Chapter 5
Solid Lipid Nanoparticles (SLN™)

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Abstract Solid lipid nanoparticles (SLN™) are a new generation of drug delivery systems being exploited for several drugs since the nineties. These particles can be composed of different types of solid lipids, such as glycerides, waxes, and fatty acids, and stabilized by a wide range of surfactants. In the present chapter, the chemical structure, production methodology, and physicochemical characterization are systematically discussed. Parameters such as particle size, distribution, polymorphic behaviour, and crystallization are required to characterize SLN and may predict their in vitro stability and in vivo profile, therefore structural parameters can influence the biopharmaceutical properties. The use of SLN for drug delivery is also dependent on their toxicological profile in vitro. Nanotoxicology is also discussed addressing the key points that may limit the clinical use of SLN.

5.1 Introduction

Nanotechnology applied to drug delivery reached the clinic within the last few decades. Nanosciences and Nanotechnologies were defined by the Royal Society and Royal Academy of Engineering *as the study of phenomena and manipulation of materials at atomic*, *molecular and macromolecular scales*, *and the design*,

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characterization, *production and application of structures*, *devices and systems by controlling shape and size at nanometer scale* (Ann 2004; Dowling et al. 2004). Lipid nanoparticles are being developed for drug delivery to overcome the limitations of traditional therapies and are providing endless opportunities in this area. Since the nineties that Solid Lipid Nanoparticles (SLN™) have captured the attention of several researchers because they combine innovation and versatility and also show major advantages for various application routes (parenteral, oral, dermal, ocular, pulmonary, rectal). The main features of SLN include their suitability to optimize drug loading and release profiles, long shelf life and low chronic toxicity, protection of incorporated labile drugs, site-specific targeting, and excellent physical stability (Souto and Muller 2010; Fangueiro et al. 2013; Wissing et al. 2004). However, some limitations may also be pointed out, such as the insufficient loading capacity, the risk of drug expulsion after polymorphic transitions during storage, and the high amount of water (70–99.9 %) required in their composition. SLN typically consist of three essential components, i.e. solid lipid, surfactant, and water. These lipid particles are composed only of solid lipids at body and room temperature, having a mean particle size of between 50 and 1,000 nm (Souto and Doktorovova 2009; Souto and Muller 2011; Souto et al. 2011a). They have the potential to load both hydrophilic and hydrophobic drugs. Depending on the location of the drug, i.e. if the drug is entrapped inside the lipid matrix, encapsulated or adsorbed onto the surface of particles, they can exist in three different morphological types. The SLN Type I is composed of a homogenous matrix in which the drug is molecularly dispersed within the lipid core or it is present in the form of amorphous clusters, also homogenously dispersed in the lipid core. The SLN Type II or drug-enriched shell model occurs when the lipid concentration is low relatively to the drug, and a drug-free (or drug-reduced) lipid core is formed when the drug reaches its saturation solubility in the remaining melt. An outer shell will solidify containing both drug and lipid with mainly drug on the surface of the particles. The SLN Type III or drug-enriched core model is formed when the drug concentration is relatively close to or at its saturation solubility in the lipid melt. The drug forms a core surrounded by a lipid-enriched shell. Types I and III are those reported to provide prolonged or/and controlled release profiles. The present chapter reviews the lipid nanoparticles chemistry, production techniques, physicochemical characterization, and their developments in drug delivery with a special focus on nanotoxicology of lipid particles.

5.2 Nanomaterials

The specific characteristic of SLN is their size, which falls in the transitional zone between individual atoms or molecules and the corresponding bulk materials. Size reduction can modify the physical and chemical properties of nanomaterials distinctively from their bulk and molecular counterparts. Independently of the particle size, the materials present in the nanoparticles in contact with cell membranes and the chemical reactivity of those materials play a dominant role when the particles react with other substances or tissues (Yang et al. 2008). Colloidally dispersed lipids

are interesting carrier systems for bioactive substances, particularly for lipophilic drugs. By definition, SLN are colloidal dispersions, since they are neither a suspension nor an emulsion (Heike 2011). Lipid compounds (of animal or vegetable resources) are generally regarded as safe (GRAS) and have proved their biocompatibility and biodegradability, since they are physiological lipids that occur naturally in the organism. The most commonly used lipids are listed in Table 5.1. The general lipid composition (e.g. fatty acids, waxes) will have a different crystallinity and capacity for accommodation of drug molecules. In addition, the organization of lipid molecules after recrystallization could result in three possible forms, mainly, an amorphous α form, an orthorhombic perpendicular β′ form, and a triclinic parallel β polymorphic form. The degree of crystallinity of the lipid matrix increases from the α form, to β′ form, and finally to β form (Westesen et al. 1993). Lipids usually show high crystallinity during storage, and not immediately after SLN production. This could lead to a problem related to drug expulsion from the lipid matrix, since less space is left for the accommodation of drug molecules with the creation of a perfect crystal lattice. The metastable form β′ is usually related to a controlled drug release profile (Wiechers and Souto 2010).

Triacylglycerols (TAGs) are widely used in pharmaceuticals as matrix materials. Physical properties, such as polymorphism, melting and solidification, density, and molecular flexibility, are influenced by the crystalline phases of TAGs. The structural properties of TAG crystals are influenced by their molecular properties, such as saturation/unsaturation of the fatty acid moieties, glycerol conformations, symmetry/asymmetry of the fatty acid compositions connected to the glycerol groups. TAGs present as fats as do diacyl- and monoacylglycerols. The physical properties of TAGs depend on their fatty acid compositions (Kaneko 2001). TAGs mostly used in SLN are trilaurin, trimyristin, tripalmitin, and tristearin (Souto et al. 2011b). These types of lipids are reported to be safe for use as colloidal carriers. Tripalmitin and tristearin are biodegradable by lipases (Olbrich et al. 2002). However, some disadvantages are associated with the use of pure TAGs, since immediately after recrystallization the α form is formed and more drug is incorporated due to the less-ordered crystal structure. During storage, other polymorphic modifications may occur (β and β′) and the reorganization does not leave space enough for the loaded drug molecules, leading to their expulsion from the lipid matrix (Souto et al. 2011b). Nevertheless, their use should not be discarded, since studies of long-term stability of SLN-based TAGs have also been reported (Souto and Muller 2006). Mono- and diacylglycerols are also broadly used in SLN production. Despite having higher solubilization capacity than TAGs, their use in lipid matrices has the advantage of forming less-ordered crystal structures leaving more space for drug accommodation (Souto et al. 2011b).

Fatty acids are also used for SLN production since these also figure in the membranes and fat tissues (Kaneko 2001). The main advantages of their applications are the acyl chains that provide flexibility and consequently lead to a molecular conformation that results in lateral packing. The crystal structure mainly depends on the physical properties, such as the presence of long chains, melting point, heat capacity, and elasticity (Kaneko 2001). Depending on the chain length of the fatty acid, fatty alcohols are suitable to be used in lipid matrices, since they show good biotolerability as the other lipids mentioned above for dermal delivery. They could

Table 5.1 Examples of common solid lipids for the production of SLN

	Trade name	Chemical terminology	Reference
TAGs	Precirol®ATO5	Mixture of monoglycerides (8–22 %), diglycerides (40–60 %), and triglycerides (25–35 %) of palmitic and stearic acid	Das et al. (2011); Sivaramakrishnan et al. (2004)
	Compritol®888ATO	Glyceryl dibehenate	Das et al. (2011); Sivaramakrishnan et al. (2004); Blasi et al. (2011); Kuo and Chung (2011); Rahman et al. (2010); Doktorovova et al. (2011); Kuo and Chen (2009)
	Dynasan®114	Glyceryl trimyristate	Martins et al. (2012); Aditya et al. (2010); Petersen et al. (2011); Noack et al. (2012)
	Dynasan®116	Glyceryl tripalmitate	Kuo and Chung (2011); Kuo and Lin (2009); Cengiz et al. (2006)
	Dynasan®118	Glyceryl tristearate	Petersen et al. (2011); Noack et al. (2012)
	Imwitor®900 K	Mono- and diglycerides based or hydrogenated fats with a glyceryl monostearate content of 40–55 %	Sivaramakrishnan et al. (2004); Doktorovova et al. (2011)
	Softisan®100	Blends of TAGs Composed by fatty acids with a chain length of C_{10}–C_{18}	Fangueiro et al. (2013); Zhang et al. (2008)
	Softisan®142	Blends of TAGs Composed by fatty acids with a chain length of C_{10}–C_{18}	Blasi et al. (2011); Nassimi et al. (2010)
Fatty acids	Stearic acid	Saturated C_{18} fatty acid	Ghadiri et al. (2012); Severino et al. (2011); Zhang et al. (2000)
Fatty alcohols	Cetyl alcohol		Sanna et al. (2010)
	Stearyl alcohol		Sanna et al. (2010); Souto et al. (2004a)
	Witepsol®E85	Mixtures of hard fats	Sarmento et al. (2011); Kuo and Chen (2009); Martins et al. (2012)
Waxes	Cacao butter		Kuo and Chung (2011); Kuo and Lin (2009); Kim et al. (2005)
	Carnauba wax		Kheradmandnia et al. (2010)
	Beeswax		Kheradmandnia et al. (2010); Attama and Müller-Goymann (2008)
	Cetyl palmitate	Ester of palmtic acid and cetyl alcohol	Carbone et al. (2012); Blasi et al. (2011); Martins et al. (2012); Ghadiri et al. (2012); Fangueiro et al. (2012)
Cationic lipids	CTAB	Cetyl trimethylammonium bromide	Carbone et al. (2012); Doktorovova et al. (2012)
	DDAB	Dimethyldioctadecylammonium bromide	Carbone et al. (2012)
	DOTAP	1,2-dioleoyl-3-trimethylammonium-propane	Tabatt et al. (2004); Carbone et al. (2012); del Pozo-Rodríguez et al. (2010)
	DODAB	Dioctadecyldimethyl ammonium bromide	Kuo and Chen (2009); Kuo and Wang (2010)

be useful as skin permeation enhancers due to the disturbance in the lipid packing order (Sanna et al. 2010). For parenteral administration, these types of lipids are also shown to be biodegradable and safe, since they are metabolized in the body via endogenous alcohol dehydrogenase enzyme systems (Dong and Mumper 2006).

Hard fats could compromise the feasibility of several types of SLN. They can be a mixture of acylglycerols based on saturated fatty acids. Their use in SLN led to a lipid matrix able to load proteins and peptides safely for parenteral administration (Fangueiro et al. 2013; Almeida et al. 1997; Sarmento et al. 2011).

Waxes are a group of lipids comprising esters of fatty acids and fatty alcohols. In opposition to acylglycerols, the alcohol represented is not glycerol. Waxes may contain free hydroxyl groups within the molecule (e.g. hydroxyoctanosyl, hydroxystearate) or free fatty acid functions (e.g. beeswax). Their polymorphism is mainly an orthorhombic form that prevails, and the polymorphic transition rate is low (Jenning and Gohla 2000). They have been proven to be able to accommodate drugs with limited solubility and also to provide a release profile depending on the amount of wax in the lipid matrix (Kheradmandnia et al. 2010).

Cationic lipids have been recently applied mainly for gene therapy (Tabatt et al. 2004), and in particular for application to negatively charged mucosal surfaces, such as the ocular mucosa (del Pozo-Rodríguez et al. 2008). Their chemical composition is mainly quaternary ammonium salts. In SLN, they are reported to be useful for monoclonal antibody adsorption and/or the absorption of DNA onto the surface of the particles and for specific targeting (Carbone et al. 2012). These lipids may be used as lipid matrices along with the other lipids already described. The anti-inflammatory effects of cationic lipids have also been reported (Filion and Phillips 1997) and they may be useful for some administration routes. A list of most used solid lipids for the production of SLN is shown in Table 5.1.

Emulsifiers have amphiphilic structure which are used to reduce the surface tension and facilitate the particle partition, i.e. with their hydrophilic groups oriented towards the aqueous phase and the hydrophobic groups oriented to the lipid (Rosen 2004). The selection of the emulsifiers mainly depends on the chosen lipid, since they need to be qualitatively and quantitatively compatible (Severino et al. 2012). The hydrophilic-lipophilic balance (HLB) is directly related to the solubility, i.e. it is the balance of the size and strength of the lipophilic and hydrophilic groups of the emulsifier and it is mandatory to form an emulsion. Thus, the required HLB (rHLB) of a final dispersion should be calculated according to the HLB of the lipid and HLB of the emulsifier (and co-emulsifier, if needed), using the following equation (Souza et al. 2012; Vieira et al. 2012):

$$rHLB = \left[\%_{Lipid} \times HLB_{Lipid}\right] = \left[\%_{Emulsifier} \times HLB_{Emulsifier}\right] + \left[\%_{Co\ \ Emulsifier} \times HLB_{Co\ \ Emulsifier}\right]$$

This equation could be useful to predict the quantities required to form an emulsion, and it is highly applied in fluid emulsions, such as SLN. However, for cream type emulsions and w/o/w emulsions it is not sufficient. Nevertheless, calculating the

rHLB is extremely relevant, since the selection of the ideal chemical emulsifier is vital to the stability of the entire formulation. The blend of the emulsifier should adjust to the tails of the lipid chains in the interface to allow the coexistence of the oil droplets in a continuous aqueous phase. The interactions between lipids and emulsifiers are mainly electrostatic, since hydrophobic moieties have attractive forces to lipid molecules. Emulsifiers are physically adsorbed onto the surface or loaded within the lipid matrix. The hydrophilic groups of the emulsifier give rise to repulsive forces, depending on the volume size and chemical nature of the hydrophilic moieties of emulsifiers (Rosen 2004).

The emulsifiers typically used in the production of SLN could be selected according to their nature (i.e. HLB) and also to the nature of the hydrophilic group. Apparently, the non-ionic emulsifiers do not show ionic charge (e.g. $RCOOCH_2CHOHCH_2OH$, monoglyceride of long-chain fatty acid). The most used are the polyoxyethylene sorbitan fatty acid esters (Tween® 20, 40, 60, 80), sorbitan fatty acid esters (Span®20, 40, 60, 80), polyoxyethylene sorbitol esters (Mirj® 45, 52, 53, 59), alkyl aryl polyether alcohols (Tyloxapol), and the triblock copolymers composed of polyoxypropylene (poloxamer, pluronic or Lutrol®F68, F127). Sugar esters could also be employed, e.g. esters of stearic, palmitic, oleic, and lauric acids.

Polyoxyethylene sorbitan fatty acid esters and triblock copolymers composed of polyoxypropylene are hydrophilic amphiphilic molecules that are dissolved in the aqueous phase of emulsions, and their lipophilic moiety is adsorbed onto the particles surface and the long polyoxypropylene chains allow the stabilization and aggregation with other particles. Sorbitan fatty acid esters are lipophilic amphiphilic molecules that are better adsorbed onto the particle surface and easily dissolved within melted lipids (Kaneko 2001).

The use of anionic or cationic emulsifiers could be important to improve the zeta potential (ZP), and thus input an electrostatic charge to avoid particle aggregation/sedimentation. The cationic emulsifiers have a molecule moiety with a positive charge (e.g. salt of a long-chain amine $RNH_3^+Cl^-$, or a quaternary ammonium salt, $RN(CH_3)_3^+Cl^-$). Stearylamine (Kuo and Chen 2009; Vighi et al. 2010; Pedersen et al. 2006), cationic lipids (quaternary ammonium salts) (Tabatt et al. 2004; Doktorovova et al. 2011, 2012), and Esterquat 1 (*N*,*N*-di-(b-stearoylethyl)-*N*,*N*-*dimethyl-ammonium* chloride) (Vighi et al. 2007, 2010) are examples used in SLN. Anionic emulsifiers have a negative moiety in the molecule (e.g. $RCOO^-Na^+$, sodium salt) and the most applied are bile salts (e.g. sodium cholate and sodium taurocholate), which improve the absorption of particles in the gastrointestinal tract (GIT). Other types of emulsifiers often applied are the phospholipids. Phospholipids derived from soy or egg phosphatidyl choline have a variable fatty chain composition. Generally, soybean phosphatidyl choline contains more saturated fatty acyl chains than egg phosphatidyl choline (Souto et al. 2011b). Its use is reported to improve the emulsions stability and it may also be applied as a permeation enhancer for topical administration (Dreher et al. 1997; Cui et al. 2006). Also, its use has resulted in a decrease in particle size due to the amphiphilic properties of phosphatidyl choline (Schubert and Muller-Goymann 2005; Schubert et al. 2006).

5.3 Production Methods of SLN

The classical production methods of SLN are very well described in the literature (Souto and Muller 2011; Souto et al. 2011a; Müller et al. 2000) and include those not requiring organic solvents and those requiring the use of solvents to solubilize the lipid materials. The selection of the ideal method mainly depends on the properties of the required drug, such as solubility, chemistry, molecular weight, and thermal stability. The most applied and simple method is the High Pressure Homogenization (HPH), either hot HPH or/and cold HPH (Müller et al. 2000). For both, the drug is dispersed or solubilized in the melted lipids. In hot HPH, an aqueous surfactant solution at the same temperature is added to the lipid phase and homogenized by high shear homogenization for 1 min at 8,000 rpm. Then, this pre-emulsion is processed by HPH to obtain a desirable particle size. Usually, 3–5 homogenization cycles at 500 bar are sufficient. After this process a nanoemulsion is obtained and following a temperature decrease there is lipid recrystallization, and consequently the formation of SLN. The cold HPH method is slightly different from the hot HPH method. After drug dispersion or solubilization in the melted lipid, this mixture is cooled using nitrogen liquid or dry ice. The obtained solid lipid mixture is ground using a mortar to produce microparticles which are suspended in an aqueous surfactant solution. Nanoparticles are formed when this microparticle suspension is homogenized by HPH at room temperature or below. The shear forces and cavitation forces in the homogenizer are able to break the microparticles into nanoparticles (Mishra et al. 2010). The main difference of these two techniques is the temperature, because cold HPH is applied when the drug is labile or heat-sensitive. The cold HPH was also developed to overcome some failures of hot HPH, such as drug distribution in the aqueous phase during homogenization and complexity of the crystallization step, which could lead to supercooled melts. However, hot HPH is the most applied process due to ease of scale-up.

Production of SLN by sonication or by high shear homogenization is applied less frequently. The lipid phase and aqueous phase are heated up to the same temperature and emulsified by mechanical stirring (high shear homogenization) or sonication. The main disadvantage of these techniques is the presence of both microparticles and nanoparticles in the final dispersion (Sinha et al. 2011).

The microemulsion technique was developed by Gasco (1997) and since then, it is being developed and modified by different research groups (Pedersen et al. 2006; Fontana et al. 2005; Patel and Patravale 2011; Ghadiri et al. 2012). The lipid and aqueous phase are heated and homogenized at a temperature above the melting point of lipid. This microemulsion is diluted with cooled water, which leads to the breaking of the microemulsion into a nanoemulsion. The obtained nanoemulsion is cooled leading to the formation of SLN. During stirring, the control of the temperature is an important factor to keep the lipid in the melted state (Sinha et al. 2011).

The double emulsion method is an alternative method for producing SLN described by Garcia-Fuentes et al. (2002). This method is appropriate for the incorporation of hydrophilic or/and labile drugs, such as proteins and peptides, since these are loaded in an inner aqueous phase avoiding chemical or enzymatic degradation. Briefly, the hydrophilic drug is solubilized in an inner aqueous phase and

added to the lipid phase containing a suitable emulsifier to form a primary w/o emulsion under high shear homogenization. The second step involves the dispersion of this primary w/o emulsion in an external aqueous surfactant phase to form the final w/o/w emulsion (Fangueiro et al. 2013).

The solvent emulsification-evaporation method described by Sjöström and Bergensåhl (1992) has been applied when the drug is not soluble in the lipid. In this case, an organic solvent, non-miscible with water (e.g. cyclohexane or chloroform) but soluble in the lipid and the lipid are dispersed in an aqueous surfactant solution to produce an o/w emulsion. The organic solvent is evaporated during stirring. This method is useful for hydrophilic drugs, such as proteins and peptides.

The solvent displacement method (also named as the nanoprecipitation method) was initially described for the production of polymeric nanoparticles (Quintanar-Guerrero et al. 1999) and has been adapted for lipid nanoparticles (Videira et al. 2002; Hu et al. 2002; Dong et al. 2012). This method requires a water-miscible organic solvent (e.g. acetone, ethanol, or methanol) that is used to dissolve the lipid phase. This lipid phase (containing the lipid and drug) is added by injection to an aqueous surfactant solution. Lipid particles are formed after complete removal of the solvent by diffusion or by distillation leading to nanoparticles precipitation (Souto and Müller 2007).

In the emulsification-diffusion method, a solution of a semi-polar organic solvent previously saturated with water to ensure thermodynamic equilibrium is used to dissolve the lipid. This solution is added to an aqueous surfactant solution obtaining a w/o emulsion. The saturated solution prevents the diffusion of the solvent from the droplets into the water phase. Formation of SLN is obtained by adding an excess of water to the emulsion, which facilitates the diffusion of solvent from the droplets (Wissing et al. 2004; Sinha et al. 2011).

The phase inversion-based method has been described by Heurtault et al. (2002). This method involves two steps. First, all components are melted and magnetically stirred using a temperature program (e.g. 25–85 °C) and cooled down to lower temperature (e.g. 60 °C). Three temperature cycles (85-60-85-60-85 °C) are applied to reach the inversion process defined by a temperature range. In the second step, an irreversible shock is applied by adding cold water. This process leads to the formation of stable nanoparticles.

More recently a new method has been described for the production of SLN. This method developed by Battaglia et al. (2010) produces SLN by coacervation in a controlled way, starting from fatty acid alkaline salts. The basis of the method is the interaction of micellar solution of sodium salts of fatty acids (e.g. sodium stearate, sodium palmitate, sodium myristate, sodium behenate) and an acid solution (coacervating solution) in the presence of an amphiphilic polymeric stabilizing agent (Battaglia et al. 2010; Corrias and Lai 2011). With the decrease of pH, the SLN can be precipitated. It is a very simple, inexpensive, and thermosensitive method that allows the incorporation of drugs and also can be applied from the laboratorial scale to the industrial scale (Battaglia and Gallarate 2012).

The different methods described here have been classified as those not using organic solvents (Fig. 5.1) and those using organic solvents (Fig. 5.2).

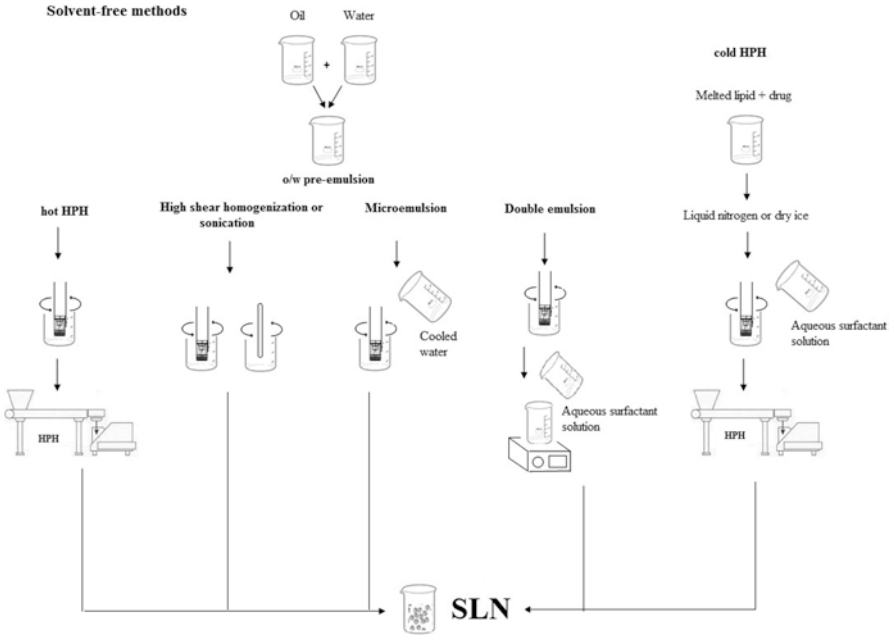

Fig. 5.1 Schematic representation of the production of SLN by solvent-free methods

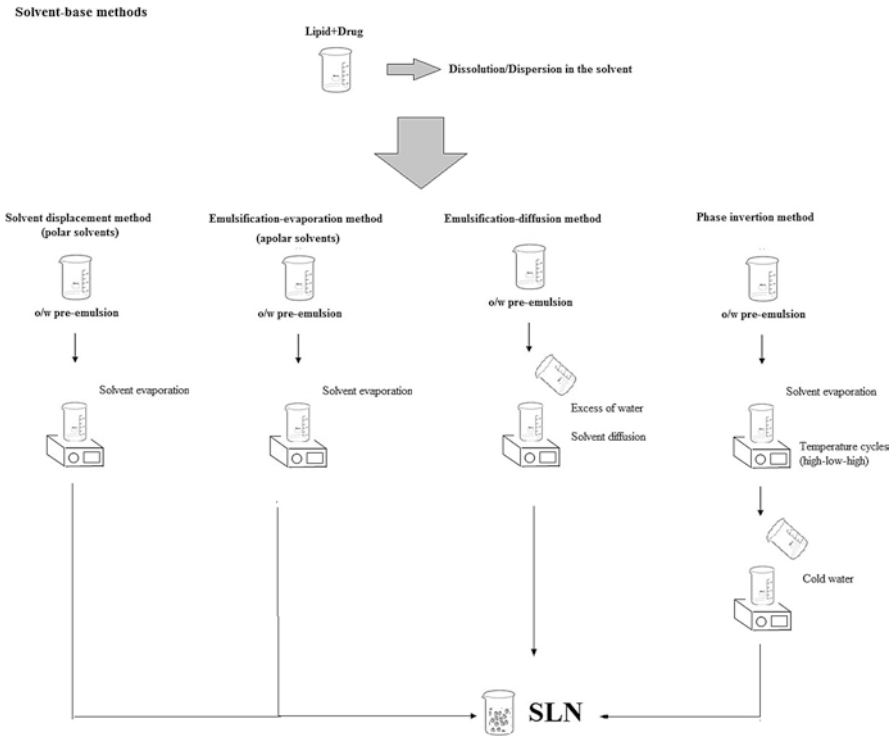

Fig. 5.2 Schematic representation of the production of SLN by solvent-based methods

5.4 Characterization and Evaluation of SLN

The physicochemical characterization of SLN is crucial to evaluate their stability, safety, and suitability for drug delivery. The physicochemical properties of SLN may compromise the administration route and the toxicological profile. A major parameter is the colloidal stability of SLN in aqueous dispersions and also the crystallization and polymorphic behaviour of the lipid matrix. The most commonly applied methods for the characterization of SLN are described in this section.

5.4.1 Mean Particle Size, Distribution, and Electrical Charge

After SLN production, the first parameter to assess should be the mean particle size and size distribution, since it is desirable to have monodispersed populations in the nanometer range. The most common techniques are the dynamic light scattering (DLS), also known as photon correlation spectroscopy (PCS), and the laser diffraction (LD). DLS is capable of determining the particle size in the submicron range based on the Brownian movement of the spherical particles in suspension. A monochromatic light beam, such as a laser, hits the moving particle, changing the wavelength of the incoming light. This change is related to the size of the particle (Berne and Pecora 2000). The frequency shifts, the angular distribution, the polarization, and the intensity of the scattered light, are determined by the size, shape, and molecular interactions in the scattering material (Gethner and Gaskin 1978). It is possible to compute the spherical size distribution and give a description of the particle's motion in the medium, measuring its diffusion coefficient and using the autocorrelation function (Berne and Pecora 2000).

LD is another technique that could be applied based on the principle that particles passing through a laser beam will scatter light at an angle that it is directly related to their size. The observed scattering angle is dependent on the shape and size of the particles and increases logarithmically with the increase of the particle size. The scattering intensity is high for larger particles and low for smaller particles (Eshel et al. 1991; Ma et al. 2000). Both techniques are broadly applied because of the advantages they show, e.g. fast data collection, relatively inexpensive, good reproducibility, low volume of samples, and automated for routine measurements. In addition, extensive experience in the technique is not required. The main differences between DLS and LD are the collected data. DLS reports the mean particle size of the entire population, whereas LD usually reports the mean particle size of 10 % (LD_{10}), 50 % (LD_{50}), and 90 % (LD_{090}) of the population of the particles. Another difference is the detection limit; DLS is not able to detect particles above 3 μm, while LD is.

Each particle develops a net charge at the surface affecting the distribution of ions in the surrounding interfacial region, resulting in an increased concentration of counter ions (ions of opposite charge to that of the particle) close to the surface. Thus, an electrical double layer is formed. In the inner region, there is the Stern layer, the ions are strongly bound, and in the outer diffuse region they are less firmly attached. The zeta potential (ZP) refers the electrical charge at the surface of the

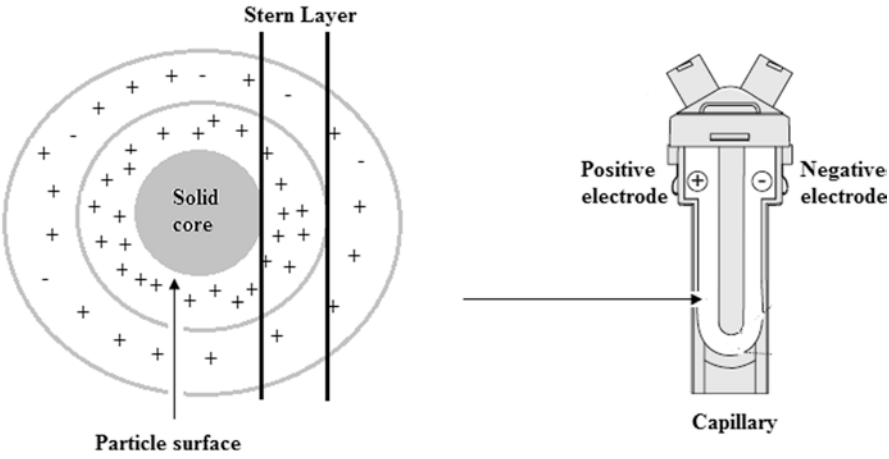

Fig. 5.3 Illustration of the ZP in the surface of particles and the esquematic representation of a capillary and the electrodes

hydrodynamic shear surrounding the colloidal particles. The magnitude of the ZP gives an indication about the long-term stability of the colloidal system (Hiemenz and Rajagopalan 1997). Thus, if all the particles in suspension have a high negative or positive value of ZP, then they will tend to repulse each other and there is no tendency to flocculate. However, if the particles have low ZP values, then there is no force to prevent the particles coming together and flocculating (Dhont 2001). The limiting values between stable and unstable suspensions are generally taken at either +30 mV or −30 mV. DLS is a technique that is used to determine the ZP, however other techniques exist, such as electrophoretic light scattering (ELS), acoustic, and electroacoustic methods. However, DLS is the most sensitive and versatile technique, but it does not directly measure the ZP. The analysis is carried out in a cell with electrodes in each end to which a potential is applied. When the voltage is applied, the particles move towards the electrode of opposite charge, and their velocity is measured and expressed as ZP (Fig. 5.3). The mobility of the particles is commonly determined by laser Doppler anemometry, which is based on the evaluation of a frequency (Doppler) shift that is observed for the light scattered from the particles motion in the electric field. DLS converts this signal directly in ZP through the Helmholtz-Swoluchowski equation (Bunjes 2005):

$$\mu = \frac{\varepsilon\zeta}{\eta\zeta}$$

where μ is the electrophoretic mobility, ε is the permittivity, ζ is the zeta potential, and η is the viscosity of the dispersion medium.

Unlike other parameters, such as particle size, ZP is affected by the surrounding environment, e.g. pH, ionic strength, and consequently the type of ions in the suspension. Therefore, the measurements should be conducted after dilution to avoid multiple scattering and for better resolution (Renliang 2008).

5.4.2 Microscopy

Microscopy is a technique used to verify the surface, morphology of the particles, their size, distribution, and shape, and other potential species that may have been produced simultaneously. Particle shape is a very important factor, since particles are preferentially required to be spherical. For controlled release, the particles should provide protection for the loaded drugs, where the contact with the aqueous environment should be minimal (Bunjes 2005). The most applied techniques are the scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM). Usually, these sophisticated techniques require sample treatment, (e.g. freeze-drying) prior to imaging, thus particles are not observed in their native state (i.e. as liquid dispersions). The major difference between TEM and SEM is the electron incidence in the sample. In SEM, electrons are scanned over the surface of the sample and can produce very high-resolution images of a sample surface, revealing details less than 1 nm in size. In TEM, electrons are transmitted through the sample providing information about the thickness and composition (crystal structure) of samples (Dubes et al. 2003). In SEM, a chemical fixation to preserve and stabilize particle structure is required. Fixation is usually performed by incubation in a buffer solution, such as glutaraldehyde, sometimes in combination with formaldehyde and other fixatives, and optionally followed by post-fixation with osmium tetroxide (Muller-Goymann 2004).

TEM is relatively fast and simple with negative staining (Friedrich et al. 2010). Samples for TEM need to be as thin as possible to allow the electron beams to travel through the sample. This technique requires a drying process and yields additional information about the internal structure of the nanoparticles. The samples are fixed with a solution containing heavy metal salts which provide high contrast in the electron microscope (e.g. uranyl acetate or phosphotungstic acid) (Kuntsche et al. 2011). Unfortunately, the resulting images have low resolution and since the freeze-drying of samples is required, they are not observed in the original state, leading to artefacts in the images (Bunjes 2005). Cryogenic microscopy is very useful for lipids, requiring low temperatures. Cryofixation may be used and low-temperature SEM/TEM performed on the cryogenically fixed samples.

AFM utilizes the force acting between a surface and a probing tip resulting in a spatial resolution of up to 0.01 nm for imaging (Mühlen et al. 1996). This technique allows imaging under hydrated conditions without pre-treatment of the samples; however samples need to be fixed, e.g. by adsorption (Muller-Goymann 2004). AFM provides a three-dimensional profile surface, which leads to a higher resolution and gives more information about the particles` surface. Also, the pre-treatment of the samples is not required. However, AFM requires longer time for analysis than SEM or TEM (Mühlen et al. 1996; Geisse 2009; Olbrich et al. 2001; Shahgaldian et al. 2003).

5.4.3 Thermal Analysis

During the recrystallization process, lipid materials could exist in a well-defined crystal, or as a mixture of different internal lattice structures. Thermal analysis could

provide information about the polymorphic modifications of lipid materials, crystallization, and thermal behaviour. The polymorphic behaviour and crystallinity of SLN can be checked by differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), thermomechanical analysis (TMA), and the differential thermal analysis (DTA). Phase transitions are followed by free energy changes, associated with changes in the enthalpy or entropy of the system. Enthalpy changes of samples are the result of an endothermic or exothermic reaction that it is translated in a signal, depending on the consumption of energy (e.g. melting of a solid), or a release of energy (e.g. recrystallization of an isotropic melt). The transition from the crystalline to amorphous phase usually requires a high input of energy. Therefore, care has to be taken to ensure that the measuring device is sensitive enough to give a sufficiently low detection limit. Entropy changes can be recognized by a change in the baseline slope, due to a change in the specific heat capacity (Ford and Mann 2012).

DSC is the most applied thermal analysis in SLN that evaluates the physical and chemical properties of the lipids as a function of time and temperature (McElhaney 1982). Two samples are usually analyzed, namely the SLN sample and the bulk material (i.e. the solid lipid used), and the temperature of both samples is raised identically over time in different containers (Gill et al. 2010). The difference in the input energy required to match both temperatures would be the amount of excess heat absorbed (endothermic) or released (exothermic) by the lipid in the SLN. During a change in temperature, DSC measures the heat quantity needed for the transition to occur, which is released or absorbed excessively by the sample on the basis of a temperature difference between the sample and the bulk material. In DSC, the amount of heat put into the systems is exactly equivalent to the amount of heat absorbed or released during a transition (Christian and O'Reilly 1986). The main advantages of this technique are the detection of polymorphism, the small sample size (e.g. 2–3 mg (solid) or ml (liquid)), relatively fast analysis times, and a sensitive and versatile procedure. TGA is also very similar to DSC; the major difference is the continuous analysis of sample and bulk material, and it is based on the thermobalance. It measures the mass variation of a sample when subjected to temperature. The source of heat is an infra-red lamp, and the atmosphere is controlled by the addition of inert gases (e.g. nitrogen, helium) or reactive gases (e.g. oxygen, hydrogen). The decomposition of effluent material can be characterized also, by coupling a gas chromatography or mass spectrometery system (McCauley and Brittain 1995). DTA is similar to DSC; however it is the more accurate of all thermal analyses procedures, because the thermocouple is inserted into the sample (McCauley and Brittain 1995). TMA measures the deformation of the sample under non-oscillating stress subjected to temperature. The sample usually is placed in a small tube connected by a quartz probe to a differential exchanger. The movements of the sample are monitored by the displacement of the exchanger. This technique enables the measurement of several characteristics, such as tensile strength, volume expansion, penetration, or elasticity (McCauley and Brittain 1995).

Figure 5.4 shows the typical DSC profiles of a solid bulk lipid, analyzed before tempering the material for 1 h at 100 °C. The differences between the two heating runs (A and B) allow the detection between different polymorphic forms in the same material. In Fig. 5.4a, the difffractogram clearly shows a sharp peak representing the β-form

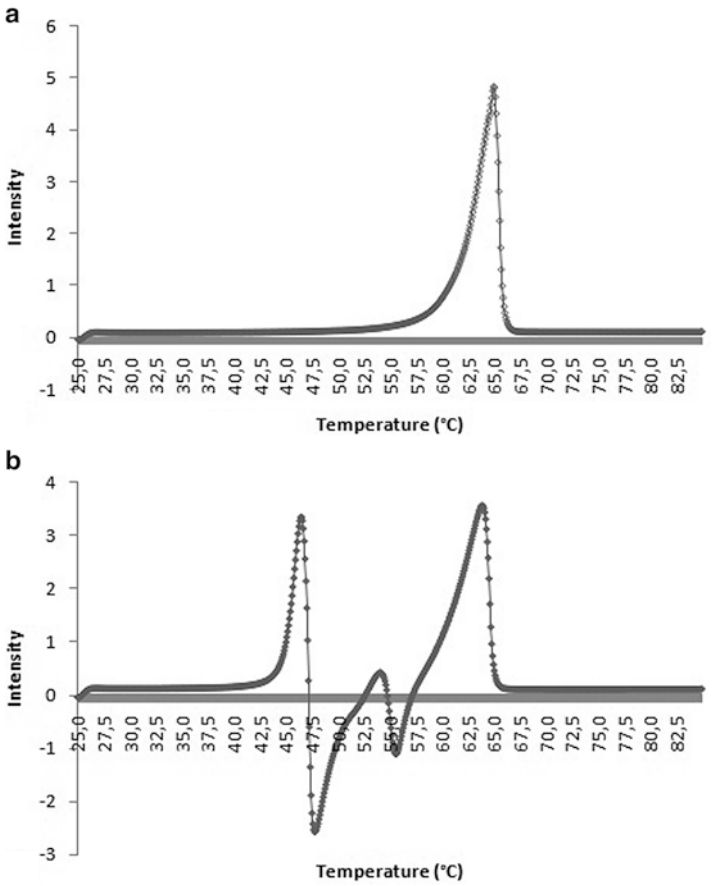

Fig. 5.4 DSC analysis of bulk lipid. (**a**) First heating run before tempering and (**b**) second heating run before tempering (modified after Souto and Müller (2006))

of the tested bulk lipid (i.e. Dynasan®116) (Souto and Muller 2006). In the second heating run (Fig. 5.4b) it is also possible to detect the metastable α-form. This type of analysis mimics the thermal stress of the lipid, since for production of SLN it is necessary to melt the lipid first and then cool the pre-emulsion for SLN formation. Analysis of SLN represents the second heating run. This explains the different polymorphic forms between bulk materials and the lipid particles (Souto and Muller 2006).

5.4.4 Crystallinity and Polymorphism

X-ray is a widely used method for the evaluation of crystallinity, since it applies wavelengths of the same magnitude as the distance between the atoms or molecules of crystal. Thus, it allows the determination of the arrangement of molecules within a crystal, i.e. the macromolecular structure of the particles (Heurtault et al. 2003). This method

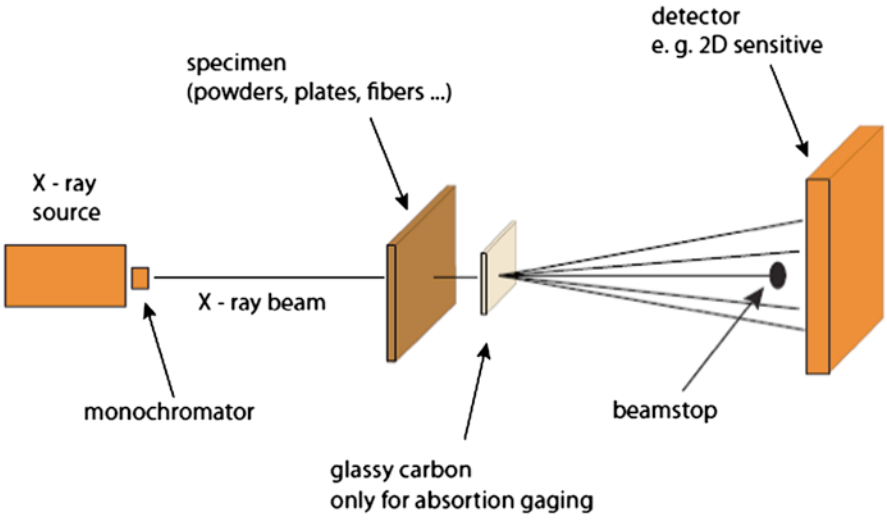

Fig. 5.5 Illustration of the X-ray instrumentation and methodology

is based on a beam of X-rays that reaches a crystal and causes the spread of light into many specific directions. This energetic radiation may arise from the removal of inner orbital electrons. These transitions are followed by the emission of an X-ray photon having energy equal to the energy difference between the two states (Krakty 1982). From the angles and intensities of these diffracted beams, a crystallographer can produce a three-dimensional picture of the density of electrons within the crystal, as shown in Fig. 5.5. From this electron density, the mean positions of the atoms in the crystal can be determined, as well as their chemical bonds, their disorder, and various other factors (Jenknins 2000). This technique can be carried out using the small angle X-ray scattering (SAXS) or the wide angle X-ray scattering (WAXS), both widely applied in the evaluation of SLN crystallinity (Schubert et al. 2006; Heurtault et al. 2003; Müller et al. 2008; Aji Alex et al. 2011). The only difference between both methods is the range of scattering angles 2θ. While standard diffractometers cover angles between about 5° and 180°, the range between 0.01° and 3° is typical for small angle instruments. These techniques are specific for studying structural features of colloidal size in bulk materials and particles loaded with drug, determining their polymorphic transitions and the quantitative determination of crystalline components in the formulation (Bunjes and Unruh 2007). It is also important to confirm the presence of the drug in the systems, i.e. if the drug is molecularly dispersed in the lipid matrix, or if it is in the amorphous state. Studies with this technique revealed the polymorphism of particles composed of acylglycerols (Westesen et al. 1997; Bunjes et al. 2003) and differences when compared to the bulk materials (Souto et al. 2006).

This technique is applied to differentiate between the crystalline and amorphous material. A typical example is shown in Fig. 5.6. The X-ray study confirms the transition rates of both liquid and solid lipids used in the lipid particles. The use of higher amounts of oil in the particles (Fig. 5.6b) decreases the crystallinity, and consequently decreases the peaks intensity in the X-ray diffractogram.

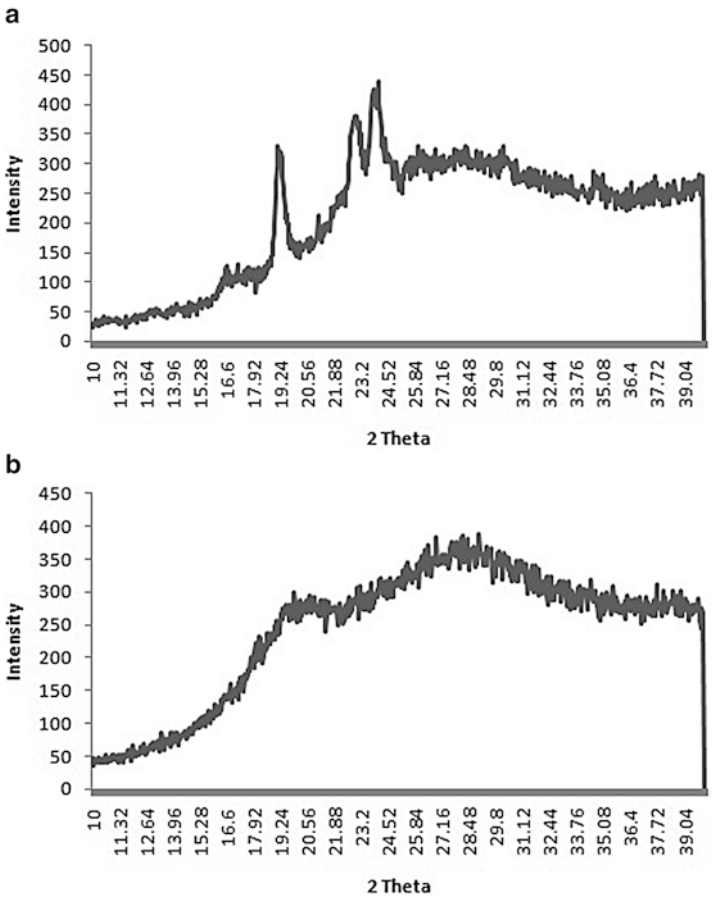

Fig. 5.6 X-ray analysis of lipid particles. (**a**) Lipid particles made of solid lipid and (**b**) lipid particles made of liquid and solid lipids (modified after Souto et al. (2004b))

5.4.5 Infra-Red Spectroscopy

Infra-red spectroscopy is applied to characterize SLN at molecular level. Each molecule absorbs at a specific wavelength that is related to its molecular and chemical structure. The usefulness of Fourier transform infra-red (FTIR) imaging in the characterization of drug delivery systems was demonstrated by Coutts-Lendon et al. (2003). FTIR could be useful to identify the lipid composition and also the presence of drug contaminants and degradants in the lipid matrix. For bulk materials, FTIR could also provide information about the functional groups. In general, this technique is very sensitive, non-destructive, and the amount of sample required for analysis is about a few nanograms. The particles are isolated by microneedle and transferred to a circular salt plate placed on the instrument. The recorded data is a three-dimensional spectral image, with spatial information in the *x*- and *y*- directions and spectral information in the *z*- direction (Barber 1993; Bhargava and Levin 2001).

5.5 Applications of SLN in Drug Delivery

SLN play an important role in the delivery of a variety of drugs, poorly water-soluble drugs, hydrophilic drugs, such as peptides and proteins (Almeida and Souto 2007). The many advantages already mentioned are mainly related with their nanometer size, but also to the protection of the drug from chemical and enzymatic degradation. In addition, the improvement on the bioavailability is also a reason for selecting SLN, independently of the administration route. SLN changes the pharmacokinetic and biodistribution profiles of the loaded drug and may offer the possibility of targeted delivery.

Depending on the administration route, SLN can overcome the many barriers for the absorption of the majority of drugs.

For oral delivery, the GIT could compromise the activity of several drugs (e.g. peptides and proteins) due to the harsh environment, as well as the transcellular pathway that particles take that leads to the uptake via M cells to the transport of the particles to the lymph (Mrsny 2012). However, some strategies to overcome these barriers are being exploited, such as the use of permeation enhancers, modification of drug solubilization/permeabilization, and the use of protease inhibitors to avoid proteolytic degradation (Sant et al. 2012).

In ocular delivery, the anatomy and physiology of the eye is an interesting challenge for drug delivery. From the anatomical point of view, the blood-retina barrier for delivering drugs into the post-segment of the eye is one of the most difficult barriers to overcome. In addition, the physiologic characteristics of the eye also limit the drug delivery, due to the short drug residence time in the eye (Araújo et al. 2009). For these reasons, new drug delivery systems based on solid lipids are required for ocular purposes to facilitate drug penetration through the corneal epithelium. New strategies report the development of positively charged SLN since the slightly anionic characteristics of the ocular mucosa above its isoelectric point (Araújo et al. 2009; Sandri et al. 2010; Başaran et al. 2010) contribute to particles' adhesion onto the ocular surface increasing drug residence time (Araújo et al. 2009).

For intravenous delivery, there are also some features, e.g. the particle size and size distribution need to be optimized to allow the circulation of particles through the vasculature. The main problem is the activation of the reticulo-endothelial system (RES), responsible for the uptake of the particles to the spleen and kidney (Yoo et al. 2011). Many strategies to overcome the particles recognition by the immune system include the use of polyethylene (PEGs) recovering the particles' surface to extend their residence time circulation in the bloodstream. This strategy also named as "stealth technology" results in long circulating particles loading active molecules with high target efficiency and activity (Moghimi et al. 2001; Cavadas et al. 2011). For brain delivery and targeting the particles have been tested, where the blood–brain barrier limits the passage of drugs. Furthermore, the surface of SLN can easily be modified offering drug targeting to tumours, the liver, and the brain with many ligands (e.g. monoclonal antibodies, DNA) (Harms and Müller-Goymann 2011; Blasi et al. 2007).

Dermal and transdermal delivery are an area of great interest for delivering drugs. The main advantages that SLN offers by this route are (1) chemical protection of drugs, (2) modulation of release profile, and (3) occlusive effect provided by SLN due a formation of an occlusive adhesive lipid film (Battaglia and Gallarate 2012).

One the major barriers of the skin is the stratum corneum, which offers a protective and effective barrier to microorganisms (Neubert 2011) and also avoids SLN permeation. SLNs show good biocompatibility and biodegradability, since they are composed of lipids that are present in the skin's structure (Souto et al. 2011b).

Table 5.2 summarizes examples of SLN formulations with respect to their therapeutic effect and administration route.

5.6 Conclusion

SLN emerged in the last decade as innovative and versatile drug delivery systems due to the many advantages they show relative to other traditional therapies. From a physicochemical point of view, SLN are simple delivery systems based on solid lipids that may be used for the delivery of many problematic drugs, such as peptides, proteins, and poorly water-soluble drugs. Knowledge about the physicochemical parameters of SLN is important due to the influence of these physicochemical parameters on biopharmaceutical behaviour. Even though this knowledge has increased considerably, over the years, it is very important to evaluate the interactions of drugs with SLN as this will aid in the prediction of the SLN in vivo pharmaceutical profile. In conclusion, intensive structural investigations are required if SLN are to be accepted as the new generation of drug carriers.

Problem Box

Question 1

(a) What are the main excipient components used in development of solid lipid nanoparticles (SLN) and how are drugs loaded within the lipid matrix?
(b) Why the determination of hydrophilic lipophilic balance (HLB) of the separate components is so important?

Answers

(a) The main components required for the development of SLN are the solid lipid, emulsifier, co-emulsifier (optional), and water. Lipophilic drugs are loaded within the lipid matrix, i.e. they need to be soluble in the solid lipid chosen in order to be molecularly dispersed and form a thermodynamically stable lipid matrix. For hydrophilic drug encapsulation, SLN are prepared using a water-in-oil in water emulsion technique, where hydrophilic drugs are loaded within the inner aqueous phase surrounded by the lipid matrix.

(continued)

Table 5.2 Examples of drugs loaded in SLN and their therapeutic effect, type of lipid used in the lipid matrix, surfactants and respective administration route

Drug	Therapeutic applications	Solid lipid	Surfactant(s)	Administration route	References
Artemether	Antimalaria	Dynasan®114	Tween® 80	Oral	Aditya et al. (2010)
Carvedilol	Antihypertensive	Imwitor®900 K	Pluronic® F 68 and, soybean phospatidylcholine	Intraduodenal	Venishetty et al. (2012)
Clotrimazole	Antifungic	Dynasan®116	Tyloxapol	Dermal	Souto and Müller (2005)
Clozapine	Antipsychotic	Dynasan®114, 116, and 118	Pluronic® F 68, soybean phospatidylcholine, and stearylamine	Intravenous Intraduodenal	Manjunath and Venkateswarlu (2005)
Cyclosporine A	Immune suppressor	Imwitor®900	Tagat S and sodium cholate	Oral	Müller et al. (2008)
		Compritol® 888 ATO	Pluronic® F 68 and Tween® 80	Ocular	Gokce et al. (2008)
Diazepam	Sedative, anticonvulsivant	Cetyl palmitate	Plantacare	Rectal	Sznitowska et al. (2001)
Fluticasone	Corticosteroid anti-inflammatory	Precirol® ATO 5	Tween® 80 and soybean phospatidylcholine	Dermal	Doktorovova et al. (2010)
Gatifloxacin	Antibiotic	Stearic acid and Compritol® 888 ATO	Pluronic® F 68	Ocular	Kalam et al. (2010)
Insulin	Diabetes mellitus	Softisan®100	Lipoid® S75 Pluronic® F 68	Oral	Fangueiro et al. (2013)
		Stearic acid and palmitic acid	Soybean phospatidylcholine and sodium cholate	Pulmonary	Liu et al. (2008)
Ketoconazole	Antifungic	Compritol® 888 ATO	Pluronic® F 68 and sodium deoxycholate	Dermal	Souto and Müller (2005)
Ketoprofen	Nonsteroidal anti-inflammatory	Beeswax and carnauba wax	Tween® 80 and egg phospatidylcholine	n.a.	Kheradmandnia et al. (2010)
Lopinavir	HIV infection	Compritol® 888 ATO	Pluronic® F 127	Intravenous	Aji Alex et al. (2011)
Paclitaxel	Chemotherapic	Precirol® ATO 5	Tween® 80	Pulmonar	Videira et al. (2012)
Paromomycin	Antimicrobial	Cetyl palmitate and stearic acid	Tween® 80, Span® 85	Dermal	Ghadiri et al. (2012)

(continued)

Table 5.2 (continued)

Drug	Therapeutic applications	Solid lipid	Surfactant(s)	Administration route	References
Rifampicin, isoniazid and pyrazinamide	Antitubercular	Stearic acid	PVA	Pulmonar	Pandey and Khuller (2005)
Saquinavir, Stavudine, and Delavirdine	HIV infection	Compritol® 888 ATO, Dynasan® 116, and cacao butter	Phospatidylcholine, cholesteryl hemisuccinate, and taurocholate	n.a.	Kuo and Chung (2011)
Taspine	Acetylcholinesterase inhibitor	Compritol® 888	Pluronic® F 68	Intravenous	Lu et al. (2008)
Timolol	Non-selective beta-adrenergic receptor blocker	Phospholipon 90G®	Tween®80	Ocular	Attama et al. (2009)
Titanium dioxide	Sunscreen	Dynasan® 116	Tyloxapol®	Dermal	Cengiz et al. (2006)
Tobramycin	Antibiotic	Stearic acid	Sodium taurocholate and Cremophor® EL	Ocular	Cavalli et al. (2002)
Tretinoin	Potential anti-cancer drug	Precirol® ATO 5 Compritol® 888 ATO Dynasan® 114 Dynasan® 118	Tween® 80	Oral	Das et al. (2011)
Vitamin E or α-tocopherol	Anti-aging	Cetyl Palmitate	Tego Care® 450	Dermal	Fangueiro et al. (2012)

Problem Box (continued)

(b) The determination of HLB is very important if one wishes to develop a long-term stable system in which the solid lipid and emulsifiers used are in thermodynamic equilibrium. The HLB value may also be useful when predicting the amount of each component to be used in the final dispersions. Since emulsifiers and solid lipids should be chemically and physically compatible, the blend of the emulsifiers should be adjusted to take account of the length of the lipid chains at the interface. This will guarantee the stability of the oil droplets in the continuous aqueous phase.

Question 2

(a) What are the main advantages associated with the use of SLN for oral drug delivery?
(b) What are the routes of administration already exploited in the use of SLN in drug delivery?

Answers

(a) The main advantages of SLN for oral drug delivery are the protection of the drug from chemical and enzymatic degradation, the improvement in drug bioavailability by optimizing drug loading and release profiles, the possibility of targeted delivery of the loaded drug, the long shelf life and low chronic toxicity of the dosage form, and the excellent physical stability of the final product.
(b) The routes of administration already exploited in the use of drug delivery SLN are the oral, pulmonary, dermal, transdermal, ocular, intravenous, and rectal routes.

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