lipids from oxidation by trapping them in the solid core [142]. In any case an investigation of the components should be performed. Radomska-Soukharev studied the chemical stability of different combinations of glycerides and surfactants during high pressure homogenization and storage. The tested excipients have shown a very good stability during the production and have exhibited good long term stability. Degradation of the lipids at ambient temperature was reported to be less than 10 % for over two years in all formulations and less than 5 % in most of them. Further data place the triglycerides as more stable than mono- and di- glycerides [143].

Physical stability

Physical instability in most of its part is related to the crystalline state of the lipids in the formulation. As we have already discussed, lipids express several crystal modifications. These modifications are α , β' and β . Supercooled melts are also possible. These different states of the lipids express different properties directly related to the stability presented in *Table 8.4*.

TABLE 8.4 Properties of supercooled melt and different lipid crystal modifications

State	Drug loading	Lipid molecules mobility	Stability	Crystal size [144]	Shape of particles [145]
Supercooled melt	Highest	Highest	Lowest	Not crystalline	Sphere
α -modification	High	High	Low	Few microns	Spheroids
β' -modification	Low	Low	High	Less than 5 μm	Spheroids
β -modification	Lowest	Lowest	Highest	20–100 μm	Platelet- and needle -like

When the formulation is rapidly cooled the lipids crystallize favorably in the alpha modification or in some cases form a supercooled melt. During storage a transition from the less stable crystalline modifications or supercooled state towards the more stable beta prime and beta modifications occurs [146]. This leads to formation of more ordered and stable crystalline structures in the lipids. As a general rule the stable modifications of the lipids usually exhibit lower EE and DL. For that reason, drug expulsion may occur after transitions of the lipid crystalline structure. Moreover, the transformation to more ordered crystalline structures cause a shape change from spherical to platelet-like or needle-like. The shape transformation results in increase in the overall surface area. Thus surfactant insufficiency can take place in the system (*Figure 8.12*).

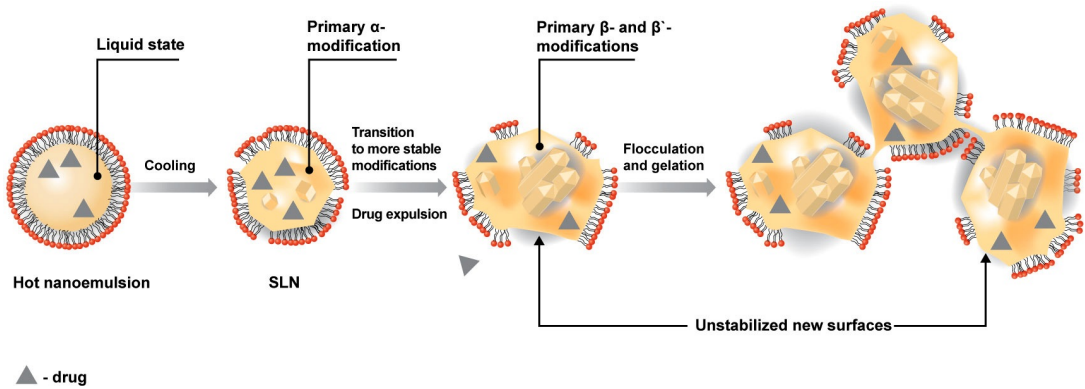


FIGURE 8.12 Changes in lipid modification and consequent instability processes of SLN during storage.

Transition from one modification into another is hard to predict and many factors may interfere. For example, presence of liquid phases may promote crystal changes by dissolving less stable crystals and further recrystallization in more stable modification [147]. Sometimes the transition may be rapid upon contact with different surfaces, stress conditions or evaporation of the solvent [148]. Rapid crystallization may result in gelation of the system and substantial difference in the properties [149]. However the colloidal state of the particles and the high amount of surfactants usually retard the crystallization into the more stable modifications [146].

Particle size and distribution should be controlled to evaluate long term stability [146]. Still, the gel formation is considered a more serious problem. Gel formation or gelation is described as the transition of the low-viscous colloidal suspension into a viscous gel. It is suggested that gel formation is connected to the crystallization of the lipids and the newly formed unstabilized surfaces (*Figure 8.12.*). Gelation is promoted upon high temperatures, light exposure and mechanical stress [150]. This is valid especially for polyethoxylated surfactants which have substantial difference in the properties at different temperatures. Other factors that influence stability are high concentrations of the particles and electrolytes [151].

Sterilization and antimicrobial preservation

Microbial stability can be achieved by different sterilization techniques. Formulations with small particle size and narrow distribution can be filtered through 0,22 micrometer filters. Other formulations are autoclavable. However, autoclaving causes melting of the particles and favors possible aggregation [152]. Formulations stabilized with polyethoxylated surfactants are not suitable for autoclaving as the high temperature may significantly decrease their HLB and stabilization ability. Still, promising results have been achieved with formulations containing lecithin and ionic surfactants [153]. Gamma-sterilization is not recommended in SLN/NLC formulations. Free radicals are formed which may undergo secondary reactions and result in chemical modification of the sample. Such changes were observed in lipid bilayer components [154].

Stabilization

Stabilization of the particles can be achieved by removal of the water as the powders are generally more stable than the suspensions.

Lyophilization is suitable technique to remove the water from SLN/NLC formulations [155]. During lyophilization the protective properties of the surfactant can be altered and the redispersion of the powder results in larger particles [156]. Modification changes, drug expulsion and change in the zeta potential are possible during the process [157]. Inclusion of suitable cryoprotectant is mandatory before lyophilization. Glucose, mannose, maltose and trehalose in concentration between 10 in 15 % find application in SLN/NLC [157]. The choice of the cryoprotectant is critical to the properties of the redispersed powder [155-158].

Spray drying is another possible process that is evaluated to form powders from SLN dispersions. Due to the high temperatures it is preferable that the lipids in the formulation have melting points higher than 70 °C. Particle aggregation can occur during the process. This restricts its uses to more diluted dispersions. Carbohydrates and ethanol mixtures instead of pure water are used during the drying process to optimize the powder properties after redispersion [159].

Another approach to stabilize SLN suspensions is to increase the viscosity of the formulation because this will reduce sedimentation and collision of the particles [160].

Despite all the problems that can be observed in the time during storage of SLN and NLC it has been shown that these system may express stability up to 3 years in their colloidal state [146].

Safety

Most of the excipients used in the preparation of SLN and NLC can be found listed in Pharmacopoeias (USP, Ph.Eur. JP etc.), Codex General Standard for Food Additives (GSFA) issued by FAO/WHO or in the current European and US cosmetics regulations [162]. Some are registered as excipients in pharmaceutical products only for certain administration routes. For example, palmitic acid is part of many food and cosmetic products but according to the FDA Inactive Ingredients Database it is only registered for oral application in pharmaceuticals. In addition, many of the lipids and especially surfactants may receive approval for certain route of administration but in lower concentrations than the ones used in SLN and NLC formulations. These circumstances reveal the limitations related to the development and the registration of these products. A careful reevaluation of safety and toxicity should be held for the new formulations. This is related to long process of development, testing and high costs. The toxicity of bulk excipients is a good starting point in the evaluation of toxicity and safety.

As was mentioned in the introduction, the lipids are physiological compounds which are presented in the everyday life. They are characterized by high safety profile and high LD50 values. Most of them are components of food sources and metabolic pathways for their degradation naturally exist. In addition, toxic effects from their degradation products are unlikely [162]. Surfactants may be a larger issue in terms of toxicity evaluation. Most of them are synthetic or semisynthetic

products and not all find application in pharmaceutical products. Often the toxicity and the failure of the final formulation is result of inappropriately selected combination of excipients.

Although the bulk excipients may have high LD50 values and GRAS status a detailed examination on the cytotoxicity of the final formulations should be performed before any in vivo tests. Proof of the safety of the lipid nanoparticles can be found in the scientific literature. Unloaded SLN composed of tripalmitin and stearic acid had no cytotoxic effect in vitro [163]. The low cytotoxicity was confirmed for SLN composed of various lipids [164]. SLN proved to be less toxic formulation compared to polylactide (PLA) and polylactide/glycolide (PLA/GA) nanoparticles [165]. Polylactide and butylcyanoacrylate nanoparticles have shown respectively 10 and 100 times higher cytotoxicity than SLN composed of glycerolbehenate and cetylpalmitate stabilized with poloxamer 407 and poloxamines [166].

Prior to in vivo tests an understanding of the possible interaction with the components of the biological fluids and tissues is mandatory. After administration in the body SLN and NLC are subject to different metabolism pathways, protein binding, distribution and elimination. Enzymes that take part in the degradation of lipids are mainly lipases and in lesser extent alcohol dehydrogenase. Non-enzymatic hydrolytic processes are also possible [167, 168]. Lipases break the ester bond in glycerol esters and partial glycerides and free fatty acids are formed. The activation of most lipases requires an oil/water interface to open the catalytic center [169]. Therefore the covering layer of the particles (the type of surfactant and its concentration) can affect the process of degradation by lipases [170]. Chemical structure of the lipids can also affect the degradation rate (e.g., longer length of the fatty acid chains in the triglycerides results in slower rates of degradation [171]; degradation of SLN composed of waxes is slower compared to SLN based on glycerides [166]). Degradation rate can also be influenced by the size of the particles because smaller size results in higher contact surface area.

The biodegradation of the SLN and NLC causes surface erosion and subsequent reduction in particle size until sufficiently small size for excretion is reached [163]. Protein binding is likely to occur and it is affected by the type of surfactant [172]. Plasma proteins that can be involved in this process are fibrinogen, IgG, IgM, apolipoproteins, transthyretin and albumin [173]. However, appropriate modifications of the particles can prevent interactions with proteins – for example, modification of the particle surface with PEG chains prevents interaction of the particles with human serum albumin.

Further fact concerning the safety is that within their size range SLN and NLC do not trigger macrophage activation and cytokine production [174]. Conclusions about the safety of the formulation cannot be based only on toxicity studies. Problems related to the physical instability of the particles (i.e., aggregation) may arise. Unexpected and uncontrolled gel formation during parenteral administration is considered extremely dangerous. Presence of aggregates and microparticles is possible in both microemulsions and SLN/NLC formulations. However, while the liquid nature of the droplets in nanoemulsion allows their deformation and lowers the chance of obstructions in small blood vessels, the rigid structure of the solid lipids increases this risk enormously. Therefore the development and testing of formulations for topical administration are much more favorable than those for parenteral.

Current applications

Parenteral

SLN were originally designed to resolve some of the problems of the parenteral nanoemulsions. However, for the past twenty years SLN and NLC formulations extended to a variety of new applications. SLN have solved some of the problems related to the fast drug expulsion from parenteral nanoemulsion. Upon appropriate surface modification (e.g., stabilization with poloxamers and poloxamines) SLN may express controlled and prolonged effect after *i.v.* administration [175]. Besides achieving controlled release for APIs like *camptothecin* the nature of SLN/NLC may increase the drug chemical stability after administration [176]. Reduction in the toxicity of parenterally administered drugs is also reported for some substances (i.e., *docetaxel* [39]). Through different superficial modifications SLN and NLC may be turned into the so called “stealth” particles for long blood circulation [177] or into targeted delivery systems – to selectively reach lung [178], spleen [179], brain [179, 180], liver [179, 181, 182], tumors or other damaged organs and tissues. SLN and NLC proved to be a suitable carrier to increase the cellular uptake and targeting efficiency and thereby to improve the therapy in cancer [183]. They can provide new solutions to problems associated with parenteral treatment of significant diseases like malaria with drugs that express low solubility [184]. A bright example is the poorly soluble anti malaria drug *artemether* which upon incorporation in NLC had lower hemolytic potential and increase in the antimalarial activity [185].

Oral

Problems related to the poor solubility and bioavailability of variety of drugs after oral administration can be solved with SLN and NLC formulations owing to the “Trojan horse effect” [110]. After intake the lipid matrices composed of triglycerides are normally digested by pancreatic lipases into mono- and di-glycerides. The monoglycerides may form micelles and mixed micelles (with bile salts) that still contain the drug. Then these lipids may undergo absorption together with the drug *via* chylomicron formation primarily into the lymphatic system. This transportation surrounds the liver and the first pass effect [186]. The lymphatic uptake of SLN was confirmed with fluorescent dyes [187]. Lymphatic uptake can be controlled with particle size as smaller size results in higher uptake [188]. In addition SLN and NLC may enhance drug absorption *via* other mechanisms – increased uptake by M-cells of Peyer’s patches in the gut [189] and adherence of the particles to the gut wall [190]. SLN and NLC show encouraging results in the improvement of the bioavailability and stability of proteins after oral administration [5, 191, 192]. In recent study *insulin* loaded SLN have shown improved stability against proteolytic enzymes *in vitro* [193].

Dermal application and cosmetic products

The dermal application of SLN and NLC gained the biggest interest among researchers. Lipid nanoparticles offer increased chemical stability of the APIs, film formation, higher occlusion, increased skin hydration and modulated drug release (*Figure 8.13.*).

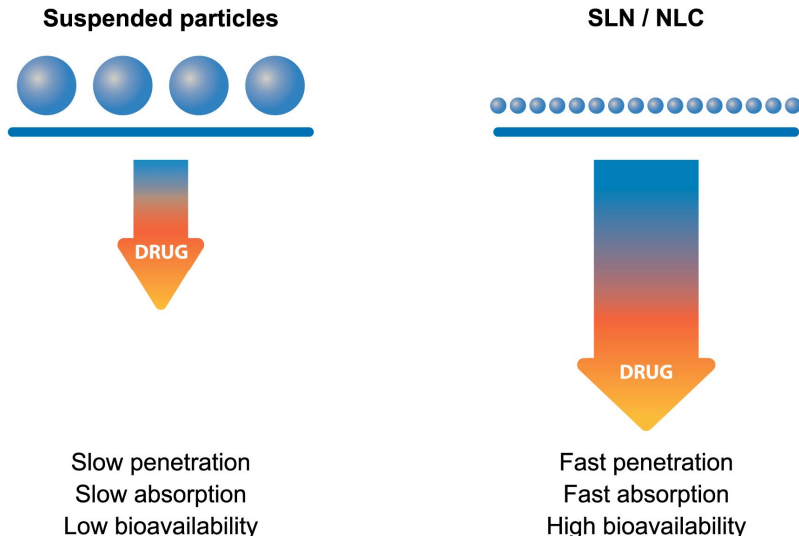


FIGURE 8.13 Tighter distribution and better occlusion of SLN/ NLC applied on skin compared to microparticles.

SLN can also be administered on damaged and inflamed skin, because they comprise non-irritant and non-toxic lipids [197].

Enhanced penetration with reduced side effects was reported [198]. Improved safety and therapeutic characteristics due to the inclusion in SLN/NLC have been reported with *calcipotriol* [199], *coenzyme Q10* [200], *celecoxib* [83], *ketoprofen* [201], *vitamin A* [202] and many more. In cases when systemic absorption must be avoided after dermal application SLN can provide epidermal targeting. Epidermal targeting *via* SLN was confirmed with fluorescence microscopy for podophyllotoxin [203]. Further SLN was found to decrease the irritant potential of retinoic acid and improved its stability [204]. NLC also offer possibilities in the treatment of significant diseases as psoriasis which was confirmed by acitrecin loaded NLC in a clinical study [40]. The broadest area of application of SLN and NLC, however, is in the cosmetics [205]. Indeed, this is expected as cosmetics are subject of less regulatory restrictions with shorter registration times compared to pharmaceuticals and the introduction of innovative technologies has become fundamental for the marketing success of the big companies. SLN and NLC are used in different anti-aging creams, sunscreen products and others. NLC loaded with Q10 show better long-term physical and chemical stability compared to nanoemulsion [206]. Tests showed that the NLC based cream has no skin irritation potential and possess a higher hydration effect compared to a conventional cream with the same composition [207].

Pulmonary, nasal and ocular application

SLN and NLC are suitable carriers with good tolerability and low toxicity for pulmonary application [208]. Their small size enables their incorporation in microparticles and drops which can effectively reach the alveoli. Moreover SLN and NLC can improve the pharmacokinetic parameters after administration *via* the lungs [208]. Various examples include *itraconazole* [22],

phenethylisothiocyanate [209], *celecoxib* [7], *beclomethasone* [210], *thymopentin* [211]. Most of these formulations are intended for use in the treatment of infectious diseases and cancer. Promising results are reported for anti-tuberculosis drugs after incorporation in SLN for pulmonary administration [213]. The lungs also offer alternative route to application of peptides and proteins. SLN showed to be a suitable carrier of insulin [212].

Nasal application is also promising for variety of drugs. SLN proved to be a suitable alternative carrier for the nasal administration of the antiemetic drug *ondansetron* [214]. Other more recent examples are the SLN formulations for intranasal administration with *budesonide* [215], *ropinirole* [216], *alprazolam* [217] and many others. Brain targeting of the SLN was achieved by intranasal administration. An example are the risperidone loaded SLN which showed promising brain bioavailability after nasal administration in mice [90].

In ocular formulations SLN and NLC exhibit increased retention time and increased absorption for different drugs - *tobramycin* [194], *cyclosporine A* [195], and *timolol* [196]. *Diclofenac* loaded SLN showed sustained release and higher permeability after tests on bioengineered human cornea [60]. SLNs formulations proved better than simple solution in the treatment of ocular infections with *tobramycin* [194].

Gene therapy

In recent years SLN and NLC have gained increasing attention in the field of gene therapy as promising alternative carriers to cationic lipids because of their rapid uptake by cells and protection of the incorporated compound against chemical degradation [218]. Furthermore SLN can be prepared by low mechanical force methods that will not damage DNA and RNA strands [110]. A positive charge that will promote the interaction with the negatively charged cell membrane and the nucleic acids can be easily induced with suitable cationic surfactants. Cationic SLNs with protamine as transfection promoter were tested and showed promising result [219].

Perspectives and conclusion

For more than 20 years of research the current and future applications of SLN and NLC seem well shaped. In parenteral formulations they will offer more possibilities for many drugs with poor aqueous solubility, short half-life and low chemical stability. Moreover SLN and NLC are likely to find more applications as targeted drug delivery systems which will "direct" the drug molecules to specific organs of interest and to reduce the systemic toxicity. Thus they can provide solutions for APIs that failed clinical tests due inappropriate tissue localization. The safety and toxicity of the excipients after oral administration is well represented by the low LD50 values of the substances making the testing and registration procedures of peroral formulations lighter. The possibility of SLN and NLC to improve bioavailability of many newly synthesized or old molecules from BCS Classes II, III and IV is a realistic challenge. Further they can resolve problems related to unpleasant taste, irritation and interactions in the gastrointestinal tract. New combinations of SLN/NLC with traditional dosage forms (e.g., tablets, pellets, capsules) are likely to be studied in terms of resolving stability, bioavailability and toxicity issues. The oral administration of peptides and proteins may also be achieved with suchlipid-based formulations. Dermal application will probably continue to be the area of the biggest interest as it is the main field of practical application of SLN and NLC at the moment. Poor skin penetration of APIs may be improved due to the better

occlusion of the nanoparticles compared to traditional creams and gels. SLN and NLC may also offer improved chemical stability towards oxidation of various lipid soluble APIs for dermal application due to the rigid nature of the particles. SLN and NLC have shown to express reduced skin irritation and reduced systemic absorption when targeted to the skin for various drugs as we discussed earlier. Similar application is likely to be used with more APIs that have irritation potential and undesired systemic effects. In the cosmetic industry SLN and NLC have already entered the market by various anti-aging and sun protection products and their number will likely continue to grow. Other routes of topical application for SLN and NLC that are less studied will induce bigger interest. Pulmonary and nasal application appear to be suitable in terms of improved safety and bioavailability. The lung as an “entrance gate” to the body is not only of particulate interest for various anti-cancer drugs and antibiotics loaded in lipid nanoparticles but also for proteins. Alongside the pulmonary administration the nasal application of SLN/NLC formulations is also promising. The reports of achieving high drug concentrations in the brain after nasal administration of SLN/NLC are very encouraging. Such approach can overcome limitations like low blood/brain uptake for certain drugs. This approach may be reliable in treatment of diseases related to CNS disorders like Parkinson’s, Alzheimer’s, multiple sclerosis and others. The increased interest in gene therapy and the suitability of cationic SLN/NLC will also expand the scope of their application. Future work will probably be focused on the improvement of the loading of the RNA and DNA and decreasing the toxicity of the final formulation. Still, SLN and NLC should not be considered as infinitely applicable. For example the highest standards set for physical stability during the storage (e.g., absolute lack of aggregation and presence of microparticles) and the probability of uncontrolled changes (e.g., gelling or aggregation) upon administration may suggest that for some routes (especially the parenteral) their administration could be an issue.

As a conclusion, we can summarize that solid lipid nanoparticles and nanostructured lipid carriers are promising drug delivery systems. Their low toxicity is one of their strongest aspects together with the advantages they offer in almost all administration routes. In addition, their production methods can be transferred to large scale without organic solvents and the dispersions can be prepared at relatively high concentrations. However, SLN and NLC cannot be regarded as the “*panacea*” to all drug delivery problems. They proved to have some drawbacks mainly related to the polymorphic modifications of the lipid matrix, physical instability (gelation, aggregation, drug expulsion) and coexisting structures (micelles, liposomes). Sterilization and antimicrobial preservation could also be problematic for many of the formulations. In future a better understanding of the colloidal state of the lipids as a result of the more sensitive and modern analytical techniques will help the researches to overcome some of the limitations we have discussed in this paper.

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References

1. Eldem T, Speiser P, Hincal A. Optimization of spray-dried and-congealed lipid micropellets and characterization of their surface morphology by scanning electron microscopy. *Pharmaceutical research* 1991;8(1): 47-54.
<http://link.springer.com/article/10.1023/A:1015874121860> (accessed 14 October 2013).
2. Siekmann B, Westesen K. Submicron-sized parenteral carrier systems based on solid lipids. *Pharm. Pharmacol. Lett* 1992;1(3) 123-126.
3. Lucks S, Müller R. WIPO Patent No. 1993005768. Geneva, Switzerland: World Intellectual Property Organization, 1993. <http://patentscope.wipo.int/search/en/WO1993005768> (accessed 14 October 2013).
4. Gasco MR. U.S. Patent No. 5,250,236. Washington, DC: U.S. Patent and Trademark Office, 1993.
<http://www.google.bg/patents?hl=en&lr=&vid=USPAT5250236&id=MU4nAAAAEBAJ&oi=fn&dq=Method+for+producing+solid+lipid+microspheres+having+a+narrow+size+distribution&printsec=abstract> (accessed 14 October 2013).
5. Martins S, Sarmiento B, Ferreira DC, Souto EB. Lipid-based colloidal carriers for peptide and protein delivery—liposomes versus lipid nanoparticles. *International journal of nanomedicine* 2007;2(4): 595-607.
<http://www.ncbi.nlm.nih.gov/pmc/articles/pmc2676808/> (accessed 14 October 2013).
6. Müller RH, Runge SA. Solid lipid nanoparticles (SLN®) for controlled drug delivery. In: Benita S. (ed.) *Submicron Emulsions in Drug Targeting and Delivery*. Amsterdam: CRC Press; 1998. p219-234
7. Patlolla RR, Chougule M, Patel AR, Jackson T, Tata PN, Singh M. Formulation, characterization and pulmonary deposition of nebulized celecoxib encapsulated nanostructured lipid carriers. *Journal of Controlled Release* 2010;144(2): 233-241.
<http://www.sciencedirect.com/science/article/pii/S0168365910001148> (accessed 14 October 2013).
8. Manjunath K, Venkateswarlu V, Hussain A. Preparation and characterization of nitrendipine solid lipid nanoparticles. *Die Pharmazie-An International Journal of Pharmaceutical Sciences* 2011;66(3): 178-186.
<http://www.ingentaconnect.com/content/govi/pharmaz/2011/00000066/00000003/art00006> (accessed 14 October 2013).
9. Nekkanti V, Venkateshwarlu V, Pillai R. Preparation, Characterization and In-Vivo Evaluation of Raloxifene Hydrochloride Solid Lipid Nanoparticles. *Pharmaceutical Nanotechnology* 2013;1(1): 68-77.

<http://www.ingentaconnect.com/content/ben/pnt/2013/00000001/00000001/art00011> (accessed 14 October 2013).

10. Jain A, Agarwal A, Majumder S, Lariya N, Khaya A, Agrawal H, Agrawal GP. Mannosylated solid lipid nanoparticles as vectors for site-specific delivery of an anti-cancer drug. *Journal of Controlled Release* 2010;148(3): 359-367. <http://www.sciencedirect.com/science/article/pii/S0168365910007467> (accessed 14 October 2013).
11. Yu DG, Williams GR, YangJH, WangX, YangJM, Li XY. Solid lipid nanoparticles self-assembled from electrosprayed polymer-based microparticles. *Journal of Materials Chemistry* 2011;21(40): 15957-15961. <http://pubs.rsc.org/en/content/articlehtml/2011/jm/c1jm12720a> (accessed 14 October 2013).
12. Jensen LB, Magnusson E, Gunnarsson L, Vermehren C, Nielsen HM, Petersson K. Corticosteroid solubility and lipid polarity control release from solid lipid nanoparticles. *International journal of pharmaceutics* 2010;390(1): 53-60. <http://www.sciencedirect.com/science/article/pii/S0378517309007315> (accessed 14 October 2013).
13. Boonme P, Souto EB, Wuttisantikul N, Jongjit T, Pichayakorn W. Influence of lipids on the properties of solid lipid nanoparticles from microemulsion technique. *European Journal of Lipid Science and Technology* 2013;115(7): 820-824. <http://onlinelibrary.wiley.com/doi/10.1002/-ejlt.201200240/full> (accessed 14 October 2013).
14. Dora CL, Putaux JL, Pignot-Paintrand I, Dubreuil F, Soldi V, Borsali R, Lemos-Senna E. Physicochemical and morphological characterizations of glyceryl tristearate/castor oil nanocarriers prepared by the solvent diffusion method. *Journal of the Brazilian Chemical Society* 2012;23(11): 1972-1981. <http://dx.doi.org/10.1590/S0103-50532012005000066> (accessed 14 October 2013).
15. Patel BD, Modi RV, Thakkar NA, Patel AA, Thakkar PH. Development and characterization of solid lipid nanoparticles for enhancement of oral bioavailability of Raloxifene. *Journal of pharmacy & bioallied sciences* 2012;4(Suppl 1): S14-S16. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3467810/> (accessed 14 October 2013).
16. Silva LFC, Kasten G, de Campos CEM, Chinelatto A L, Lemos-Senna E. Preparation and characterization of quercetin-loaded solid lipid microparticles for pulmonary delivery. *Powder Technology* 2013;239: 183-192. <http://www.sciencedirect.com/science/article/pii/S003259101300065X> (accessed 14 October 2013).
17. Helgason T, Awad TS, Kristbergsson K, Decker EA, McClements DJ, Weiss J. Impact of surfactant properties on oxidative stability of β -carotene encapsulated within solid lipid nanoparticles. *Journal of agricultural and food chemistry* 2009;57(17): 8033-8040. <http://pubs.acs.org/doi/abs/10.1021/jf901682m> (accessed 14 October 2013).

18. Gokce EH, Korkmaz E, Dellera E, Sandri G, Bonferoni MC, Ozer O. Resveratrol-loaded solid lipid nanoparticles versus nanostructured lipid carriers: evaluation of antioxidant potential for dermal applications. *International journal of nanomedicine* 2012;7: 1841-1850. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3352689/> (accessed 14 October 2013)
19. K uchler S, Radowski MR, Blaschke T, Dathe M, Plendl J, Haag R, Kramer KD. Nanoparticles for skin penetration enhancement—a comparison of a dendritic core-multishell-nanotransporter and solid lipid nanoparticles. *European Journal of Pharmaceutics and Biopharmaceutics* 2009;71(2): 243-250. <http://www.sciencedirect.com/science/article/pii/S0939641108003226> (accessed 14 October 2013).
20. Zhuang CY, Li N, Wang M, Zhang XN, Pan WS, Peng JJ, Tang X. Preparation and characterization of vincapocetine loaded nanostructured lipid carriers (NLC) for improved oral bioavailability. *International journal of pharmaceutics* 2010;394(1): 179-185. <http://www.sciencedirect.com/science/article/pii/S0378517310003480> (accessed 14 October 2013).
21. Kim JK, Park JS, Kim CK. Development of a binary lipid nanoparticles formulation of itraconazole for parenteral administration and controlled release. *International journal of pharmaceutics* 2010;383(1): 209-215. <http://www.sciencedirect.com/science/article/pii/S0378517309006218> (accessed 14 October 2013).
22. Pardeike J, Weber S, Haber T, Wagner J, Zarfl HP, Plank H, Zimmer A. Development of an itraconazole-loaded nanostructured lipid carrier (NLC) formulation for pulmonary application. *International journal of pharmaceutics* 2011;419(1) 329-338. <http://www.sciencedirect.com/science/article/pii/S037851731100754X>
23. Shah KA, Joshi MD, Patravale VB. Biocompatible microemulsions for fabrication of glyceryl monostearate solid lipid nanoparticles (SLN) of tretinoin. *Journal of biomedical nanotechnology* 2009;5(4): 396-400. <http://www.ingentaconnect.com/content/asp-jbn/2009/-00000005/-00000004/art00009> (accessed 14 October 2013).
24. Hu L, Xing Q, Meng J, Shang C. Preparation and enhanced oral bioavailability of cryptotanshinone-loaded solid lipid nanoparticles. *AAPS PharmSciTech* 2010;11(2): 582-587. <http://link.springer.com/article/10.1208/s12249-010-9410-3> (accessed 14 October 2013).
25. Shah M, Pathak K. Development and statistical optimization of solid lipid nanoparticles of simvastatin by using 23 full-factorial design. *AAPS PharmSciTech* 2010;11(2): 489-496. <http://link.springer.com/article/10.1208/s12249-010-9414-z> (accessed 14 October 2013).
26. Bhalekar MR, Pokharkar V, Madgulkar A, Patil N, Patil N. Preparation and evaluation of miconazole nitrate-loaded solid lipid nanoparticles for topical delivery. *AAPS PharmSciTech* 2009;10(1): 289-296. <http://link.springer.com/article/10.1208/s12249-009-9199-0> (accessed 14 October 2013).

27. Kalam MA, Sultana Y, Ali A, Aqil M, Mishra AK, Chuttani K. Preparation, characterization, and evaluation of gatifloxacin loaded solid lipid nanoparticles as colloidal ocular drug delivery system. *Journal of drug targeting* 2010;18(3): 191-204. <http://informahealthcare.com/doi/-abs/10.3109/10611860903338462> (accessed 14 October 2013).
28. Soares S, Fonte P, Costa A, Andrade J, Seabra V, Ferreira D, Sarmiento B. Effect of freeze-drying, cryoprotectants and storage conditions on the stability of secondary structure of insulin-loaded solid lipid nanoparticles. *International journal of pharmaceutics* 2013;456(2): 370-381. <http://www.sciencedirect.com/science/article/pii/S0378517313008090> (accessed 14 October 2013).
29. Doktorovová S, Araújo J, Garcia ML, Rakovský E, Souto EB. Formulating fluticasone propionate in novel PEG-containing nanostructured lipid carriers (PEG-NLC). *Colloids and Surfaces B: Biointerfaces* 2010;75(2): 538-542. <http://www.sciencedirect.com/science/article/pii/S0927776509004664> (accessed 14 October 2013).
30. Sarmiento B, Mazzaglia D, Bonferoni MC, Neto AP, do Céu Monteiro M, Seabra V. Effect of chitosan coating in overcoming the phagocytosis of insulin loaded solid lipid nanoparticles by mononuclear phagocyte system. *Carbohydrate Polymers* 2011;84(3): 919-925. <http://www.sciencedirect.com/science/article/pii/S0144861710010283> (accessed 14 October 2013).
31. Fonte P, Nogueira T, Gehm C, Ferreira D, Sarmiento B. Chitosan-coated solid lipid nanoparticles enhance the oral absorption of insulin. *Drug Delivery and Translational Research* 2011;1(4): 299-308. <http://link.springer.com/article/10.1007/s13346-011-0023-5> (accessed 14 October 2013).
32. Abdelbary G, Fahmy RH. Diazepam-loaded solid lipid nanoparticles: design and characterization. *Aaps Pharmscitech* 2009;10(1): 211-219. <http://link.springer.com/article/10.1208/s12249-009-9197-2> (accessed 14 October 2013).
33. Battaglia L, Gallarate M, Cavalli R, Trotta M. Solid lipid nanoparticles produced through a coacervation method. *Journal of microencapsulation* 2010;27(1): 78-85. <http://informahealthcare.com/doi/abs/10.3109/02652040903031279> (accessed 14 October 2013).
34. Chirio D, Gallarate M, Peira E, Battaglia L, Serpe L, Trotta M. Formulation of curcumin-loaded solid lipid nanoparticles produced by fatty acids coacervation technique. *Journal of microencapsulation* 2011;28(6): 537-548. http://www.researchgate.net/publication/51247427_Formulation_of_curcumin-loaded_solid_lipid_nanoparticles_produced_by_fatty_acids_coacervation_technique/file/60b7d517f7dddaf0b7.pdf (accessed 14 October 2013)
35. Xie S, Zhu L, Dong Z, Wang Y, Wang X, Zhou W. Preparation and evaluation of ofloxacin-loaded palmitic acid solid lipid nanoparticles. *International journal of*

- nanomedicine2011;6: 547-555. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3065800/> (accessed 14 October 2013)
36. Kuo YC, Ko HF. Targeting delivery of saquinavir to the brain using 83-14 monoclonal antibody-grafted solid lipid nanoparticles. *Biomaterials* 2013;34(20): 4818-4830. <http://www.sciencedirect.com/science/article/pii/S0142961213002950> (accessed 14 October 2013).
 37. Kuo YC, Chung JF. Physicochemical properties of nevirapine-loaded solid lipid nanoparticles and nanostructured lipid carriers. *Colloids and Surfaces B: Biointerfaces* 2011;83(2): 299-306. <http://www.sciencedirect.com/science/article/pii/S0927776510006685> (accessed 14 October 2013).
 38. Xue HY, Wong HL. Tailoring nanostructured solid-lipid carriers for time-controlled intracellular siRNA kinetics to sustain RNAi-mediated chemosensitization. *Biomaterials* 2011;32(10): 2662-2672. <http://www.sciencedirect.com/science/article/pii/S0142961210016029> (accessed 14 October 2013).
 39. Liu D, Liu Z, Wang L, Zhang C, Zhang N. Nanostructured lipid carriers as novel carrier for parenteral delivery of docetaxel. *Colloids and Surfaces B: Biointerfaces* 2011;85(2): 262-269. <http://www.sciencedirect.com/science/article/pii/S0927776511001184> (accessed 14 October 2013).
 40. Agrawal Y, Petkar KC, Sawant KK. Development, evaluation and clinical studies of Acitretin loaded nanostructured lipid carriers for topical treatment of psoriasis. *International journal of pharmaceutics* 2010;401(1): 93-102. <http://www.sciencedirect.com/science/article/pii/S0378517310007088> (accessed 14 October 2013).
 41. Farboud ES, Nasrollahi SA, Tabbakhi Z. Novel formulation and evaluation of a Q10-loaded solid lipid nanoparticle cream: in vitro and in vivo studies. *International journal of nanomedicine* 2011;6: 611-617. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3107720/> (accessed 14 October 2013).
 42. Siddiqui A, Alayoubi A, El-Malah Y, Nazzal S. Modeling the effect of sonication parameters on size and dispersion temperature of solid lipid nanoparticles (SLNs) by response surface methodology (RSM). *Pharmaceutical development and technology* 2013;(0): 1-5. <http://informahealthcare.com/doi/abs/10.3109/10837450.2013.784336> (accessed 14 October 2013).
 43. Siddiqui A, Gupta V, Liu YY, Nazzal S. Doxorubicin and MBO-asGCS oligonucleotide loaded lipid nanoparticles overcome multidrug resistance in adriamycin resistant ovarian cancer cells (NCI/ADR-RES). *International Journal of Pharmaceutics* 2012;431(1): 222-229. <http://www.sciencedirect.com/science/article/pii/S0378517312003961> (accessed 14 October 2013).

44. Siddiqui A, Alayoubi A, Nazzal S. The effect of emulsifying wax on the physical properties of CTAB-based solid lipid nanoparticles (SLN). *Pharmaceutical development and technology* 2013;(0): 1-4. <http://informahealthcare.com/doi/abs/10.3109/10837450.2012.751401> (accessed 14 October 2013).
45. Chawla V, Saraf SA. Rheological studies on solid lipid nanoparticle based carbopol gels of aceclofenac. *Colloids and Surfaces B: Biointerfaces* 2012;92: 293-298. <http://www.sciencedirect.com/science/article/pii/S0927776511007417> (accessed 14 October 2013).
46. Nasrollahi SA, Abbasian AR, Farboud ES. In vitro comparison of simple tretinoin-cream and cream loaded with tretinoin-SLN. *Journal of Pharmaceutical Technology & Drug Research* 2013;2: 13. <http://www.hoajonline.com/journals/pdf/2050-120X-2-13.pdf> (accessed 14 October 2013).
47. Sanna V, Caria G, Mariani A. Effect of lipid nanoparticles containing fatty alcohols having different chain length on the ex vivo skin permeability of Econazole nitrate. *Powder Technology* 2010;201(1): 32-36. <http://www.sciencedirect.com/science/article/pii/S003259101000118X> (accessed 14 October 2013).
48. Kovacevic A, Savic S, Vuleta G, Müller RH, Keck CM. Polyhydroxy surfactants for the formulation of lipid nanoparticles (SLN and NLC): Effects on size, physical stability and particle matrix structure. *International Journal of Pharmaceutics* 2011;406(1): 163-172. <http://www.sciencedirect.com/science/article/pii/S0378517311000081> (accessed 14 October 2013).
49. Severino P, Santana MHA, Souto EB. Optimizing SLN and NLC by 2² factorial design: Effect of homogenization technique. *Materials Science and Engineering: C* 2012; 32(6): 1375-1379. <http://www.sciencedirect.com/science/article/pii/S0928493112001361> (accessed 14 October 2013).
50. Zhang J, Smith E. Percutaneous permeation of betamethasone 17-valerate incorporated in lipid nanoparticles. *Journal of pharmaceutical sciences* 2011;100(3): 896-903. <http://onlinelibrary.wiley.com/doi/10.1002/jps.22329/full> (accessed 14 October 2013).
51. Nguyen HM, Hwang IC, Park JW, Park HJ. Enhanced payload and photo-protection for pesticides using nanostructured lipid carriers with corn oil as liquid lipid. *Journal of microencapsulation* 2012;29(6): 596-604. <http://informahealthcare.com/doi/abs/10.3109/02652048.2012.668960> (accessed 14 October 2013).
52. Kheradmandnia S, Vasheghani-Farahani E, Nosrati M, Atyabi F. Preparation and characterization of ketoprofen-loaded solid lipid nanoparticles made from beeswax and carnauba wax. *Nanomedicine: Nanotechnology, Biology and Medicine* 2010;6(6): 753-759.

- <http://www.sciencedirect.com/science/article/pii/S1549963410001644> (accessed 14 October 2013).
53. Lacerda SP, Cerize NNP, Ré MI. Preparation and characterization of carnauba wax nanostructured lipid carriers containing benzophenone-3. *International journal of cosmetic science* 2011;33(4): 312-321. <http://onlinelibrary.wiley.com/doi/10.1111/j.1468-2494.2010.00626.x/full> (accessed 14 October 2013).
 54. Xie S, Wang F, Wang Y, Zhu L, Dong Z, Wang X, Zhou W. Acute toxicity study of tilmicosin-loaded hydrogenated castor oil-solid lipid nanoparticles. *Particle and fibre toxicology* 2011;8(1): 1-10. <http://www.biomedcentral.com/content/pdf/1743-8977-8-33.pdf> (accessed 14 October 2013).
 55. Han C, Qi CM, Zhao BK, Cao J, Xie SY, Wang SL, Zhou WZ. Hydrogenated castor oil nanoparticles as carriers for the subcutaneous administration of tilmicosin: in vitro and in vivo studies. *Journal of Veterinary Pharmacology and Therapeutics* 2009;32(2): 116-123. <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2885.2008.01009.x/full> (accessed 14 October 2013).
 56. Xie S, Pan B, Wang M, Zhu L, Wang F, Dong Z, Zhou W. Formulation, characterization and pharmacokinetics of praziquantel-loaded hydrogenated castor oil solid lipid nanoparticles. *Nanomedicine* 2010;5(5): 693-701. <http://www.futuremedicine.com/doi-abs/10.2217/nnm.10.42> (accessed 14 October 2013).
 57. How CW, Abdullah R, Abbasalipourkabir R. Characterization and Cytotoxicity of Nanostructured Lipid Carriers Formulated with Olive Oil, Hydrogenated Palm Oil and Polysorbate 80. *NanoBioscience, IEEE Transactions on* 2013;12(2): 72-78. http://ieeexplore.ieee.org/-xpls/abs_all.jsp?arnumber=6384803 (accessed 14 October 2013).
 58. Rahman HS, Rasedee A, How CW, Abdul AB, Zeenathul NA, Othman HH, Yeap SK. Zerumbone-loaded nanostructured lipid carriers: preparation, characterization, and antileukemic effect. *International journal of nanomedicine* 2013;8: 2769-2781. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3739459/> (accessed 14 October 2013).
 59. Kuo YC, Chung CY. Solid lipid nanoparticles comprising internal Compritol 888 ATO, tripalmitin and cacao butter for encapsulating and releasing stavudine, delavirdine and saquinavir. *Colloids and Surfaces B: Biointerfaces* 2011;88(2): 682-690. <http://www.sciencedirect.com/science/article/pii/S0927776511004656> (accessed 14 October 2013).
 60. Attama AA, Reichl S, Müller-Goymann CC. Diclofenac sodium delivery to the eye: In vitro evaluation of novel solid lipid nanoparticle formulation using human cornea construct. *International journal of pharmaceutics* 2008;355(1): 307-313.

61. Zhang L, Hayes DG, Chen G, Zhong Q. Transparent Dispersions of Milk Fat-Based Nanostructured Lipid Carriers for Delivery of β -Carotene. *Journal of agricultural and food chemistry* 2013;61(39): 9435-9443. <http://www.sciencedirect.com/science/article/pii/S0378517307010162> (accessed 14 October 2013).
62. Zhang L, Hayes DG, Chen G, Zhong Q. Transparent Dispersions of Milk Fat-Based Nanostructured Lipid Carriers for Delivery of β -Carotene. *Journal of agricultural and food chemistry* 2013;61(39): 9435-9443. <http://pubs.acs.org/doi/abs/10.1021/jf403512c> (accessed 14 October 2013).
63. Allen LV, Popovich NG. *Ansel's pharmaceutical dosage forms and drug delivery systems* (Vol. 306, p. 300). New York: Lippincott Williams & Wilkins; 2005.
64. Webster AJ, Cates ME. Osmotic stabilization of concentrated emulsions and foams. *Langmuir* 2001;17(3): 595-608. <http://pubs.acs.org/doi/abs/10.1021/la000699m> (accessed 14 October 2013).
65. Solans C, Solé I. Nano-emulsions: Formation by low-energy methods. *Current Opinion in Colloid & Interface Science* 2012;17(5): 246-254. <http://www.sciencedirect.com/science/article/-pii/S1359029412000787> (accessed 14 October 2013).
66. Shafiq S, Faiyaz S, Sushma T, Farhan JA, Khar RK, Ali M. Development and bioavailability assessment of ramipril nanoemulsion formulation. *Eur J Pharm Biopharm* 2007;66: 227–243. <http://www.sciencedirect.com/science/article/pii/S0939641106002839> (accessed 14 October 2013).
67. Schoenitz M, Joseph S, Nitz A, Bunjes H, Scholl S. Controlled Polymorphic Transformation of Continuously Crystallized Solid Lipid Nanoparticles in a Microstructured Device: A Feasibility Study. *European Journal of Pharmaceutics and Biopharmaceutics* 2013; In press. <http://www.sciencedirect.com/science/article/pii/S0939641113002889> (accessed 14 October 2013).
68. Triplett II MD, Rathman JF. Optimization of β -carotene loaded solid lipid nanoparticles preparation using a high shear homogenization technique. *Journal of Nanoparticle Research* 2009;11(3): 601-614. <http://link.springer.com/article/10.1007/s11051-008-9402-3> (accessed 14 October 2013).
69. Müller RH, Runge SA, Ravelli V, Thünemann AF, Mehnert W, Souto EB. Cyclosporine-loaded solid lipid nanoparticles (SLN[®]): Drug–lipid physicochemical interactions and characterization of drug incorporation. *European Journal of Pharmaceutics and Biopharmaceutics* 2008;68(3): 535-544. <http://www.sciencedirect.com/science/article/pii/S0939641107002603> (accessed 14 October 2013).

70. Cerreto F, Paolicelli P, Cesa S, Amara HMA, D'Auria FD, Simonetti G, Casadei MA. Solid Lipid Nanoparticles as Effective Reservoir Systems for Long-Term Preservation of Multidose Formulations. *AAPS PharmSciTech* 2013;14(2): 847-853. <http://link.springer.com/article/-10.1208/s12249-013-9972-y> (accessed 14 October 2013).
71. Paolicelli P, Cerreto F, Cesa S, Feeney M, Corrente F, Marianecci C, Casadei MA. Influence of the formulation components on the properties of the system SLN-dextran hydrogel for the modified release of drugs. *Journal of microencapsulation* 2009;26(4): 355-364. <http://informahealthcare.com/doi/abs/10.1080/02652040802372899> (accessed 14 October 2013).
72. Gramdorf S, Kumpugdee-Vollrath M, Bilek H, Perlich J. Influence of emulsifiers on the crystallization of Solid Lipid Nanoparticles (SLN). *Annual Report HASYLAB, DESY* 2010. http://photon-science.desy.de/annual_report/files/2009/2009622.pdf (accessed 14 October 2013).
73. Kwon TK, Lim KB, Kim JC. Solid lipid nanoparticles coated with silk fibroin. *Journal of Industrial and Engineering Chemistry* 2011;17(1): 10-13. <http://www.sciencedirect.com/science/-article/pii/S1226086X10002534> (accessed 14 October 2013).
74. Rodríguez Gascón A, Solinís AMA, Del Pozo RA, San D, Diego V, Pedraz Muñoz JL. European Patent No. EP 2460516. Munich, Germany: European Patent Office 2012. <http://www.freepatentsonline.com/EP2460516.html> (accessed 14 October 2013).
75. Rosenberger V, Moldavski N, Flashner-barak M, Lerner IE. European Patent No. EP 1680087. Munich, Germany: European Patent Office 2006. <http://www.freepatentsonline.com/-EP1680087.html> (accessed 14 October 2013).
76. Montenegro L, Sarpietro MG, Ottimo S, Puglisi G, Castelli F. Differential scanning calorimetry studies on sunscreen loaded solid lipid nanoparticles prepared by the phase inversion temperature method. *International journal of pharmaceuticals* 2011;415(1): 301-306. <http://www.sciencedirect.com/science/article/pii/S037851731100528X> (accessed 14 October 2013).
77. Montenegro L, Trapani A, Latrofa A, Puglisi G. In vitro evaluation on a model of blood brain barrier of idebenone-loaded solid lipid nanoparticles. *Journal of Nanoscience and Nanotechnology* 2012;12(1): 330-337. <http://www.ingentaconnect.com/content/asp/-jnn/2012/00000012/-00000001/art00040> (accessed 14 October 2013).
78. Zhang JQ, Liu J, Li XL, Jasti BR. Preparation and characterization of solid lipid nanoparticles containing silibinin. *Drug Delivery* 2007;14(6): 381-387. <http://informahealthcare.com/doi/abs/-10.1080/10717540701203034> (accessed 14 October 2013).

79. Lv Q, Yu A, Xi Y, Li H, Song Z, Cui J, Zhai G. Development and evaluation of penciclovir-loaded solid lipid nanoparticles for topical delivery. *International journal of pharmaceutics* 2009;372(1): 191-198. <http://www.sciencedirect.com/science/article/pii/S0378517309000374> (accessed 14 October 2013).
80. Ma P, Dong X, Swadley CL, Gupte A, Leggas M, Ledebur HC, Mumper RJ. Development of idarubicin and doxorubicin solid lipid nanoparticles to overcome Pgp-mediated multiple drug resistance in leukemia. *Journal of biomedical nanotechnology* 2009;5(2): 151. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2805476/> (accessed 14 October 2013).
81. Uner M, Wissing SA, Yener G, Muller RH. Influence of surfactants on the physical stability of solid lipid nanoparticle (SLN) formulations. *Die Pharmazie-An International Journal of Pharmaceutical Sciences* 2004;59(4): 331-332. <http://www.ingentaconnect.com/content/-govi/pharmaz/2004/00000059/00000004/art00024> (accessed 14 October 2013).
82. Kristl J, Teskac K, Milek M, Mlinaric-Rascan I. Surface active stabilizer tyloxapol in colloidal dispersions exerts cytostatic effects and apoptotic dismissal of cells. *Toxicology and applied pharmacology* 2008;232(2): 218-225. <http://www.sciencedirect.com/science/article/pii/S0041008X08002627> (accessed 14 October 2013).
83. del Pozo-Rodríguez A, Delgado D, Solinís MÁ, Pedraz JL, Echevarría E, Rodríguez JM, Gascón AR. Solid lipid nanoparticles as potential tools for gene therapy: In vivo protein expression after intravenous administration. *International journal of pharmaceutics* 2010;385(1): 157-162. <http://www.sciencedirect.com/science/article/pii/S0378517309007297> (accessed 14 October 2013).
84. Joshi M, Patravale V. Nanostructured lipid carrier (NLC) based gel of celecoxib. *International journal of pharmaceutics* 2008;346(1): 124-132. <http://www.sciencedirect.com/science/article/pii/S0378517307004875> (accessed 14 October 2013).
85. Mandawgade SD, Patravale VB. Development of SLNs from natural lipids: application to topical delivery of tretinoin. *International journal of pharmaceutics* 2008;363(1): 132-138. <http://www.sciencedirect.com/science/article/pii/S0378517308004651> (accessed 14 October 2013).
86. Xie S, Wang S, Zhao B, Han C, Wang M, Zhou W. Effect of PLGA as a polymeric emulsifier on preparation of hydrophilic protein-loaded solid lipid nanoparticles. *Colloids and Surfaces B: Biointerfaces* 2008;67(2): 199-204. <http://www.sciencedirect.com/science/article/pii/S0927776508003032> (accessed 14 October 2013).

87. Sharma P, Ganta S, Denny WA, Garg S. Formulation and pharmacokinetics of lipid nanoparticles of a chemically sensitive nitrogen mustard derivative: Chlorambucil. *International Journal of Pharmaceutics* 2009;367(1): 187-194. <http://www.sciencedirect.com/science/-article/pii/S0378517308006571> (accessed 14 October 2013).
88. Aji Alex MR, Chacko AJ, Jose S, Souto EB. Lopinavir loaded solid lipid nanoparticles (SLN) for intestinal lymphatic targeting. *European Journal of Pharmaceutical Sciences* 2011;42(1): 11-18. <http://www.sciencedirect.com/science/article/pii/S0928098710003477> (accessed 14 October 2013).
89. Sznitowska M, Janicki S, Gajewska M, Kulik M. Investigation of diazepam lipospheres based on Witexol and lecithin intended for oral or rectal delivery. *Acta Pol Pharm* 2000;57(1): 61-64. http://www.ptfarm.pl/pub/File/wydawnictwa/acta_pol_2000/pdf-y%202000_1/061-064.pdf (accessed 14 October 2013).
90. Li H, Zhao X, Ma Y, Zhai G, Li L, Lou H. Enhancement of gastrointestinal absorption of quercetin by solid lipid nanoparticles. *Journal of Controlled Release* 2009;133(3): 238-244. <http://www.sciencedirect.com/science/article/pii/S0168365908006299> (accessed 14 October 2013).
91. Patel S, Chavhan S, Soni H, Babbar AK, Mathur R, Mishra AK, Sawant K. Brain targeting of risperidone-loaded solid lipid nanoparticles by intranasal route. *Journal of Drug Targeting* 2011;19(6): 468-474. <http://informahealthcare.com/doi/abs/10.3109/1061186X.2010.523787> (accessed 14 October 2013).
92. Ahlin P, Kristl J, Smid-Korbar J. Optimization of procedure parameters and physical stability of solid lipid nanoparticles in dispersions. *Acta pharmaceutica* 1998;48(4) 259-267.
93. Trotta M, Cavalli R, Trotta C, Bussano R, Costa L. Electro spray technique for solid lipid-based particle production. *Drug development and industrial pharmacy* 2010;36(4): 431-438. <http://informahealthcare.com/doi/abs/10.3109/03639040903241817> (accessed 14 October 2013).
94. Charcosset C, El-Harati A, Fessi H. Preparation of solid lipid nanoparticles using a membrane contactor. *Journal of controlled release* 2005;108(1): 112-120. <http://www.sciencedirect.com/science/article/pii/S0168365905003366> (accessed 14 October 2013).
95. Izquierdo P, Esquena J, Tadros TF, Dederen C, Garcia MJ, Azemar N, Solans C. Formation and stability of nano-emulsions prepared using the phase inversion temperature method. *Langmuir* 2002;18(1): 26-30. <http://pubs.acs.org/doi/abs/10.1021/la010808c> (accessed 14 October 2013).

96. Heurtault B, Saulnier P, Pech B, Proust JE, Benoit JP. A novel phase inversion-based process for the preparation of lipid nanocarriers. *Pharmaceutical research* 2002;19(6): 875-880. <http://link.springer.com/article/10.1023/A:1016121319668> (accessed 14 October 2013).
97. Carbone C, Tomasello B, Ruozi B, Renis M, Puglisi G. Preparation and optimization of PIT solid lipid nanoparticles via statistical factorial design. *European journal of medicinal chemistry* 2012;49: 110-117. <http://www.sciencedirect.com/science/article/pii/S0223523412000025> (accessed 14 October 2013).
98. Heurtault B, Saulnier P, Benoit JP, Proust JE, Pech B, Richard J. U.S. Patent No. 8,057,823. Washington, DC: U.S. Patent and Trademark Office, 2011. <http://www.google.bg/patents?hl=en&lr=&vid=USPAT8057823&id=Ckz9AQAABAJ&oi=fnd&dq=8,057,823&prints ec=abstract> (accessed 14 October 2013).
99. Li Z, Yu L, Zheng L, Geng F. Studies on crystallinity state of puerarin loaded solid lipid nanoparticles prepared by double emulsion method. *Journal of thermal analysis and calorimetry* 2010;99(2): 689-693. <http://www.akademai.com/index/N138607180635287.pdf> (accessed 14 October 2013).
100. Varshosaz J, Minayian M, Moazen E. Enhancement of oral bioavailability of pentoxifylline by solid lipid nanoparticles. *Journal of liposome research* 2010;20(2), 115-123. <http://informahealthcare.com/doi/abs/10.3109/08982100903161456> (accessed 14 October 2013).
101. Lopes R, Eleutério CV, Gonçalves LMD, Cruz MEM., Almeida AJ. Lipid nanoparticles containing oryzalin for the treatment of leishmaniasis. *European Journal of Pharmaceutical Sciences* 2012;45(4): 442-450. <http://www.sciencedirect.com/science/article/pii/S0928098711003216> (accessed 14 October 2013).
102. Urbán-Morlán Z, Ganem-Rondero A, Melgoza-Contreras LM, Escobar-Chávez JJ, Nava-Arzaluz MG, Quintanar-Guerrero D. Preparation and characterization of solid lipid nanoparticles containing cyclosporine by the emulsification-diffusion method. *International journal of nanomedicine* 2010;5: 611-620. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2939706/> (accessed 14 October 2013).
103. Wang T, Wang N, Zhang Y, Shen W, Gao X, Li T. Solvent injection-lyophilization of tert-butyl alcohol/water cosolvent systems for the preparation of drug-loaded solid lipid nanoparticles. *Colloids and Surfaces B: Biointerfaces* 2010;79(1): 254-261. <http://www.sciencedirect.com/science/-article/pii/S0927776510002080> (accessed 14 October 2013).
104. Campardelli R, Cherain M, Perfetti C, Iorio C, Scognamiglio M, Reverchon E, Porta GD. Lipid Nanoparticles production by supercritical fluid assisted emulsion-diffusion. *The Journal of Supercritical Fluids* 2013;82: 34-40. <http://www.sciencedirect.com/science/-article/pii/S0896844613002295> (accessed 14 October 2013).

105. Chen YJ, Jin RX, Zhou YQ, Zeng J, Zhang H, Feng QR. Preparation of solid lipid nanoparticles loaded with Xionggui powder-supercritical carbon dioxide fluid extraction and their evaluation in vitro release. *China journal of Chinese materia medica* 2006;31(5): 376-379. <http://www.ncbi.nlm.nih.gov/pubmed/16711418> (accessed 14 October 2013).
106. Mehnert W, Mäder K. Solid lipid nanoparticles Production, characterization and applications. *Advanced drug delivery reviews* 2012;64(S): 83-101. <http://www.sciencedirect.com/-science/article/pii/S0169409X12002815> (accessed 14 October 2013).
107. Stoye I, Schröder K, Müller-Goymann CC. 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. *European journal of pharmaceutics and biopharmaceutics* 1998;46(2): 191-200. <http://www.sciencedirect.com/science/article/pii/S093964119800023X> (accessed 14 October 2013).
108. Andrews S, Nover D, Schladow S. Using laser diffraction data to obtain accurate particle size distributions: the role of particle composition. *Limnology and Oceanography: Methods* 2010;8: 507-526. <https://www.aslo.org/lomethods/free/2010/0507.html> (accessed 14 October 2013).
109. Merkus HG. Particle size measurements: fundamentals, practice, quality (Vol. 17). Springer; 2009.
110. Liu J, Gong T, Wang C, Zhong Z, Zhang Z. Solid lipid nanoparticles loaded with insulin by sodium cholate-phosphatidylcholine-based mixed micelles: preparation and characterization. *International journal of pharmaceutics* 2007; 340(1): 153-162. <http://www.sciencedirect.com/-science/article/pii/S0378517307002426> (accessed 14 October 2013).
111. Kumar S, Randhawa JK. High melting lipid based approach for drug delivery: Solid lipid nanoparticles. *Materials Science and Engineering: C* 2013;33(4): 1842-1852. <http://www.sciencedirect.com/science/article/pii/S0928493113000507> (accessed 14 October 2013).
112. Dubes A, Parrot-Lopez H, Abdelwahed W, Degobert G, Fessi H, Shahgaldian P, Coleman AW. Scanning electron microscopy and atomic force microscopy imaging of solid lipid nanoparticles derived from amphiphilic cyclodextrins. *European journal of pharmaceutics and biopharmaceutics* 2003;55(3): 279-282. <http://www.sciencedirect.com/science/article/pii/S0939641103000201> (accessed 14 October 2013).
113. Zur Mühlen A, Zur Mühlen E, Niehus H, Mehnert W. Atomic force microscopy studies of solid lipid nanoparticles. *Pharmaceutical research* 1996;13(9): 1411-1416. <http://link.springer.com/article/10.1023/A:1016042504830> (accessed 14 October 2013).

114. Unruh T, Bunjes H, Westesen K, Koch MH. Observation of size-dependent melting in lipid nanoparticles. *The Journal of Physical Chemistry B* 1999;103(47): 10373-10377. <http://pubs.acs.org/doi/abs/10.1021/jp9912612> (accessed 14 October 2013).
115. Saupé A, Gordon KC, Rades T. Structural investigations on nanoemulsions, solid lipid nanoparticles and nanostructured lipid carriers by cryo-field emission scanning electron microscopy and Raman spectroscopy. *International journal of pharmaceutics* 2006;314(1): 56-62. <http://www.sciencedirect.com/science/article/pii/S0378517306000639> (accessed 14 October 2013).
116. Müller RH. Zetapotential und Partikelladung in der Laborpraxis: Einführung in die Theorie, praktische Messdurchführung. Dateninterpretation. Stuttgart: Wissenschaftliche Verlagsgesellschaft mbH; 1996.
117. Li J, Guo X, Liu Z, Okeke CI, Li N, Zhao H, Wu T. Preparation and evaluation of charged solid lipid nanoparticles of tetrandrine for ocular drug delivery system: pharmacokinetics, cytotoxicity and cellular uptake studies. *Drug development and industrial pharmacy* 2013; (0): 1-8. <http://informahealthcare.com/doi/abs/10.3109/03639045.2013.795582> (accessed 14 October 2013).
118. Ye J, Wang A, Liu C, Chen Z, Zhang N. Anionic solid lipid nanoparticles supported on protamine/DNA complexes. *Nanotechnology* 2008;19 (28): 285708. <http://iopscience.iop.org/0957-4484/19/28/285708> (accessed 14 October 2013).
119. Lim SJ, Kim CK. Formulation parameters determining the physicochemical characteristics of solid lipid nanoparticles loaded with all-trans retinoic acid. *International journal of pharmaceutics* 2002;243(1): 135-146. <http://www.sciencedirect.com/science/article/pii/S0378517302002697> (accessed 14 October 2013).
120. Westesen K, Bunjes H. Do nanoparticles prepared from lipids solid at room temperature always possess a solid lipid matrix?. *International journal of pharmaceutics* 1995;115(1): 129-131. <http://www.sciencedirect.com/science/article/pii/S0378517394003478> (accessed 14 October 2013).
121. Bunjes H, Siekmann B, Westesen K. (1998). 7. Emulsions of supercooled melts – a novel drug delivery system. In: Benita S (ed.) *Submicron emulsions in drug targeting and delivery*. CRC Press; 1998. p175.
122. Höhne G, Hemminger WF, Flammersheim HJ. *Differential scanning calorimetry*. Springer; 2003.
123. Almeida EDP, Costa AA, Serafini MR, Rossetti FC, Marchetti JM, Sarmiento VHV, Lira AAM. Preparation and characterization of chloroaluminum phthalocyanine-loaded solid lipid nanoparticles by thermal analysis and powder X-ray diffraction techniques. *Journal of*

- thermal analysis and calorimetry 2012;108(1): 191-196. <http://www.akademai.com/index/-35620R77470213U7.pdf> (accessed 14 October 2013).
124. Martins SM, Sarmiento B, Nunes C, Lúcio M, Reis S, Ferreira DC. Brain targeting effect of camptothecin-loaded solid lipid nanoparticles in rat after intravenous administration. *European Journal of Pharmaceutics and Biopharmaceutics* 2013; In press. <http://www.sciencedirect.com/-science/article/pii/S0939641113002907> (accessed 14 October 2013).
125. Noack A, Hause G, Mäder K. Physicochemical characterization of curcuminoid-loaded solid lipid nanoparticles. *International Journal of Pharmaceutics* 2012;423(2): 440-451. <http://www.sciencedirect.com/science/article/pii/S0378517311011379> (accessed 14 October 2013).
126. Okonogi S, Riangjanapatee P. Potential technique for tiny crystalline detection in lycopene-loaded SLN and NLC development. *Drug development and industrial pharmacy* 2013;(0): 1-8. <http://informahealthcare.com/doi/abs/10.3109/03639045.2013.828215> (accessed 14 October 2013).
127. Westesen K, Bunjes H, Koch MHJ. Physicochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential. *Journal of Controlled Release* 1997;48(2): 223-236. <http://www.sciencedirect.com/science/-article/pii/S0168365997000461>(accessed 14 October 2013).
128. Zimmermann E, Souto EB, Muller RH. Physicochemical investigations on the structure of drug-free and drug-loaded solid lipid nanoparticles (SLNTM) by means of DSC and ¹H NMR. *Die Pharmazie-An International Journal of Pharmaceutical Sciences* 2005;60(7): 508-513. <http://www.ingentaconnect.com/content/govi/pharmaz/2005/00000060/00000007/art00005> (accessed 14 October 2013).
129. Jores K, Haberland A, Wartewig S, Mäder K, Mehnert W. Solid lipid nanoparticles (SLN) and oil-loaded SLN studied by spectrofluorometry and Raman spectroscopy. *Pharmaceutical research* 2005;22(11): 1887-1897. <http://link.springer.com/article/10.1007/s11095-005-7148-5>(accessed 14 October 2013).
130. Jores K, Mehnert W, Mäder K. Physicochemical investigations on solid lipid nanoparticles and on oil-loaded solid lipid nanoparticles: a nuclear magnetic resonance and electron spin resonance study. *Pharmaceutical research* 2003;20(8): 1274-1283. <http://link.springer.com/-article/10.1023/A:1025065418309> (accessed 14 October 2013).
131. Yassin AEB, Anwer MK, Mowafy HA, El-Bagory IM, Bayomi MA, Alsarra IA. Optimization of 5-fluorouracil solid-lipid nanoparticles: a preliminary study to treat colon cancer. *International journal of medical sciences* 2010;7(6): 398-408. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2990076/> (accessed 14 October 2013).

132. Ren J, Zou M, Gao P, Wang Y, Cheng G. Tissue distribution of borneol-modified ganciclovir-loaded solid lipid nanoparticles in mice after intravenous administration. *European Journal of Pharmaceutics and Biopharmaceutics* 2012;83(2): 141-148. <http://www.sciencedirect.com/-science/article/pii/S093964111200344X> (accessed 14 October 2013).
133. Lukowski G, Pfliegel P. Electron diffraction of solid lipid nanoparticles loaded with aciclovir. *Pharmazie* 1997;52(8): 642-643.
134. zur Mühlen A, Schwarz C, Mehnert W. Solid lipid nanoparticles (SLN) for controlled drug delivery—drug release and release mechanism. *European journal of pharmaceutics and biopharmaceutics* 1998;45(2): 149-155. <http://www.sciencedirect.com/science/article/pii/S0939641197001501> (accessed 14 October 2013).
135. Silva AC, Lopes CM, Fonseca J, Soares ME, Santos D, Souto EB, Ferreira D. Risperidone release from solid lipid nanoparticles (SLN): validated HPLC method and modelling kinetic profile. *Current Pharmaceutical Analysis* 2012;8(4): 307-316. <http://www.ingentaconnect.com/-content/ben/cpa/2012/00000008/00000004/art00001> (accessed 14 October 2013).
136. Ying XY, Cui D, Yu L, Du YZ. Solid lipid nanoparticles modified with chitosan oligosaccharides for the controlled release of doxorubicin. *Carbohydrate Polymers* 2011;84(4): 1357-1364. <http://www.sciencedirect.com/science/article/pii/S0144861711000701> (accessed 14 October 2013).
137. Aditya NP, Shim M, Lee I, Lee Y, Im MH, Ko S. Curcumin and genistein coloaded nanostructured lipid carriers: In vitro digestion and antiprostata cancer activity. *Journal of agricultural and food chemistry* 2013;61(8): 1878-1883. <http://pubs.acs.org/doi/full/10.1021/jf305143k> (accessed 14 October 2013).
138. Waraho T, McClements DJ, Decker EA. Mechanisms of lipid oxidation in food dispersions. *Trends in Food Science & Technology* 2011;22(1): 3-13. <http://www.sciencedirect.com/science/article/pii/S092422441000261X> (accessed 14 October 2013).
139. McClements DJ, Decker EA. Lipid Oxidation in Oil-in-Water Emulsions: Impact of Molecular Environment on Chemical Reactions in Heterogeneous Food Systems. *Journal of Food Science* 2000;65(8): 1270-1282. <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2621.2000.tb10596.x/abstract> (accessed 14 October 2013).
140. Dimakou CP, Kiokias SN, Tsaprouni IV, Oreopoulou V. Effect of processing and storage parameters on the oxidative deterioration of oil-in-water emulsions. *Food Biophysics* 2007; 2(1): 38-45. <http://link.springer.com/article/10.1007/s11483-007-9027-6> (accessed 14 October 2013).

141. Nuchi CD, McClements DJ, Decker EA. Impact of Tween 20 hydroperoxides and iron on the oxidation of methyl linoleate and salmon oil dispersions. *Journal of agricultural and food chemistry* 2001;49(10): 4912-4916. <http://pubs.acs.org/doi/abs/10.1021/jf010370m> (accessed 14 October 2013).
142. Waraho T, Cardenia V, Rodriguez-Estrada MT, McClements DJ, Decker EA. Prooxidant mechanisms of free fatty acids in stripped soybean oil-in-water emulsions. *Journal of agricultural and food chemistry* 2009;57(15): 7112-7117. <http://pubs.acs.org/doi/abs/10.1021/jf901270m> (accessed 14 October 2013).
143. McClements DJ, Decker EA, Weiss J. Emulsion-based delivery systems for lipophilic bioactive components. *Journal of Food Science* 2007;72(8): R109-R124. <http://onlinelibrary.wiley.com/doi/10.1111/j.1750-3841.2007.00507.x/full> (accessed 14 October 2013).
144. Radomska-Soukharev A. Stability of lipid excipients in solid lipid nanoparticles. *Advanced drug delivery reviews* 2007;59(6): 411-418. <http://www.sciencedirect.com/science/article/pii/S0169409X07000385> (accessed 14 October 2013).
145. Garti N, Sato K. *Crystallization and polymorphism of fats and fatty acids* (Vol. 31). Marcel Dekker Inc.; 1988.
146. Westesen K, Siekmann B. Investigation of the gel formation of phospholipid-stabilized solid lipid nanoparticles. *International journal of pharmaceutics* 1997;151(1): 35-45. <http://www.sciencedirect.com/science/article/pii/S0378517397048904> (accessed 14 October 2013).
147. Freitas C, Müller RH. Correlation between long-term stability of solid lipid nanoparticles (SLN™) and crystallinity of the lipid phase. *European journal of pharmaceutics and biopharmaceutics* 1999;47(2): 125-132. <http://www.sciencedirect.com/science/article/pii/S0939641198000745> (accessed 14 October 2013).
148. Yoshino H, Kobayashi M, Samejima M. Influence of the fatty acid composition on the properties and polymorphic transition of fatty suppository bases, *Chem.Pharm. Bull.* 1983;31(1): 237-246.
149. Jenning V, Schäfer-Korting M, Gohla S. Vitamin A-loaded solid lipid nanoparticles for topical use: drug release properties. *Journal of Controlled Release* 2000;66(2): 115-126. <http://www.sciencedirect.com/science/article/pii/S0168365999002230> (accessed 14 October 2013).
150. Awad TS, Helgason T, Kristbergsson K, Decker EA, Weiss J, McClements DJ. Effect of cooling and heating rates on polymorphic transformations and gelation of tripalmitin solid lipid nanoparticle (SLN) suspensions. *Food Biophysics* 2008;3(2): 155-162. <http://link.springer.com/article/10.1007/s11483-008-9057-8> (accessed 14 October 2013).

151. Freitas C, Müller RH. Effect of light and temperature on zeta potential and physical stability in solid lipid nanoparticle (SLN™) dispersions. *International journal of pharmaceutics* 1998;168(2): 221-229. <http://www.sciencedirect.com/science/article/pii/S0378517398000921> (accessed 14 October 2013).
152. Choi KO, Aditya NP, Ko S. Effect of aqueous pH and electrolyte concentration on structure, stability and flow behaviour of non-ionic surfactant based solid lipid nanoparticles. *Food Chemistry* 2013; In press. <http://www.sciencedirect.com/science/article/pii/S0308814613013605> (accessed 14 October 2013).
153. Schwarz C, Mehnert W, Lucks JS, Müller RH. Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization. *Journal of Controlled Release* 1994;30(1): 83-96. <http://www.sciencedirect.com/science/article/pii/0168365994900477> (accessed 14 October 2013).
154. Basaran E, Demirel M, Sirmagül B, Yazan Y. Cyclosporine-A incorporated cationic solid lipid nanoparticles for ocular delivery. *Journal of microencapsulation* 2010;27(1): 37-47. <http://informahealthcare.com/doi/abs/10.3109/02652040902846883> (accessed 14 October 2013).
155. Sculier JP, Coune A, Brassinne C, Laduron C, Atassi G, Ruyschaert JM, Frühling J. Intravenous infusion of high doses of liposomes containing NSC 251635, a water-insoluble cytostatic agent. A pilot study with pharmacokinetic data. *Journal of clinical oncology* 1986;4(5): 789-797. <http://jco.ascopubs.org/content/4/5/789.short> (accessed 14 October 2013).
156. del Pozo-Rodríguez A, Solinís MA, Gascón AR, Pedraz JL. Short-and long-term stability study of lyophilized solid lipid nanoparticles for gene therapy. *European Journal of Pharmaceutics and Biopharmaceutics* 2009;71(2): 181-189. <http://www.sciencedirect.com/science/-article/pii/S0939641108003779> (accessed 14 October 2013).
157. Cavalli R, Caputo O, Carlotti ME, Trotta M, Scarnecchia C, Gasco MR. Sterilization and freeze-drying of drug-free and drug-loaded solid lipid nanoparticles. *International journal of pharmaceutics* 1997;148(1): 47-54. <http://www.sciencedirect.com/science/article/pii/S0378517396048223> (accessed 14 October 2013).
158. Schwarz C, Mehnert W. Freeze-drying of drug-free and drug-loaded solid lipid nanoparticles (SLN). *International journal of pharmaceutics* 1997;157(2): 171-179. <http://www.sciencedirect.com/science/article/pii/S0378517397002226> (accessed 14 October 2013).

159. Ohshima H, Miyagishima A, Kurita T, Makino Y, Iwao Y, Sonobe T, Itai S. Freeze-dried nifedipine-lipid nanoparticles with long-term nano-dispersion stability after reconstitution. *International journal of pharmaceutics* 2009;377(1): 180-184. <http://www.sciencedirect.com/science/article/pii/S0378517309002907> (accessed 14 October 2013).
160. Freitas C, Müller RH. Spray-drying of solid lipid nanoparticles (SLN). *European journal of pharmaceutics and biopharmaceutics* 1998;46(2): 145-151. <http://www.sciencedirect.com/-science/article/pii/S0939641197001720> (accessed 14 October 2013).
161. Souto EB, Wissing SA, Barbosa CM, Müller RH. Evaluation of the physical stability of SLN and NLC before and after incorporation into hydrogel formulations. *European journal of pharmaceutics and biopharmaceutics* 2004;58(1): 83-90. <http://www.sciencedirect.com/-science/article/pii/S093964110400061X> (accessed 14 October 2013).
162. Wissing SA, Kayser O, Müller RH. Solid lipid nanoparticles for parenteral drug delivery. *Advanced drug delivery reviews* 2004;56(9): 1257-1272. <http://www.sciencedirect.com/-science/article/pii/S0169409X04000456> (accessed 14 October 2013).
163. Müller RH, Mäder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery—a review of the state of the art. *European journal of pharmaceutics and biopharmaceutics* 2000;50(1): 161-177. <http://www.sciencedirect.com/science/article/pii/S0939641100000874> (accessed 14 October 2013).
164. Miglietta A, Cavalli R, Bocca C, Gabriel L, Rosa Gasco M. Cellular uptake and cytotoxicity of solid lipid nanospheres (SLN) incorporating doxorubicin or paclitaxel. *International journal of pharmaceutics* 2000;210(1): 61-67. <http://www.sciencedirect.com/science/article/pii/S0378517300005627> (accessed 14 October 2013).
165. Yuan H, Miao J, Du YZ, You J, Hu FQ, Zeng S. Cellular uptake of solid lipid nanoparticles and cytotoxicity of encapsulated paclitaxel in A549 cancer cells. *International journal of pharmaceutics* 2008;348(1): 137-145. <http://www.sciencedirect.com/science/article/pii/S0378517307005960> (accessed 14 October 2013).
166. Müller RH, Maaßen S, Weyhers H, Specht F, Lucks JS. Cytotoxicity of magnetite-loaded polylactide, polylactide/glycolide particles and solid lipid nanoparticles. *International journal of pharmaceutics* 1996;138(1): 85-94. <http://www.sciencedirect.com/science/article/pii/S0378517396045395> (accessed 14 October 2013).
167. Müller RH, Olbrich C. Solid lipid nanoparticles: phagocytic uptake, in vitro cytotoxicity and in vitro biodegradation: 2nd communication. *Pharmazeutische Industrie* 1999;61(6): 564-569. <http://cat.inist.fr/?aModele=afficheN&cpsid=1890462> (accessed 14 October 2013).

168. Olbrich C, Kayser O, Müller RH. Enzymatic degradation of Dynasan 114 SLN—effect of surfactants and particle size. *Journal of Nanoparticle Research* 2002;4(1-2): 121-129. <http://link.springer.com/article/10.1023/A:1020159331420> (accessed 14 October 2013).
169. Dong X, Mumper RJ. The metabolism of fatty alcohols in lipid nanoparticles by alcohol dehydrogenase. *Drug development and industrial pharmacy* 2006;32(8): 973-980. <http://informahealthcare.com/doi/abs/10.1080/03639040600640006> (accessed 14 October 2013).
170. Borgström B, Donnér J. The polar interactions between pancreatic lipase, colipase and the triglyceride substrate. *FEBS letters* 1977;83(1) 23-26.
171. Müller RH, Rühl D, Runge SA. Biodegradation of solid lipid nanoparticles as a function of lipase incubation time. *International journal of pharmaceutics* 1996;144(1): 115-121. <http://www.sciencedirect.com/science/article/pii/S037851739604731X> (accessed 14 October 2013).
172. Olbrich C, Mehnert W, Müller RH. In vitro degradation properties of solid lipid nanoparticles. In: *Proc. 2nd World Meeting APGI/APV 1998, Paris; 1998.*
173. Cavalli R, Bocca C, Miglietta A, Caputo O, Gasco MR. Albumin adsorption on stealth and non-stealth solid lipid nanoparticles. *STP pharma sciences* 1999;9(2): 183-189. <http://cat.inist.fr/?aModele=afficheN&cpsidt=1302192> (accessed 14 October 2013).
174. Goppert TM, Muller RH. Plasma protein adsorption of Tween 80-and poloxamer 188-stabilized solid lipid nanoparticles. *Journal of drug targeting* 2003;11(4): 225-231. <http://www.ingentaconnect.com/content/apl/gdrt/2003/00000011/00000004/art00004> (accessed 14 October 2013).
175. Schöler N, Olbrich C, Tabatt K, Müller RH, Hahn H, Liesenfeld O. Surfactant, but not the size of solid lipid nanoparticles (SLN) influences viability and cytokine production of macrophages. *International journal of pharmaceutics* 2001;221(1), 57-67. <http://www.sciencedirect.com/science/article/pii/S0378517301006603> (accessed 14 October 2013).
176. Joshi MD, Müller RH. Lipid nanoparticles for parenteral delivery of actives. *European Journal of Pharmaceutics and Biopharmaceutics* 2009;71(2): 161-172. <http://www.sciencedirect.com/science/article/pii/S0939641108003391> (accessed 14 October 2013).
177. Yang SC, Lu LF, Cai Y, Zhu JB, Liang BW, Yang CZ. Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. *Journal of controlled release* 1999;59(3): 299-307. <http://www.sciencedirect.com/science/article/pii/S0168365999000073> (accessed 14 October 2013).

178. Pignatello R, Leonardi A, Pellitteri R, Carbone C, Caggia S, Graziano ACE, Cardile, V. Evaluation of new amphiphilic PEG derivatives for preparing stealth lipid nanoparticles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 2013;434: 136-144. <http://www.sciencedirect.com/science/article/pii/S0927775713004421> (accessed 14 October 2013).
179. Xiang QY, Wang MT, Chen F, Gong T, Jian YL, Zhang ZR, Huang Y. Lung-targeting delivery of dexamethasone acetate loaded solid lipid nanoparticles. *Archives of pharmacal research* 2007;30(4): 519-525. <http://link.springer.com/article/10.1007/BF02980228> (accessed 14 October 2013).
180. Luo CF, Yuan M, Chen MS, Liu SM, Zhu L, Huang BY, Xiong W. Pharmacokinetics, tissue distribution and relative bioavailability of puerarin solid lipid nanoparticles following oral administration. *International journal of pharmaceutics* 2011;410(1): 138-144. <http://www.sciencedirect.com/science/article/pii/S0378517311001992> (accessed 14 October 2013).
181. Bondi ML, Craparo EF, Giammona G, Drago F. Brain-targeted solid lipid nanoparticles containing riluzole: preparation, characterization and biodistribution. *Nanomedicine* 2010;5(1): 25-32. <http://www.futuremedicine.com/doi/abs/10.2217/nnm.09.67> (accessed 14 October 2013).
182. Xu Z, Chen L, Gu W, Gao Y, Lin L, Zhang Z, Li, Y. The performance of docetaxel-loaded solid lipid nanoparticles targeted to hepatocellular carcinoma. *Biomaterials* 2009;30(2): 226-232. <http://www.sciencedirect.com/science/article/pii/S0142961208006856> (accessed 14 October 2013).
183. Jia L, Zhang D, Li Z, Duan C, Wang Y, Feng F, Zhang Q. Nanostructured lipid carriers for parenteral delivery of silybin: Biodistribution and pharmacokinetic studies. *Colloids and Surfaces B: Biointerfaces* 2010;80(2): 213-218. <http://www.sciencedirect.com/science/article/pii/S0927776510003255> (accessed 14 October 2013).
184. Mosallaei N, Jaafari MR, Hanafi-Bojd MY, Golmohammadzadeh S, Malaek-Nikouei B. Docetaxel-loaded solid lipid nanoparticles: Preparation, characterization, in vitro, and in vivo evaluations. *Journal of pharmaceutical sciences* 2013;102(6): 1994-2004. <http://onlinelibrary.wiley.com/doi/10.1002/jps.23522/full> (accessed 14 October 2013).
185. Nayak AP, Tiyaaboonchai W, Patankar S, Madhusudhan B, Souto EB. Curcuminoids-loaded lipid nanoparticles: novel approach towards malaria treatment. *Colloids and Surfaces B: Biointerfaces* 2010;81(1): 263-273. <http://www.sciencedirect.com/science/article/pii/S092777651000384X> (accessed 14 October 2013).
186. Patil S, Joshi M, Pathak S, Sharma S, Patravale V. Intravenous β -artemether formulation (ARM NLC) as a superior alternative to commercial artesunate formulation. *Journal of*

- Antimicrobial Chemotherapy 2012;67(11): 2713-2716.
<http://jac.oxfordjournals.org/content/67/11/2713.short> (accessed 14 October 2013).
187. Tsai MJ, Huang YB, Wu PC, Fu YS, Kao YR, Fang JY, Tsai YH. Oral apomorphine delivery from solid lipid nanoparticles with different monostearate emulsifiers: Pharmacokinetic and behavioral evaluations. *Journal of pharmaceutical sciences* 2011;100(2): 547-557.
<http://onlinelibrary.wiley.com/doi/10.1002/jps.22285/full> (accessed 14 October 2013).
188. Yuan H, Chen J, Du YZ, Hu FQ, Zeng S, Zhao HL. Studies on oral absorption of stearic acid SLN by a novel fluorometric method. *Colloids and Surfaces B: Biointerfaces* 2007;58(2): 157-164.
<http://www.sciencedirect.com/science/article/pii/S0927776507001166> (accessed 14 October 2013).
189. He J, Hou SX, Feng JF, Cai BQ. Effect of particle size on oral absorption of silymarin-loaded solid lipid nanoparticles. *China journal of Chinese materia medica* 2005;30(21): 1651-1653.
<http://europepmc.org/abstract/MED/16400939> (accessed 14 October 2013).
190. Harde H, Das M, Jain S. Solid lipid nanoparticles: an oral bioavailability enhancer vehicle. *Expert Opinion on Drug Delivery* 2011;8(11): 1407-1424.
<http://informahealthcare.com/-doi/abs/10.1517/17425247.2011.604311> (accessed 14 October 2013).
191. Aungst BJ. Intestinal permeation enhancers. *Journal of pharmaceutical sciences* 2000;89(4): 429-442.
[http://onlinelibrary.wiley.com/doi/10.1002/\(SICI\)15206017\(200004\)89:4%3C429::AID-JPS1%3E3.0.CO;2-J/full](http://onlinelibrary.wiley.com/doi/10.1002/(SICI)15206017(200004)89:4%3C429::AID-JPS1%3E3.0.CO;2-J/full) (accessed 14 October 2013).
192. Basu B, Garala K, Bhalodia R, Joshi B, Mehta K. Solid lipid nanoparticles: a promising tool for drug delivery system. *J Pharm Res* 2010;3: 84-92.
193. Almeida AJ, Souto E. Solid lipid nanoparticles as a drug delivery system for peptides and proteins. *Advanced drug delivery reviews* 2007; 59(6): 478-490.
<http://www.sciencedirect.com/-science/article/pii/S0169409X07000439> (accessed 14 October 2013).
194. Zhang N, Ping Q, Huang G, Xu W, Cheng Y, Han X. Lectin-modified solid lipid nanoparticles as carriers for oral administration of insulin. *International journal of pharmaceutics* 2006;327(1): 153-159.
<http://www.sciencedirect.com/science/article/pii/S0378517306006016> (accessed 14 October 2013).
195. Cavalli R, Gasco MR, Chetoni P, Burgalassi S, Saettone MF. Solid lipid nanoparticles (SLN) as ocular delivery system for tobramycin. *International journal of pharmaceutics* 2002;238(1): 241-245.
<http://www.sciencedirect.com/science/article/pii/S0378517302000807> (accessed 14 October 2013).

196. Shen J, Deng Y, Jin X, Ping Q, Su Z, Li L. Thiolated nanostructured lipid carriers as a potential ocular drug delivery system for cyclosporine A: Improving in vivo ocular distribution. *International journal of pharmaceutics* 2010;402(1): 248-253. <http://www.sciencedirect.com/-science/article/pii/S0378517310007702> (accessed 14 October 2013).
197. Attama AA, Reichl S, Müller-Goymann CC. Sustained release and permeation of timolol from surface-modified solid lipid nanoparticles through bioengineered human cornea. *Current Eye Research* 2009; 34(8): 698-705. <http://informahealthcare.com/doi/abs/10.1080/-02713680903017500> (accessed 14 October 2013).
198. Pardeike J, Hommoss A, Müller RH. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. *International journal of pharmaceutics* 2009;366(1): 170-184. <http://www.sciencedirect.com/science/article/pii/S0378517308006996> (accessed 14 October 2013).
199. Štecová J, Mehnert W, Blaschke T, Kleuser B, Sivaramakrishnan R, Zouboulis CC, Schäfer-Korting M. Cyproterone acetate loading to lipid nanoparticles for topical acne treatment: particle characterisation and skin uptake. *Pharmaceutical research* 2007;24(5): 991-1000. <http://link.springer.com/article/10.1007/s11095-006-9225-9> (accessed 14 October 2013).
200. Lin YK, Huang ZR, Zhuo RZ, Fang JY. Combination of calcipotriol and methotrexate in nanostructured lipid carriers for topical delivery. *International journal of nanomedicine* 2010;5: 117-128. <http://www.ncbi.nlm.nih.gov/pmc/articles/pmc2841490/> (accessed 14 October 2013).
201. Obeidat WM. Investigation of temperature-induced physical instability of preserved coenzyme Q10-loaded (NLC): A comparative study at different temperatures. *African Journal of Pharmacy and Pharmacology* 2012;6(32): 2413-2423. <http://www.academicjournals.org/-AJpp/PDF/pdf2012/29Aug/Obeidat.pdf> (accessed 14 October 2013).
202. Cirri M, Bragagni M, Mennini N, Mura P. Development of a new delivery system consisting in “drug-in cyclodextrin-in nanostructured lipid carriers” for ketoprofen topical delivery. *European Journal of Pharmaceutics and Biopharmaceutics* 2012;80(1): 46-53. <http://www.sciencedirect.com/science/article/pii/S0939641111002219> (accessed 14 October 2013).
203. Dingler A, Blum RP, Niehus H, Muller RH, Gohla S. Solid lipid nanoparticles (SLNTM/LipopearlTM) a pharmaceutical and cosmetic carrier for the application of vitamin E in dermal products. *Journal of microencapsulation* 1999;16(6): 751-767. <http://informahealthcare.com/doi/abs/10.1080/026520499288690> (accessed 14 October 2013).

204. Chen H, Chang X, Du D, Liu W, Liu J, Weng T, Yang X. Podophyllotoxin-loaded solid lipid nanoparticles for epidermal targeting. *Journal of controlled release* 2006;110(2): 296-306. <http://www.sciencedirect.com/science/article/pii/S0168365905005201> (accessed 14 October 2013).
205. Castro GA, Coelho ALL, Oliveira CA, Mahecha GA, Oréface RL, Ferreira LA. Formation of ion pairing as an alternative to improve encapsulation and stability and to reduce skin irritation of retinoic acid loaded in solid lipid nanoparticles. *International journal of pharmaceuticals* 2009;381(1): 77-83. <http://www.sciencedirect.com/science/article/pii/S0378517309004979> (accessed 14 October 2013).
206. Puglia C, Blasi P, Rizza L, Schoubben A, Bonina F, Rossi C, Ricci M. Lipid nanoparticles for prolonged topical delivery: An in vitro and in vivo investigation. *International journal of pharmaceuticals* 2008;357(1): 295-304. <http://www.sciencedirect.com/science/article/pii/S0378517308000951> (accessed 14 October 2013).
207. Junyaprasert VB, Teeranachaideekul V, Souto EB, Boonme P, Müller RH. Q10-loaded NLC versus nanoemulsions: Stability, rheology and in vitro skin permeation. *International journal of pharmaceuticals* 2009;377(1): 207-214. <http://www.sciencedirect.com/science/article/pii/S0378517309003044> (accessed 14 October 2013).
208. Pardeike J, Schwabe K, Müller RH. Influence of nanostructured lipid carriers (NLC) on the physical properties of the Cutanova Nanorepair Q10 cream and the in vivo skin hydration effect. *International journal of pharmaceuticals* 2010;396(1): 166-173. <http://www.sciencedirect.com/science/article/pii/S0378517310004151> (accessed 14 October 2013).
209. Pilcer G, Amighi K. Formulation strategy and use of excipients in pulmonary drug delivery. *International journal of pharmaceuticals* 2010;392(1): 1-19. <http://www.sciencedirect.com/science/article/pii/S0378517310001912> (accessed 14 October 2013).
210. Dharmala K, Yoo JW, Lee CH. Development of Chitosan-SLN Microparticles for chemotherapy: In vitro approach through efflux-transporter modulation. *Journal of Controlled Release* 2008;131(3): 190-197. <http://www.sciencedirect.com/science/article/pii/S0168365908004215> (accessed 14 October 2013).
211. Jaafar-Maalej C, Andrieu V, Elaissari A, Fessi H. Beclomethasone-loaded lipidic nanocarriers for pulmonary drug delivery: preparation, characterization and in vitro drug release. *Journal of Nanoscience and Nanotechnology* 2011;11(3): 1841-1851. <http://www.ingentaconnect.com/content/asp/jnn/2011/00000011/00000003/art00003> (accessed 14 October 2013).

212. Li YZ, Sun X, Gong T, Liu J, Zuo J, Zhang ZR. Inhalable microparticles as carriers for pulmonary delivery of thymopentin-loaded solid lipid nanoparticles. *Pharmaceutical research* 2010;27(9): 1977-1986. <http://link.springer.com/article/10.1007/s11095-010-0201-z> (accessed 14 October 2013).
213. Liu J, Gong T, Fu H, Wang C, Wang X, Chen Q, Zhang Z. Solid lipid nanoparticles for pulmonary delivery of insulin. *International Journal of Pharmaceutics* 2008;356(1): 333-344. <http://www.sciencedirect.com/science/article/pii/S0378517308000215> (accessed 14 October 2013).
214. Pandey R, Khuller GK. Solid lipid particle-based inhalable sustained drug delivery system against experimental tuberculosis. *Tuberculosis* 2005;85(4): 227-234. <http://www.sciencedirect.com/-science/article/pii/S1472979205000119> (accessed 14 October 2013).
215. Joshi AS, Patel HS, Belgamwar VS, Agrawal A, Tekade AR. Solid lipid nanoparticles of ondansetron HCl for intranasal delivery: development, optimization and evaluation. *Journal of Materials Science: Materials in Medicine* 2012;23(9): 2163-2175. <http://link.springer.com/article/10.1007/s10856-012-4702-7> (accessed 14 October 2013).
216. Chavan SS, Ingle SG, Vavia PR. Preparation and characterization of solid lipid nanoparticle-based nasal spray of budesonide. *Drug Delivery and Translational Research* 2012;3(5): 402-408. <http://link.springer.com/article/10.1007/s13346-012-0105-z> (accessed 14 October 2013).
217. Pardeshi CV, Rajput PV, Belgamwar VS, Tekade AR, Surana SJ. Novel surface modified solid lipid nanoparticles as intranasal carriers for ropinirole hydrochloride: application of factorial design approach. *Drug delivery* 2013;20(1): 47-56. <http://informahealthcare.com/doi/abs/-10.3109/10717544.2012.752421> (accessed 14 October 2013).
218. Singh AP, Saraf SK, Saraf SA. SLN approach for nose-to-brain delivery of alprazolam. *Drug Delivery and Translational Research* 2012;2(6): 498-507. <http://link.springer.com/article/10.1007/s13346-012-0110-2> (accessed 14 October 2013).
219. Vighi E, Montanari M, Ruozi B, Iannuccelli V, Leo E. The role of protamine amount in the transfection performance of cationic SLN designed as a gene nanocarrier. *Drug Delivery* 2012;19(1): 1-10. <http://informahealthcare.com/doi/abs/10.3109/10717544.2011.621989> (accessed 14 October 2013).
220. Vighi E, Montanari M, Hanuskova M, Iannuccelli V, Coppi G, Leo E. Design flexibility influencing the in vitro behavior of cationic SLN as a nonviral gene vector. *International Journal of Pharmaceutics* 2012;440(2): 161-169. <http://www.sciencedirect.com/science/article/pii/S0378517-312008630> (accessed 14 October 2013).