Review Article

# Lipid Nanoparticles at the Current Stage and Prospects – A Review Article

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**ABSTRACT**

Lipid nanoparticles are developing rapid in the field of the constantly demanding modern medicine. This article focuses on the main features related to the theoretical and practical development of the two main types of lipid nanoparticles – solid lipid nanoparticles (SLN) and nano-structured lipid carriers (NLC). A list of the basic parameters that should be evaluated when developing a new formulation is presented alongside with comment on the benefits of using lipid nanoparticles via different administration routes, evidences of their low toxicity and the most commonly used production methods.

**Keywords:** Solid lipid nanoparticles, Nano-structured lipid carriers, administration, perspectives.

## INTRODUCTION

During the past years nanotechnology became one of the most innovative and perspective areas in medicine. The increasing demand for achieving higher bioavailability levels of "difficult" molecules due to their poor water solubility, low stability or other drawbacks resulted in introduction of new dedicated technologies. Different types of nanoparticles are developed and many of them already attract the commercial attention for their health benefits in medicine and cosmetics. Amongst these – inorganic, polymeric nano-particles, liposomes, nanocrystals, nanotubes, dendrimers and lipid nanoparticles are of biggest interest[1].

Lipid Nanoparticles (LN) are the subject of this article (*Fig.1*). They can be subdivided into two big groups – *Solid Lipid Nanoparticles* (SLN) and *Nanostructured Lipid Carriers* (NLC).

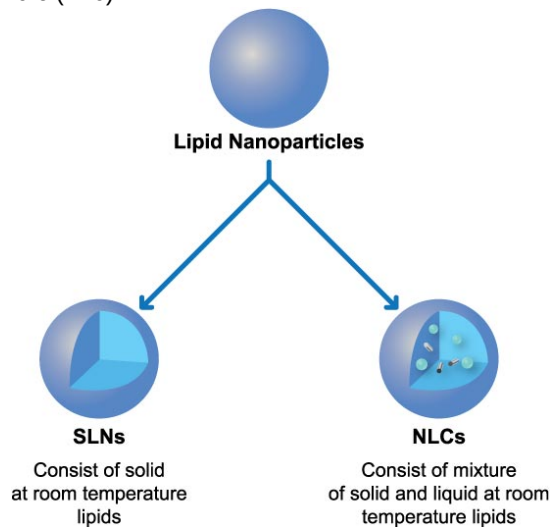


**Figure 1:** *Difference between the two basic types of lipid nanoparticles*

The first SLN were formulated in the early 1990s and since then the number of scientific groups and patents related to them is increasing rapidly[2]. Obtained from solid at room temperature lipids, SLN combine 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[3-5].

NLC consist of mixture obtained from solid and liquid at room temperature lipids. The use of such mixtures aims to achieve better physicochemical properties compared to compositions that contain only solid lipids. Because of the closeness between the two types of LN some scientific groups consider that NLC are just the next step in the development of SLN[6].

LN outmatch other nanoparticles by important parameters.

- *Better stability* compared to the liposomes due to their rigid core that is solid at room and body temperatures[2,7,8] (*Fig.2*).
- *Lower toxicity* and *better tolerability* as an alternative to polymeric systems[6], which is considered to correlate with biodegradable nature of the lipid carrier[9].

LN offer many other opportunities, possibilities and potential benefits. In this article we will try to summarise the most important of them and the critical factors to develop, produce and test a successful formulation.

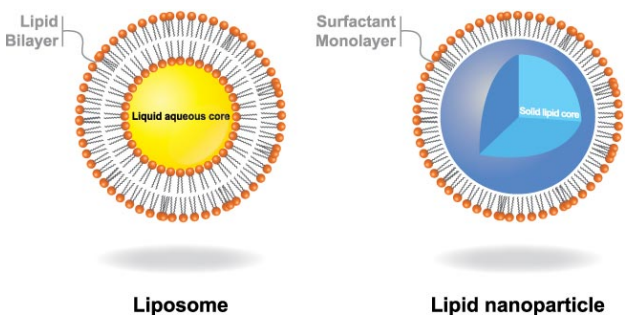


**Figure 2:** *Comparison between liposomes and lipid nanoparticles*

## CHARACTERISTICS OF LIPID NANOPARTICLES

Different physicochemical factors of LN (e.g. composition, size, shape, entrapment efficiency, drug loading capacity, drug localisation etc.) can affect the drug delivery properties - ability to pass through biological membranes, rate of release, drug bioavailability, stability, toxicity, elimination, circulation time in the bloodstream etc.

### Composition

LN can be regarded as obtained by replacing the liquid lipid phase in nanoemulsion with solid one. They consist of drug trapped in biocompatible solid lipid core and surfactant at the outer shell[6,7]. Most of the excipients used for their production are recognised as safe worldwide for pharmaceutical use and listed in the EU and US pharmacopoeias. Amongst the most frequently used are free fatty acids, different glycerol esters, natural oils, surfactants, coating materials. Their suitability is predetermined by their high chemical stability[10], relatively low melting point[5], and relatively low chemical and physiological reactivity[10].

Examples of combinations of different lipids, surfactants and compounds are presented in *Table 1* at the end of the article.

The main component in this type of nanoparticles is the lipid or lipid blend. Naturally their properties will strongly depend on the type of used lipids and on their characteristics. For example the lipid droplets in nano-emulsion are spherical in result of their liquid state tending to acquire the smallest possible thermodynamically favourable surface. When the nano-emulsion is cooled it turns into solid state and a polymorphic transition can occur. The lipids then can undergo polymorph transformations (e.g. from alpha to beta' or/and to beta forms etc.). Such crystallinity can further lead to changes in the particles shape, e.g. from spherical to needle, platelet or other[11]. A shape change can potentially result in surfactant insufficiency to cover the increased surface and cause particle aggregation and instability. It is important these potential events to be studied and predicted in the light of a successful formulation development. For instance, in such cases when the shape change leads to insufficiency of surfactant, an excess of surfactant can be added after solidification to increase the stability[11].

The other basic components in LN are the surface active compounds (SAC). They play significant importance to the LN properties (e.g. size, stability, zeta potential)[12]. SAC from almost all groups (both ionic and non-ionic) are used. Combinations are often preferred to increase stability, supported by the knowledge that a competition for binding interface sites between surfactants from same class may occur[13]. An individual approach is needed when choosing SAC in different formulations to ensure the formation of stable LN.

Recent years different reports on improved stability after applying a coating polymer layer are published. This approach is popular in the production of liposomes but may be applied to LN as a way to improve characteristics (e.g. improved stability, extended blood circulation, higher bioavailability, targeting etc.). Different types of coated LN are reported including – PEG-(Poly ethylene glycol)-ylated[14], mannosylated[15] and coated with silk fibroin[16]. In a formulation with coated LN, the outer shell can be regarded as another very important part of their structure.

### Size

LN are defined as colloidal particles in the range between 50nm and 1000nm[17]. The size depends on the type and amount of the excipients, method of preparation, stability, storage conditions, and chemical nature of drug. For example the type and concentration of surfactants appear to have major effect on particle size[18].In general the average particle size decreases with increasing SAC concentration in the formulation (*Fig. 3*).

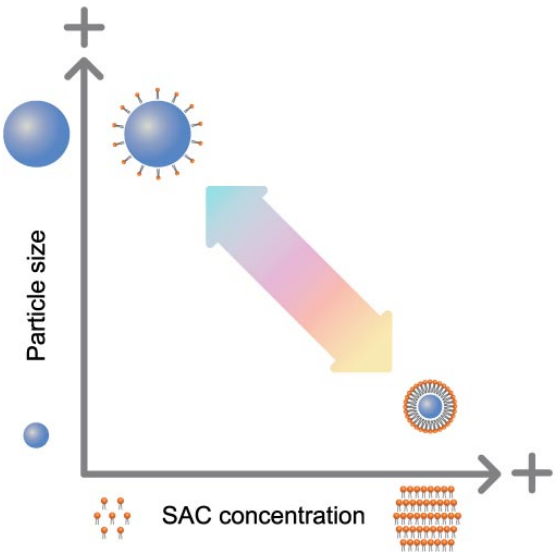


**Figure 3:** *Dependence of particle size and SAC concentration*

Size consistency is also an important sign for stability of the system. Often instability is related with an increase in particle size (aggregation) during storage. Examples of different formulations, production methods and size of the particles can be found in *Table 1* at the end of the article.

### Shape

Particle shape can influence the particles contact surface area. Therefore the amount of surfactant needed to cover it, the surface that is exposed to degradation processes, the surface that is available for association with plasma proteins etc. will strongly depend on the intrinsic shape of the particle.

Shape depends on different factors. The main factor affecting the shape is the composition of carrier with its structure forming ingredients in LN, i.e. the lipids. As was stated earlier they can exhibit polymorphic transitions during solidification which depend on the specific properties of the used lipid or lipid blend.

Shape can also depend on other factors – e.g. size. In one survey the effect of size on shape was studied. LN larger than 200 nm were mostly spherical while the smaller particles – less than 100 nm had more pronounced isometric shape[19].

### Drug localisation

Depending on their nature and the properties of the carrier drugs can be located in different regions[4], illustrated on *Fig. 4*. Drug localisation is an important characteristic that influences the release rate from the particles.

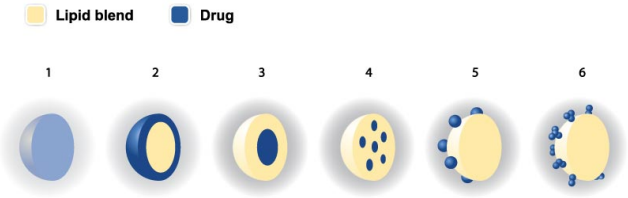


**Figure 4:** *Different possibilities of drug localisation in lipid nanoparticles after preparation. The drug can: (1) homogenously dissolve in the full volume of the particle; (2) migrate to surface layers; (3) migrate to the centre; (4) disperse in the particle; (5) attach to the particle surface; (6) attach in the form of clusters to the particle surface.*

Loading into the superficial layers of the LN or on its surface can result in burst release whereas drug location into the core will tend to a slower release[7,20].

Numerous factors can affect the drug localisation within the LN – type of lipid, lipophilicity of the drug, size of the drug molecule, type of surfactant etc. In most cases accurate prediction is hard to make.

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

Two critical parameters to measure the effectiveness and ability of the LN to encapsulate and hold the drug are commonly used, namely *Entrapment efficiency* and *Drug loading capacity*.

EE is expressed as the amount of drug included in the LN, related to the quantity used in the process (Eq. 1)[21]. It is an important indicator in case of creating effective formulations. EE can be influenced by different factors, for example solubility of the drug in the lipid melt[22].

$$EE\% = \frac{Amount\ of\ drug\ in\ the\ LN}{Amount\ of\ drug\ added\ to\ the\ formulation} * 100 \qquad \textbf{(Eq. 1)}$$

DL is another parameter that serves to estimate the reliability of the production process (Eq. 2):

$$DL\% = \frac{Amount\ of\ drug\ in\ the\ LN}{Amount\ of\ drug\ added\ to\ the\ formulation + Amount\ of\ excipients\ added} * 100 \qquad \textbf{(Eq. 2)}$$

Both parameters possess important value in prediction the uptake capacity of the lipid phase for a given drug.

### Melting interval

The melting interval of the lipid blend as well as the melting interval of the LN is critical in the cases when hot preparation methods are chosen or when stability issues due to the melting temperature exist (e.g. degradation). Melting temperature also influences the encapsulation rate and drug release. The effects of hardness and melting point on encapsulation rate of different oils (e.g. coconut, jojoba oil and macadamia oil) used to prepare LN were examined. Encapsulation increased with oil hardness (coconut oil) while the drug release rate from the SLN showed inverse tendency (macadamia oil)[23]. In another survey LN with relatively low melting temperature also exhibited rapid release of the loaded drug[24]. The melting interval also has effect over the chance of alpha to beta and beta` transitions of the lipid. Usually lipids with high melting point are less likely to undergo transition. Some scientists consider that a temperature of 50°C is optimal for obtaining SLN[25]. We suggest that every formulation needs an individual approach.

At *Fig.5* can be observed that at high melting temperature drug encapsulation increases but the release rate is low due to more rigid structure of the particles. At the opposite at low melting temperatures drug release rate is high, but drug encapsulation is low due to the increased softness. It can be concluded that the selection of carrier with proper melting temperature is important prerequisite for obtaining the anticipated encapsulation and release rate. However for every different lipid blend and drug the optimal relationship between melting interval, drug encapsulation, and drug release should be examined.

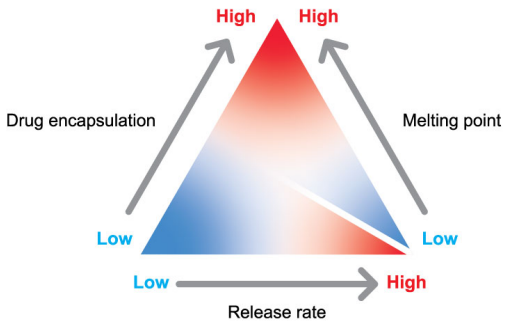


**Figure 5:** *Relationships between melting point, release rate and drug encapsulation*

### Drug release

The detailed mechanism of drug release from LN *in vivo* is poorly understood. There are suggestions that the two main possibilities are diffusion of the drug or/and degradation of the lipid matrix. These processes are not well studied. *In vitro* method(s) to observe the drug release from the carrier should be established[26-28]. The method itself should be appropriate to test LN formulation as well as the administration route for which it is intended. *In vitro* test is also important in cases of

predicting the release profile of lipid nanoparticle formulations intended for sustained release.

The basic parameters in these tests should be type of receptor medium, pH, temperature, presence of enzymes etc. For example - phosphate buffer with pH=7,4 and temperature of 37°C were used for characterisation of release rate of LN loaded with doxorubicin[29].

**Stability**

Stability is one of the most important issues in the process of developing a new formulation. It can be affected by physical, chemical, microbiological or other factors. The main technological problem with lipid nanoparticles is the gel-formation[8]. Agglomeration also occurs but it is considered a less important problem[2]. Different parameters can predict to some point the stability of the formulation – zeta potential, particle size, surfactant concentration, storage temperature. High temperature, light, and shear stress promote the gelation of amorphous lipid particles due to modification of the lipid crystals to beta-modification which result in crystallisation. Hence, the particle surface can increase due to the preferred formation of platelets[30]. Depending on the parameters that affect stability there are different approaches to stabilise the system – for example the gel formation can be limited by adding co-emulsifying agents[24], lyophilisation[29] and storage at low temperatures[29]. Despite the expressed tendencies to gel-formation and agglomeration, stability of over two and three years was reported[31,32].

**Zeta potential**

Zeta potential is known as one of the indicators for the stability of suspensions[33]. It can serve as a prediction parameter of the storage stability of LN. Usually relatively high values of zeta potential indicate that the nanoparticles are charged and the agglomeration is suppressed due to electric repulsion[34].

## BENEFITS OF USING LIPID NANOPARTICLE FORMULATIONS IN VARIOUS ADMINISTRATION ROUTES

The human body offers a variety of opportunities for drug application. The U.S. Food and Drugs Administration (FDA) has approved more than hundred administration routes. The most popular are the oral, parenteral, dermal, pulmonary, nasal, ocular etc. Lipid nanoparticles find application in almost every one of them, offering substantial benefits.

**Administration of lipid nanoparticles via the oral route**

The oral route is considered the safest, least expensive, easy and convenient to the patients. Drugs from almost all pharmacological groups are administrated by that way. However in some cases it has some disadvantages related mainly to the acidic environment of the stomach and the variety of drug metabolism enzymes localised in the gastro-intestinal tract (GIT). These factors may lead to degradation and/or inactivation, respectively low and insufficient bioavailability.

Improved biopharmaceutical parameters after oral administration of lipid nanoparticle formulations were reported by various authors[35-37]. This is explained with better stability in GIT and increased absorption[38].

Solubility and permeability of the active compounds are as important as the stability. Poor soluble molecules are eliminated from the gastro-intestinal tract before they fully dissolve and reach the blood[39]. LN give the opportunity to dissolve the lipophilic drugs into their lipid matrix. This way they can release the drug in a molecular state on the site of application or even on the site of action. Particle size also has impact on solubility. Smaller size refers to bigger contact surface and faster dissolution, respectively increased bioavailability[40].

The oral administration of proteins is one of the biggest challenges of modern medicine. Lipid nanoparticles give encouraging results related to improved bioavailability and reduced degradation of peptides by proteolytic enzymes[41-44]. Insulin loaded SLN with lecithin showed improved stability against proteolytic enzymes *in vitro*[45]. Another study with insulin loaded lipid nanoparticles showed promising results in the efforts of creating a sustained drug release system for non-parenteral administration[46].

**Parenteral administration of LN**

The parenteral route ensures fastest and full bioavailability, it does not influence or interact with the digestive system, it bypasses the liver first pass metabolism, and is used in cases when the patient`s mental or physical state makes other routes impossible.

Literature reports demonstrate that LN can be sterilized[7]. LN are also good candidates for lyophilisation[47] thus suitable as aseptic drug carrier. Different life saving drugs for parenteral administration are successfully incorporated in lipid nanoparticles aiming to prolong the drug effect, to improve biopharmaceutical characteristics, to lower side effects[29] of antifungal[48], antibiotic[49], antiviral agents[50] etc.

**Dermal administration of lipid nanoparticles**

Dermal route at present is the major area of interest for LN application. The skin is a complex structure with alternating hydrophilic and lipophilic layers including mostly water, lipids and proteins. This organisation (particularly *stratum corneum*) is difficult to overcome especially for large molecules, poorly soluble drugs and highly lipophilic/hydrophilic substances.

Main advantages and possibilities that LN formulations for dermal application offer are: epidermal targeting, follicular delivery, controlled drug delivery, prolonged effect, increased photo stability of the active ingredients and increased skin hydration (due to a great occlusivity – *Fig.6*) [51,52].

Important advantage of LN to the conventional dermal forms is improved dermal uptake after topical application[53] and high accumulation in skin *in vivo*[54]. A

good chance to exchange lipids with the skin surface also occurs, which appears to be very promising approach to enhance drug penetration[55]. Another benefit of LN is that they contain mainly natural lipids which are harmless to the skin.

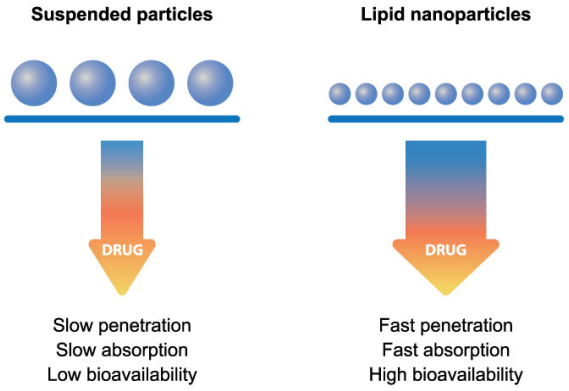


**Figure 6:** *Distribution of suspended and nanoparticles on skin. Nanoparticles have tighter distribution because of their small size and cause greater occlusion effect.*

Varieties of opportunities for combination with different traditional vehicles are possible. A promising approach to increase the drug penetration and absorption through skin was demonstrated with the combination of SLN with foams[56]. Combinations with other frequently used vehicles are also possible.

LN offer promising alternative for delivery of actives used in cosmetics[51]. A recent study compares the NLC formulation of Coenzyme Q10 to nanoemulsion[57]. The Q10 loaded NLC show good long-term physical and chemical stability. It becomes evident from the accelerated tests that Q10 is rather more stable when entrapped in NLC than in nanoemulsion. As the liposomes at the beginning of their usage LN start to find application in cosmetics. And the first NLC based cosmetic product - the Cutanova Nanorepair Q10 cream is already available on the market[58] followed by rising number of new LN based cosmetic individual products and cosmetic series[52]. 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[58].

**Pulmonary administration of lipid nanoparticles**

The pulmonary route is gaining popularity as an alternative possibility for administration. It is non-invasive, can be used for both local and systemic drug delivery, thanks to the large absorptive mucosal surface and the rich blood supply of the lungs. Particles with diameter less than five micrometres can reach to the alveoli[59], which makes the lipid nanoparticles with their submicron sizes to be regarded as attractive delivery system to the lower respiratory way.

Counting on the fact that metabolic enzymes are present in the lungs but yet different and less than those observed in gastrointestinal tract the therapists consider pulmonary route very promising[60], particularly for proteins[61]. Insulin loaded SLN showed promising results[62]. Beside proteins of interest for pulmonary delivery could be other molecules- e.g. paclitaxel[63].

For successful development of pulmonary drug delivery systems with LN, several unique challenges still remain. The major issue is related to ensure sufficient stability and appropriate particle size in the final pressurized spray package[64], as well as the eventual agglomeration due to collisions after the spray actuation.

**Nasal administration of lipid nanoparticles**

The nasal route is non-invasive, safe, easy to use and acceptable for self-administration. Particles with size up to one micrometre applied on the nasal mucosa rapidly enter the bloodstream[65]. LN as a non-toxic and biodegradable carriers have a huge potential in terms of nasal administration. LN formulations can be applicable for either local or systemic effect. Nanostructured lipid carriers showed the ability to increase immune responses when used to deliver nasally antigenic molecules[66]. However, they often require the addition of polymeric coatings, co-administration with other adjuvants or the presence of immunopotentiators in their composition.

Interesting part in this case are the reports for direct nose to brain drug delivery[67]. A survey concluded that nanoparticles may spread into the neurones and supporting cells by a number of endocytic mechanisms[67]. The upper size limit of nanoparticles is 100 nm in order to reach the brain via intraaxonal route of the axons in *filia olfactoria*. Detailed studies for direct nose-to-brain transport mechanism with lipid nanoparticles are still to be made. Nasal administration of risperidone lipid nanoparticles showed to be more effective in brain targeting after nasal administration compared to intravenous one[68].

**Ocular administration of lipid nanoparticles**

The ocular drug delivery is a subject of great interest because of the unique anatomical structure of the eyes. They are easily accessible for self-administration of drugs due to their localisation in the body. However some precorneal loss factors - tear dynamics, non-productive absorption, transient residence time in the cul-de-sac, and poor permeability of the cornea result in low bioavailability of the conventional preparations (e.g. eye drops, eye lotions, eye powders and semi-solid eye preparations). A suitable ocular formulation should release the drug in a way to overcome the protective barriers of the eye without causing permanent tissue damage. Lipid nanoparticles are ideal candidates to deliver drugs to the eyes due to their high penetration abilities, stability, lack of irritation and possibility to be surface modified and sterilized[7]. Drugs from different groups have yet successfully passed *in vitro* and *in vivo* tests – NSAIDs flurbiprofen[69]and diclofenac sodium[70], immunosuppressant Cyclosporine A[71] and others.

## TOXICITY, METABOLISM AND ELIMINATION OT LIPID NANOPARTICLES

One of the most important questions staying behind the developers is the evaluation of eventual toxicity. The specific type of drug carrier may have a tremendous effect on stability, absorption, tissue distribution, elimination, adverse effects etc.

### Toxicity

Most of the excipients used to obtain lipid nanoparticles are already utilised in preparation of parenteral emulsions since the 60`s and proved to be save[72]. It is notable that toxic effects from their degradation products are unlikely[7].

*In vitro* cytotoxicity is evaluated on several cell lines – human granulocytes, HL 60 cells (Human promyelocytic leukemia cells) and MCF-7 cells (breast cancer cell line). Studies show that LN are ten-fold[73] to twenty-fold[74] less toxic to human granulocytes if compared to alternative nanoparticles. Compared to polymeric nanoparticles consisted of poly lactic acid (PLA) and co poly lactic acid/glycolic acid (PLGA) recognised as safe polymers, SLN are 20 times less toxic on human granulocytes and HL 60 cells[74]. Tripalmitin and stearic acid based SLN in concentrations 0.10-0.25% are reported to be non-toxic and highly safe to HL60 or MCF-7 cells[35,75]. *In vitro* toxicity of lipid nanoparticles is studied and evidences for extremely low levels or lack of toxicity are demonstrated by variety of scientific teams[2,35,75-77].

Further fact concerning the safety is that with its size range LN do not trigger macrophage activation and cytokine production[77].

### Metabolism

LN consist mainly of lipids that can undergo biodegradation. The process includes different types of enzymes.

Degradation by lipases, alcohol dehydrogenase and in lesser extent by non-enzymatic hydrolytic processes are the main mechanisms of LN metabolism[78,79]. Surface erosion is also observed[78]. The degradation rate can be influenced by the particle size e.g. the smaller particles will have bigger contact surface thus faster surface erosion.

Protein binging of LN is also subject of interest. Survey reports demonstrate that the plasma proteins taking part in this process are fibrinogen, IgG, IgM, apolipoproteins, transthyretin, and albumin[80]. Some factors like type and amount of the used surfactant can affect the type and rate of protein binging of LN[80].

### Elimination

Nanoparticles are excreted fully only when their size is sufficiently small[35]. This statement is addressed mainly for the non-degradable particles. LN consists of bio-degradable lipids that undergo *in vivo* reduction in size over time thus resulting in full elimination over time.

## PREPARATION TECHNIQUES

Presently two basic approaches, mechanical (top-down) and chemical (bottom-up) are used in the preparation of LN[81]. The top-down techniques use external energy to break down larger agglomerates into smaller nano - aggregates. Chemical processes refer to techniques where chemical reaction and/or self-assembly is used. Here opposite to the first approach the input of mechanical energy is limited to prevent unwanted processes (e.g. chemical or physical instability). Organic solvents can be eventually employed in the preparation. In cases when organic solvent is used issues with its safety and toxicity may arise.

Other classification divides the techniques to high-energy and low-energy depending on the external amount of energy used for the preparation of LN[82].

Different techniques for the production of lipid nanoparticles are known and plenty of sub-modifications are described in the literature. Below are summarised the most commonly used of them.

### High pressure homogenisation (HPH) technique

This technique is one of the most frequently used[72,83,84]. The pre-formulation is passed through a minute gap in the homogenising valve. This way high turbulence and shear, combined with compression, acceleration, pressure drop, and impact are induced. As a result disintegration of particles and dispersion throughout the product occur. HPH has some advantages over the other methods including easy scale up, avoidance of organic solvents and short production time. High pressure homogenisers are widely used, that is why this technique is considered as being industrially the most feasible one. In the industry 50 to 150 kg of SLN dispersions per hour can be obtained by placing two homogenisers in series[85].

High pressure homogenisation divides into cold and hot techniques (*Fig.7*).

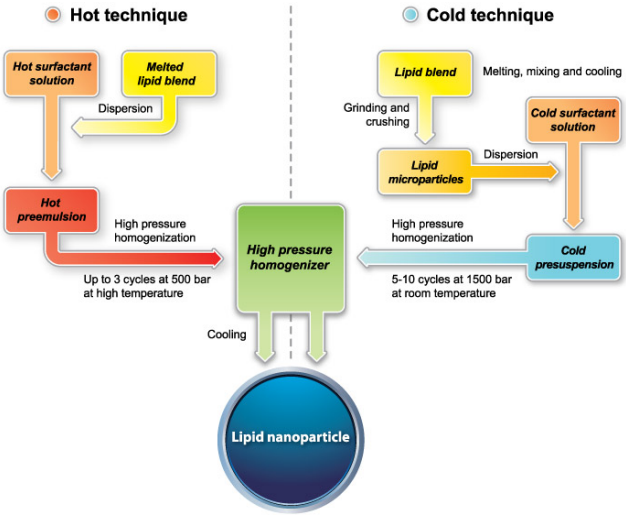


**Figure 7:** *Scheme of hot and cold high pressure homogenization technique*

In the hot technique the lipid blend and the surfactant solution are heated 5-10$^0$C above the melting point of the lipid blend. Then the lipid blend is dispersed in surfactant solution by high speed stirring. The obtained emulsion is passed through a high pressure homogeniser adjusted at the same temperature. Usually three cycles at 500 bars or two cycles at 800 bars are enough. Then the formulation is cooled down to solidify the lipid nanoparticles.

In the cold technique the lipid melt is mixed at higher temperature and then cooled down. After solidification it is crushed and grinded to obtain lipid micro particles. Then the particles are dispersed in cold surfactant solution to form a cold pre-suspension. Then the cold pre-suspension is passed through a high pressure homogeniser at room temperature usually for 5-10 cycles at 1500 bar. Although the cold technique gives larger particles it is appropriate for thermo labile drugs or in cases when the hot method is inapplicable due to drug migration in the aqueous phase during homogenisation.

### High shear homogenisation technique

Technique widely used in the production of nano-dispersions[84] (*Fig. 8*). The melted lipid blend and hot surfactant aqueous solution are homogenised in high shear mixer[86]. Although it is easy to handle the dispersion quality is often poor. It is most often combined with ultra-sonication to obtain better results[87]. The influence of optimisation of different parameters in this method is on an early stage of investigation[88].

### Ultrasonication technique

This is a mechanical method based on the use of powerful ultrasound (20 kHz; 10 MHz) that causes acoustic cavitation in an aqueous solution of the surfactant in which the melted lipid blend is dispersed[24,52] (*Fig. 8*). This cavitation causes disintegration of the lipid phase into smaller particles. The compounds are subjected to ultrasound by using sonication bath or sonication probe. Usually this technique is combined with another type of homogenisation. Preparation of the primary emulsion carries risk of metal contamination, which necessitates a filtration of the primary emulsion through 0.45µm filter[28].

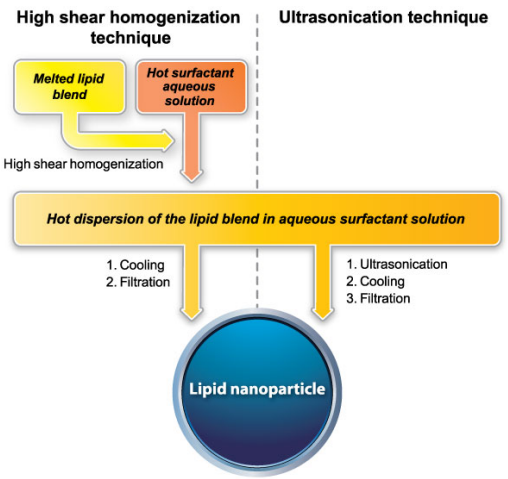


**Figure 8:** *Scheme of similarities and differences between high shear homogenization and ultra-sonication technique*

### Membrane contractor technique

A relatively novel approach to obtain LN in which the lipid blend is heated to a temperature above its melting point and passed through membrane pores[89] (*Fig. 9*). This leads to formation of small droplets. The aqueous phase is stirred and circulates inside the membrane module, it swaps away the droplets.

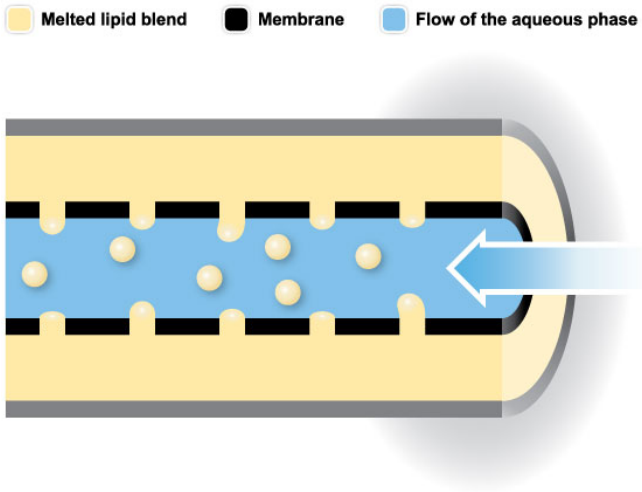


**Figure 9:** *Membrane contractor technique*

The LN are formed after cooling the aqueous phase down to room temperature. Usually nitrogen is used to create pressure on the melted lipid blend. The method has several advantages such as control of the particle size by altering the pore size and its scaling up ability[89].

### Microemulsion technique

First developed by Gasco in the early 90s, this method includes preparation of warm microemulsion by using melted lipids, surfactants and co-surfactants. In subsequent stage the hot microemulsion is dispersed in cold water (2$^0$C -3$^0$C) under stirring, causing the nanodroplets to transform into solid particles[90]. LN obtained by this technique are spherical in shape and have a narrow size distribution. Ratios of the warm micro emulsion to the cold water can vary but usually are in the range from 1:25 to 1:50.

### Double emulsion technique

Approach primary used to encapsulate hydrophilic drugs or peptides based on the preparation on W/O/W emulsion[91] (*Fig. 10*) First the drug is dissolved in an aqueous solution and then emulsified in melted lipid blend to form a primary emulsion. This primary emulsion is stabilised with gelatine, Poloxamer 407 or other suitable excipients. Then it is dispersed in aqueous solution of hydrophilic emulsifier to form a double emulsion. Then the double emulsion is stirred and isolated by filtration[92].

Relatively large particles are obtained with this technique but an advantage is the possibility of surface modification (e.g. PEG-ylation)[91].

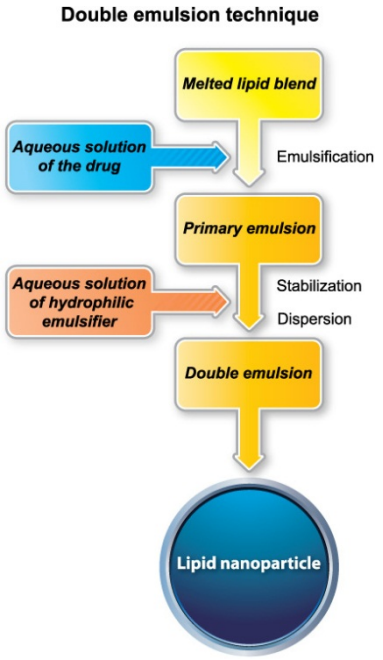


**Figure 10:** *Scheme of double emulsion technique*

### Emulsification-solvent evaporation technique

This technique is based on solvent emulsification-evaporation method previously used to obtain polymeric nanoparticles[93]. The lipid blend is dissolved in organic solvent. Then this mixture is emulsified in surfactant water solution. Next the organic solvent is evaporated and the lipid precipitates as nanoparticles in the water. The mean particle size is function of the lipid and surfactant type and their ratio. Most often it varies from 30 to 100 nm. Main disadvantage of the method is the use of organic solvents which are toxic and difficult to be fully evaporated.

### Emulsification-solvent diffusion technique

This approach uses a micro emulsion obtained by partially water-soluble solvent such as ethylformate, methylethyl ketone or benzyl alcohol[94]. The method is based on the water miscibility with these solvents. Once the transient O/W emulsion (containing lipid phase into water saturated organic solvent and surfactants) is mixed with excess of water, the lipid phase solidifies instantly due to diffusion of the organic solvent into the continuous phase. Thus the nanoparticles are obtained by adding water to the primary emulsion and extracting the solvent. LN with size below 100 nm can be produced with this technique. By varying the lipid/surfactant ratio, concentration and the type of the solvent - particles with different size can be obtained[94].

### Solvent injection (or solvent displacement) technique

Technique in which a solvent that distributes very rapidly in water (DMSO, ethanol) is used[95]. First the lipid is dissolved in the solvent and then it is transferred into surfactant/water solution. The solvent migrates rapidly in the water and lipid particles precipitate in the aqueous solution. Particle size depends on the velocity of distribution processes. Higher velocity results in smaller particles. The more lipophilic solvents give larger particles which may become an issue. The method offers advantages such as low temperatures and low shear stress.

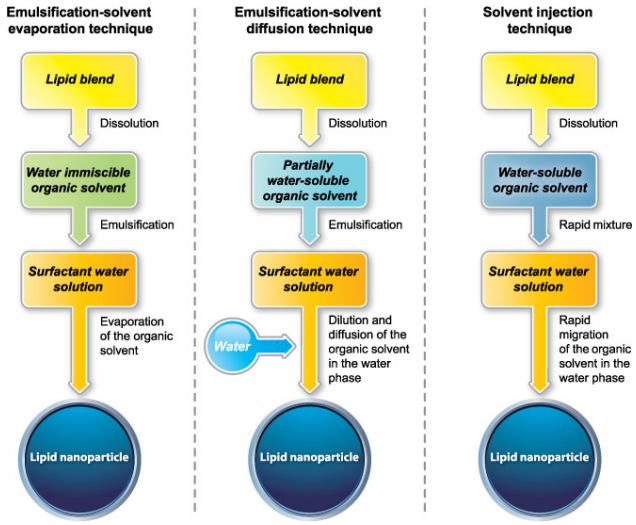


**Figure 11:** *Schemes of techniques employing organic solvents*

### Supercritical fluid technique (SCF)

A novel technique, in which a supercritical fluid is employed. The supercritical fluid has unique thermo-physical properties. It can be either gas or liquid. Critical temperature of the fluid is the temperature above which it is not possible to liquefy a gas. As the pressure raises the density and the ability of the fluid to dissolve compounds increases while the viscosity remains relevantly constant. Thus under high pressure and appropriate temperature in the supercritical range the fluid can dissolve or liquefy the compound (for example drug containing lipid). Gases used in this method are CO2, CHCl2, and CH2FCF3 etc. CO2 is one of the best options because it is safe, cheap, non-irritable, and relatively inert and has a low critical point [31.5°C, 75.8 bars][96]. SCF technology can be used in variety of processes for micro/nanoparticle production: rapid expansion of supercritical solution (RESS), particles from gas saturated solutions (PGSS), gas/supercritical antisolvent (GAS/SAS), aerosol solvent extraction system (ASES), solution enhanced dispersion by supercritical fluids (SEDS), which can all find application in LN production depending on the drug solubility in SCF[96,97].

### Phase inversion technique

Phase inversion technique is based on the inversion in the phases of an emulsion[98]. The method uses a ternary diagram to determine lipid, surfactants and water concentrations. In this technique the proportion of hydrophilic surfactant has major influence on the average diameter and the size distribution of the particles.

## CHARACTERISATION AND ANALYSIS OF LIPID NANOPARTICLES

One of the most important parameters in the characterisation of nanoparticles classifying them as such

is their size. Different techniques are used to measure lipid particles size and their distribution but the most frequently used are the dynamic laser light scattering and the laser diffraction technique. The dynamic laser light scattering is based on time dependent fluctuations in scattering intensity of monochromatic light that hits the particles. These time dependent fluctuations depend on particles size and can be transformed into a size distribution[99]. The laser diffraction technique on the other hand is based on the theory that the particles scatter the light at angle proportional to their size when a laser beam is passed through them. This method is good for measuring sizes between 0,1 and 3,000 micrometres. Particle size distribution is calculated after the intensity of the scattered light from a sample is measured as a function of the angle. This information is compared with a scattering model in order to calculate size distribution.

Other analytical methods that find application to determine different characteristics of the LN are spectrofluorimetry, fluorescent spectroscopy, parelectric spectroscopy, fluorescent measurement, optical transmittance, plate tensiometry to determine aqueous phase surfactant concentration, infrared Fourier transforms spectroscopy, nuclear magnetic resonance, combination of filtration and HPLC, infrared and Raman spectroscopy. In order to examine the distribution processes at phase interfaces spectral–spatial ESR imaging can be used.

## PERSPECTIVES AND CONCLUSION

LN as carriers with rigid lipid core can improve the stability of pharmaceutical compounds[100]. They can become solution for common problems with plenty of actives and excipients related to instability to light, oxidation and hydrolytic attacks, incompatibilities and interactions in drug products and cosmetics. They can also improve the drug stability after administration[91].

Alongside with the improved stability LN offer a variety of opportunities for modified release. Compared to alternative forms this potential is even augmented in terms of easiness by correction of some of the production parameters to adjust the release rate. For example the release of prednisolone from SLN can be adjusted by change in the surfactant/lipid ratio or by change in the production temperature[101].

LN are regarded as targeted carriers to particulate physiological systems[10]. The distribution in the body is strongly influenced by their composition and surface properties. Specific modification in production parameters and composition can control and direct LN to specific organs. For example uncoated, PEG-ylated and Polysorbate coated lipid nanoparticles show extremely different pharmacokinetic behaviour. The uncoated LN after interactions with components of the human plasma distribute mainly in the liver and spleen, while the PEG-ylated and Polysorbate coated distribute in the blood circulation and the brain respectively[10]. Thus modifications of LN can result in brain or liver targeted delivery while in other cases a formulation for prolonged blood circulation can be obtained.

Lipid nanoparticles can furthermore offer many opportunities for combinations with different traditional formulations like carbopol based gels[102] and foams[56]. Combinations with different polymers and polysaccharides – e.g. complex structures with chitosan[103], coating with mucoadhesive polymers[71] are also doable which may additionally increase their potential as drug carriers.

LN are sterilizable[7] and good candidates for lyophilisation[47] which makes them applicable in parenteral and ophthalmic preparations and in surgery.

Other positives and possibilities that LN offer are: LN can serve as non-viral vectors which can be successfully used in gene therapy[104]; radiolabelled SLN showed significant lymphatic uptake after inhalation in rats[105]; LN are non-toxic, non irritative and can be used on damaged of inflamed skin[106]; Lipid micro particles and respectively LN can be prepared with inherent for the body homolipids[107].

Regardless of all the opportunities a very detailed consideration is needed before commencing LN project (Fig. 12). The selection of administration route, production technology, drug substance and its chemical form, excipients, analytical procedures, *in vitro* and *in vivo* tests must be carefully planned and performed. The most important characteristics of the product must be appropriate drug size, high DL and EE, long-term stability, sufficient release of the active and low toxicity.

As a consequence of all the advantages, prospects and challenges that LN offer as a drug delivery system with its opportunities for cheap reproducible fabrication[108] we can hopefully observe their place in the future of pharmacy, medicine and cosmetics. The appearance of the first registered drug on the market based on LN is not very far. Such our optimism additionally lies in the fact that not less than thirty cosmetic products based on LN currently take place on the market and their popularity turns into fashion among users and specialists.

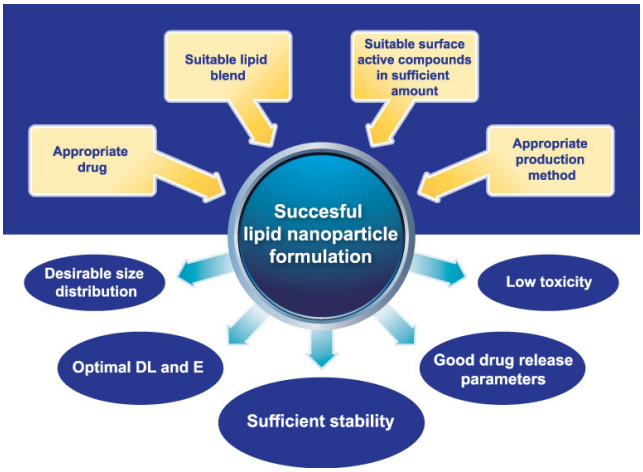


**Figure 12:** *Critical parameters in producing a successful lipid nanoparticle formulation*

**Table 1:** Size of lipid nanoparticles in various formulations

| Compound | Lipid matrix | Surfactant | Preparation techniques | Part. size [nm] | Ref. |
|---|---|---|---|---|---|
| α-tocopherol | Stearic acid | Tween 20 and sodium taurocholate | Microemulsion | 195 | 109 |
| β-carotene | | | | 187 | |
| 5-Fluorouracil | Glyceryl monostearate | Soybean phosphatidylcholine | Solvent injection-lyophylization | 189 | 47 |
| | | Tween 80 and soybean phosphatidylcholine | | 125 | |
| Actarit | Stearic acid | Soya lecithin and Poloxamer 188 | Solvent diffusion–evaporation | 241 | 110 |
| Amphotericin B | 1,2-dipalmitoyl-glycero-3 phosphocholine and cholesterol | No additional surfactant | Spontaneous emulsification and solvent evaporation | 159 | 48 |
| | 1,2-dipalmitoyl-glycero-3-phosphocholine, cholesterol and 1,2-dipalmitoyl-glycero-3-phosphate | | | 73 | |
| | 1,2-dipalmitoyl-glycero-3-phosphocholine, Cholesterol and 1,2-dipalmitoyl-glycero-3-phosphate | PEG 2000 | | 95 | |
| Artemisia arborescense oil | Glyceryl behenate | Poloxamer 188 | Hot high-pressure homogenization | 223 | 54 |
| | | Sodium lauroapho acetate | | 219 | |
| Baclofen | Stearic acid | Phosphatidylcholine and sodium taurocholate | Multiple (w/o/w) warm microemulsion | 161 | 111 |
| Bovine serum albumin | Hydrogenated castor oil | Poly (lactic-co-glycolic acid) | Double (w/o/w) emulsion–solvent evaporation | 150 | 26 |
| Carvedilol phosphate | Stearic acid | Sodium taurocholate | Solvent emulsification-evaporation | 320 | 112 |
| Chlorambucil | Stearic acid | Poloxamer and sodium glycocholate | Controlled temperature probe sonication | 270 | 113 |
| | Stearic acid and oleic acid | Tyloxapol and sodium glycocholate | | 250 | |
| Cinnarizine | Glyceryl monostearate | Soybean phosphatidylcholine | Solvent injection-lyophylization | 195 | 47 |
| | | Tween80 | | 119 | |
| Clobetasol-17-propionate | Soya lecithin | Chitosan (as a coating agent) | Solvent-injection | 248 | 114 |
| Clotrimazole | Glyceryl tripalmitate | - | Hot high-pressure homogenization | - | 115 |
| Curcuminoids | Trymyristin | Poloxamer 188 and Tween 80 | Nanoemulsion technique employing high-speed homogenizer and ultrasonic probe | 237 | 116 |
| | Tristearin | | | 314 | |
| | Glyceryl monostearate | | | 111 | |
| | Trymyristin,Tristearin, glyceryl monostearate with medium chain triglycerides | | | 178 | |
| Cyclosporine A | Glyceryl palmitostearate and propylene glycol dicaprylate/dicaprate | Tween 80 | Microemulsion | 59 | 71 |
| Dexamethasone | Stearic acid | Sodium taurocholate and soy phosphatidylcholine | Microemulsion | 80 | 117 |
| Diazepam | Cetylpalmitate | Plantacare | Hot high-pressure homogenization | 203 | 118 |
| Diazepam | Witepsol H37 | Egg lecithin | Hot high-pressure homogenization | 650 | 24 |
| Diclofenac sodium | Phosphatidylcholine and goat fat | Tween 80 | Hot high-pressure homogenization | 165 | 70 |
| DNA-naked plasmid | Glycerol distearate | Tween 80 | Solvent emulsification –evaporation | 276 | 119 |
| Doxorubicin | Monostearin | No additional surfactant | Solvent diffusion | 195 | 120 |
| Econazole | Glycerol distearate in combination with different fatty alcohols | Tween 80 | High shear homogenization | 150 | 121 |
| Enrofloxacin | Tetradecanoic acid | No additional surfactant | Hot homogenization and ultrasonication | 116 | 49 |
| | Palmitic acid | | | 111 | |
| | Stearic acid | | | 217 | |
| Flurbiprofen | Glyceryl behenate and caprylic/capric triglycerides | Macrogol-15-hydroxystearate and Tween 80 | Microemulsion and ultrasonication | 55 | 69 |
| Insulin | Hydrogenated castor oil | Poly (lactic-co-glycolic acid) | Double emulsion (w/o/w) solvent evaporation technique | 144 | 26 |
| Insulin | Stearic and palmitic acid | Soybean phosphatidylcholine and sodium cholate | Reverse micelle-double emulsion | 110 | 62 |
| Ketoprofen | Beeswax and carnauba wax | Egg lecithin and Tween 80 | Microemulsion | 75 | 122 |
| Lopinavir | Glyceryl behenate | Pluronic F 127 | Hot homogenization and ultrasonication | 230 | 37 |
| Lutein | Glyceryl palmitostearate and corn oil | Myverol 18-04K and Pluronic F68 | Microemulsion and ultrasonication | 130 | 123 |
| Lysozyme | Hydrogenated castor oil | Poly (lactic-co-glycolic acid) | Double emulsion (w/o/w) solvent evaporation technique | 135 | 26 |
| Nateglinide | Hydrogenated soya phosphatidyl choline | Triton X-100 | Solvent injection technique | 173 | 124 |
| Nifedipine | Hydrogenated soybean Phosphatidylcholine and dipalmitoylphosphatidylglycerol | - | Combination of co-grinding by a roll mill and high-pressure homogenization | 53 | 125 |
| Paclitaxel | Glyceryl tricaprylate | Soybean lecithin and Macrogol-15-hydroxystearate | Phase inversion | 49 | 63 |
| Paclitaxel | Monostearin | Poloxamer 188 | Solvent diffusion | 437 | 126 |
| | Stearic acid | | | 391 | |
| | Glycerotristearate | | | 185 | |
| | Glycerol behenate | | | 401 | |
| Quercetin | Glyceryl monostearate | Soya lecithin, Tween 80 and PEG 400 | Emulsification and low-temperature solidification | 155 | 127 |
| Risperidone | Glyceryl behenate | Sodium lauryl sulphate | Emulsion-solvent evaporation | 220 | 27 |
| Risperidone | Glyceryl monostearate, | PEG fatty acid esters and sodium deoxycholate | Hot high-pressure homogenization | 154 | 128 |
| | | | Ultrasonication | 98 | |
| Risperidone | Glyceryl behenate | Pluronic F-127 | Solvent emulsification and solvent evaporation | 148 | 68 |
| Salmon calcitonin | Tripalmitin | Lecithin | Double emulsion-solvent emulsification | 200 | 129 |
| | Tripalmitin | Lecithin and PEG | | 226 | |
| | Tripalmitin and medium chain triglycerides | Lecithin and PEG | | 207 | |
| | Tripalmitin | Lecithin and Chitosan coating | | 537 | |
| Simvastatin and Tocotrienol | Glyceryl behenate | Poloxamer 188 | Microemulsion and high-shear homogenization | 107 | 130 |
| Stavudine | Trimyrystin | Macrogol-15-hydroxystearate, Tween 80, polyglyceryl-3 dioleate and Poloxamer 188 | Hot high-pressure homogenization | 84 | 50 |
| Sylibin | Glycerol monostearate and medium chain | Pluronic F68 | Emulsion evaporation at high temperature | 232 | 131 |

| | | | | | |
|---|---|---|---|---|---|
| | triglycerides | | and solidification at low temperature | | |
| Tamoxifen | Hydrogenated palm oil and hydrogenated lecithin | No additional surfactant | Hot high-pressure homogenization | 104 | 132 |
| Taspine | Glyceryl behenate | Poloxamer 188 | Film evaporation - extrusion method | 173 | 133 |
| Topotecan | Stearic acid | Soya lecithin and Sodium taurodeoxycholate | Microemulsion | 160 | 134 |
| | Stearic and oleic acid | | | 138 | |
| Tretionin | Glyceryl monostearate | Tween 80 | Emulsification-solvent diffusion | 410 | 135 |
| Verapamil | Cacao butter | Curdlan and Tween 80 | Microemulsion and pH change | 170 | 28 |
| Vinpocetine | Glyceryl monostearate | Tween 80 and soya lecithin | Ultrasonic-solvent emulsification | 109 | 136 |
| | | Polyoxyethylene and soya lecithin | | 122 | |

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