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The Effects of Solid and Liquid Lipids on the Physicochemical Properties of Nanostructured Lipid Carriers

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Abstract

The aim of this work was to identify from a review of current literature the effects of lipids used in the development of Nanostructured Lipid Carriers (NLCs) on the physicochemical properties of the resulting formulation. The size of the solid lipid, affected by the molecular weight and the complexity of the structure, tends to affect the particle size of the final formulation proportionally; the higher the molecular weight and the more complex the molecular structure, the bigger the particle size of the NLCs. However, there is no straight correlation between the size and the structure of the liquid lipid and the particle size. Moreover, there seems to be a correlation of the solid to liquid lipid ratio which affects the particle size; there has been a trend of increasing particle size when more solid lipid was used. Regarding the entrapment efficiency, it is highly affected by the drug and its interaction with the lipids, as its solubility in the lipids needs to be high so the drug can stay entrapped within the lipid core. There was no direct correlation between the type of lipid used or the ratio and the zeta potential, which affects the stability of the NLCs.

Keywords: Nanostructured lipids carriers; Solid lipid; Liquid lipid; Ratio of solid to liquid lipid; Particle size; Entrapment efficiency; Zeta potential; Liposomes

Introduction

Lipid-based drug delivery systems have been developed[1] over the past few years in order to overcome the challenges associated with poor bioavailability of various drugs and their delivery to specific sites to achieve pharmacological action[2]. For this reason, nanoparticles had been employed to carry the drug to the desirable site of action so that the medicine would have reduced toxicity and high efficacy. For example, in case of cancer therapies a drug delivery system would target only the cancer cells whilst not affecting the healthy cells surrounding the cancer ones. It could also provide greater safety, precision and biocompatibility[3], whereas traditional medications that had been widely used for cancer treatment affect healthy cells while trying to cure the tumours, which ultimately leads to a range of side effects, including extreme fatigue. The optimal approach would be to create a drug delivery system which would target the specific site of cancer, would have good bioavailability and would not affect healthy cells[4]. Furthermore, targeted delivery systems offer better patient compliance, since few of their applications, such as inhaled formulations[5] or transcutaneous injections[6] are non-invasive, compared to other therapies (i.e. traditional chemotherapy or traditional injectable treatments)[7]. Lipid-based drug delivery systems mainly consist of liposomes, transfersomes, solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) (Figure 1).

Liposomes and transfersomes are self-assembled delivery systems and they mainly consist of phospholipid bilayer(s) as a key constituent. Based on phospholipid and surfactant selection these delivery systems can be either neutral or charged with or without the presence of cholesterol[8-10]. Their main difference is the addition of surfactant in the preparation of the transfersomes which adds elasticity to the transfersomes[11,12]. SLNs and NLCs are a different category of drug delivery systems consisting of lipids and surfactants. Their main difference is that for the SLNs preparation, solid lipids are only required, while liquid lipids are mixed with solid lipids during the preparation of the NLCs, adding more flexibility and stability to the system[13]. Furthermore, the composition of each of these drug delivery systems, including liposomes, transfersomes, SLNs and NLCs, with their advantages and disadvantages, are further explained in Table 1.

Lipids

The term lipid refers to fats, phospholipids, oils and fat-like substances which can be also found in living organisms[35]. They have very limited to almost no solubility in water[36]. Lipids are either hydrophobic or amphiphilic molecules that come from a carbanion-based condensation of thioester or isoprene units. Lipids are divided into two major categories, simple and complex lipids. Simple lipids are those which yield two groups of products upon hydrolysis, whereas complex lipids are those which yield three or more groups of products upon hydrolysis. Each of the simple and complex categories is further divided into sub-categories (Table 2)[37-40].

Simple and complex lipids classification

Fatty acyls are further divided into free fatty acids and fatty acid esters[41]. Free fatty acids are classified into short, medium and long chain fatty acids. *Short chain fatty acids* consist of less than 6 carbon atoms on their

main hydrocarbon chain, *medium chain fatty acids* consist of 6-12 carbon atoms and *long chain fatty acids* consist of more than 12 carbon atoms[42]. The chain length can affect the phase transition temperature (T_m) of the lipid. Tm is the temperature at which each lipid transforms from its solid state to the liquid state, and it can be referred to as a melting point temperature[36]. The longer the chain length is, the more interactions are in place, therefore the molecule requires higher energy to transform into the liquid state, hence the higher Tm for longer chain lipids[43]. Furthermore, fatty acids can also be grouped based on their saturation and unsaturation; they can be either *saturated* (contain only single bonds), *monounsaturated* (contain only one double bond) or *polyunsaturated* (contain more than one double bond). Fatty acid esters include N-acyl glycine, acyl carnitines and fatty acyl amino acids[41].

Glycerolipids are further divided into three classes based on the fatty acid(s) which is/are attached to the glycerol molecule[38]. *Monoglycerides* consist of one fatty acid linked to a glycerol molecule via esterification[44], *diglycerides* consist of two fatty acids which are esterified to a glycerol molecule[45], and *triglycerides* consist of three fatty acids esterified to the glycerol molecule (Figure 2)[38].

Regarding the complex lipids, the classification is mostly done based on some additional components and substitution groups. Glycerophospholipids can be further divided based on the amino alcohol group which can be esterified; the two mains groups are the lecithins and the cephalins, which contain choline and ethanolamine respectively[46] (Figure 3). Sphingolipids are mainly classed into ceramides, sphingomyelins and cerebrosides. Ceramides consist of a fatty acid and sphingosine, while sphingomyelins result from the esterification of the 1-hydroxyl group of ceramide with phosphoric acid esterified with choline/ethanolamine. Last, cerebrosides are glycosphingolipids where a glycosidic linkage exists[47] (Figure 4). Regarding the sterol lipids, there are two main categories, the steroids and the secosteroids. The steroids consist of the same four fused carbon ring and they can be classified in the C18 steroids, i.e. estrogen, C19 steroids, i.e. androgens such as testosterone and C21 steroids, i.e. progestogens. The secosteroids are characterized by a cleavage in one of the four rings[48]. Last, the number of isoprene units further divides the prenol lipids in various categories. In general, all prenol lipids which consist of more than 4 isoprene units are called polyprenols and they are further classified into isoprenoids and quinones. Bactoprenols have 10-12 isoprene units and dolichols consist of 18-22 isoprene units[49]. There is no further classification for saccharolipids and polyketides.

Classification of lipids based on the saturation and state

The main difference between solid and liquid lipids is whether the lipid is saturated or unsaturated. Saturated lipids consist of a chain that has only single bonds between carbon atoms whilst the unsaturated lipids consist of a chain that has at least one double bond between the carbon atoms[50]. Unsaturated lipids with more than one double bond on their main hydrocarbon chain are called polyunsaturated lipids[36]. Saturated lipids are solid in room temperature due to their high melting point, whereas unsaturated lipids are liquid in room temperature owing to their lower melting points. In addition, unsaturated lipids in turn introduce bends and kinks to the chain of the lipid (making more complex structure). Therefore, it is more difficult for these molecules to crystallize

and this is why the melting point is lower[36]. For example, stearic acid is a saturated fatty acid and its melting point is around 70°C[51], whereas oleic acid is an unsaturated fatty acid with a melting point of around 15°C[52].

Below the phase transition temperature, lipid can be found in a solid state while above this temperature, the lipid transforms to its liquid phase. There are various factors affecting the phase transition temperature[43]. One of the factors is the position of the double bonds in unsaturated lipids. When it is located in the middle of the chain that makes it more difficult to crystallize compared to the bond(s) being closer to the end of the chain. Consequently, lipids that have double bonds in the middle of their hydrocarbon chain have a lower Tm than those having double bonds closer to the terminal of the chain[43].

Nanostructured lipid carriers (NLCs)

Preparation method of NLCs

NLCs were developed and introduced in late 1990s by Muller & Dingler by modifying the composition of SLNs to improve their biocompatibility, stability and drug loading; they have replaced some of the solid lipid composition with liquid lipid, resulting in a formulation consisting of *solid lipid, liquid lipid, surfactant and drug*[53,54]. NLCs are mostly used to improve the oral bioavailability of poorly aqueous-soluble drugs[55]. However, they have been used even for hydrophilic drugs such as tobramycin[56], gentiopicrin[57] and rosuvastatin[58]. Several methods have been developed and optimized in order to prepare NLCs[59-61], including the following:

- Hot high-pressure homogenisation (Hot HPH): The lipid phase (solid and liquid) is mixed and heated above the melting point of the solid lipid, then the drug is added to the lipid mixture. At the same time, the aqueous phase is prepared by mixing water with surfactant; the aqueous phase is also heated at the same temperature as the lipid mixture. As a next step, both phases (i.e. lipid and aqueous) are mixed and homogenized using high shear (around 10,000-20,000 rpm) at higher temperature for a short time to obtain a pre-emulsion, which is further passed through the high pressure homogenizer for a number of cycles. The number of cycles for which the pre-emulsion is passing through the homogenizer reduces the particle size into nano-emulsion. Finally, the nano-emulsion is constantly stirred in ambient conditions until it reaches the room temperature. This process allows the solidification of the particles because the solid lipid recrystallizes[56,62-68].
- Cold high-pressure homogenisation (Cold HPH): This method is used as an alternative to the Hot HPH because some hydrophilic/lipophilic drugs can undergo decomposition at higher temperatures. The lipid phase is subjected to HPH and then immediately cooled down using dry ice or liquid nitrogen. Then, the micro-particles obtained from grounding the solid mass are dispersed in the aqueous phase. Lastly, the mixture of lipid and aqueous phase is subjected to high shear homogenisation or ultrasonication to form NLCs[69,70].
- Emulsification-ultrasonication: This method is similar to the Hot HPH. The aqueous phase is added to the lipid phase and the obtained pre-emulsion is homogenized using high-speed mixing. Finally, the emulsion is ultra-sonicated and cooled down to room temperature to form NLCs[71-74].

- Solvent diffusion: The active ingredient and the lipids are added to a mixture or a single phase consisting of water-miscible organic solvents such as methanol, the solution is sonicated at high temperature to get a clear lipid phase. The aqueous phase is prepared using water and surfactant and the same temperature as for the lipid phase is used during mixing. Then, the lipid phase is added to the aqueous phase under constant mixing using high temperature. Afterwards, the final dispersion is cooled down to room temperature under constant mixing, so that the organic solvent evaporates to generate NLCs[75-78].
- Solvent emulsification evaporation: This method is similar to the solvent diffusion, but instead of water-miscible organic solvents, water-immiscible organic solvents are used such as chloroform[74,79-81].
- Film-ultrasonication: In this method, lipid phase consists of both solid and liquid lipids and the drug is dissolved in ethanol. The aqueous phase consists of water and surfactant, which are mixed, employing high temperature. The organic phase is evaporated from the mix via rotary evaporator. Upon evaporation, a thin film is formed which is collected and dispersed in the hot aqueous phase under sonication. The dispersion is cooled down at room temperature and NLCs are formed[82-84].
- Micro-emulsion: The liquid lipid is initially heated alone, followed by the addition of melted solid lipid and, once mixed, the drug is added to the mixture. The aqueous phase is prepared, as in all methods, using surfactant and water. Both lipid and aqueous phases are heated at high temperature. Then, the lipid phase is added to the aqueous phase; mechanical stirring is being used for this step and the solution is maintained at the same high temperature. Once the micro-emulsion has been formed, it is added to cold water under constant stirring; the dilution with cold water allows the formation of NLCs[85-88].
- Hot melt extrusion technology: This method has been developed for commercialisation or large-scale manufacturing of NLCs, as the above-mentioned methods are difficult to commercialize since they involve many steps. Hot melt extrusion technology uses a twin screw extruder which consists of three feeding ports: one for the addition of the solid lipid with the drug, second for the heated liquid lipid and third for the aqueous phase. All the materials are sonicated with probe sonicator to form NLCs[89].
- Supercritical fluid technology: Here lipids are melted and the supercritical fluid which is normally carbon dioxide is dissolved in the lipid matrix. This results in either a gas suspension or a solution (depending on the solubility of the materials in the fluid). And lastly, suspension/solution is atomized and sprayed into a chamber, where the gas evaporates and NLCs are formed[90].

Applications of NLCs

There are various applications for which NLCs have been investigated, such as topical, oral, pulmonary, brain and ocular delivery[91]. Topical delivery of NLCs has been examined for the treatment of various skin diseases such as fungal infections[92], inflammation[86], acne vulgaris[71] and psoriasis[88]. Topical delivery offers controlled release of the drug and the nanoparticles enhance its permeability. NLCs that are delivered via topical delivery also protect the active ingredients as well as demonstrate reduced irritation compared to conventional creams and gels[91].

Oral delivery of NLCs has been investigated in a few studies since the oral route is the most convenient drug administration route; however, it has the major drawback of poor bioavailability of hydrophobic drugs. NLCs delivered through the oral route can provide longer circulation time in the gastrointestinal tract and have reduced clearance[91]. Jain and Ram have investigated the preparation of glipizide-loaded NLCs for the treatment of type II diabetes[93]. Moreover, Shah et al. have developed raloxifene-loaded NLCs formulation for the treatment of osteoporosis and proved that the NLCs enhance the bioavailability of the drug[77]. The effect of lercanidipine hydrochloride-loaded NLCs formulation for the treatment of hypertension was explored; the results demonstrated NLCs as a promising delivery system[74].

Another use of NLCs which has been widely investigated is their drug delivery to the pulmonary system. Pulmonary delivery lacks the ability to deliver the drug to the specific site of action, especially when the drug is required to be delivered and deposited into the lower respiratory tract, detect and kill cancer cells. The use of NLCs in the pulmonary system produces localized effect and avoids their clearance until they reach the desired site of action, possessing non-toxic or irritant properties[91]. NLCs have been studied for the treatment of lung cancer[94-97], cystic fibrosis[56], chronic obstructive pulmonary disease (COPD)[58], lung fungal infections[67] and other pulmonary disorders[98]. In general, NLCs seem to be a promising alternative as a drug delivery system offering less invasive route when compared to the traditional chemotherapy used for lung cancers and disorders, improving patient compliance[7].

NLCs have also been studied for drug delivery to the brain. Madane and Mahajan have studied the effect of curcumin-loaded NLCs on brain cancer cells via nasal administration[62]. The permeability of the traditional drugs through this route into the brain is limited due to the protective functions of the blood-brain barrier. The use of NLCs affords reduced drug expulsion and enhances drug effect due to the NLCs' lipid nature that facilitates penetration into the blood-brain barrier[99].

Ocular delivery is one of the other routes that NLCs have been examined for. Ocular delivery is used for various eye diseases such as cataract, glaucoma or diabetic retinopathy and it can be administered via eye drops, eye gels, or even eye injections, which might not favour patient compliance[100]. During ocular delivery, there are anatomical barriers as well as corneal absorption issues that impair the bioavailability of the drug, such that less drug can be delivered to the desirable site of action[100]. NLCs offer better corneal permeation, therefore they afford better bioavailability, and they also offer a non-invasive alternative to injections, which enhances patient compliance[91]. Seyfoddin et al. evaluated the use of acyclovir-loaded NLCs for ocular delivery in order to treat blindness and successfully developed a drug delivery system that demonstrated promising corneal permeation and bioavailability[85].

Categories of NLCs

There are three distinct types of NLCs, which are classified based on their internal structure[61,91,101]. These are imperfect type, amorphous type and multiple type (Figure 5).

The imperfect type of NLCs includes various lipids consisting of fatty acids such as glycerides. The drug loading can be increased by extending the imperfection of the structure; this can be performed by mixing glycerides with various hydrocarbon chain lengths and saturations[102-104]. Furthermore, they have a higher solid lipid concentration than the liquid lipid[105]. The amorphous type of NLCs consists of a specific lipid such as isopropyl myristate, hydroxyoctacosanyl hydroxystearate or medium chain triglycerides such as Miglyol mixed with solid lipids[56,63,66,68,94,106]. The multiple type of NLCs consists of various liquid lipid compartments, which are distributed within the solid matrix of the core, as they have a high liquid lipid concentration, thus enhancing drug dissolution as well as drug loading. Multiple type NLCs offer extended release, as the oil compartments are protected by the solid lipid matrix[107,108] and they also consist of higher liquid lipid concentration[105].

Lipids in NLCs

One of the most significant factors that affect the preparation and development of NLCs is the type of lipids used[109]. Drug solubility in the lipid matrix is significant and the encapsulation efficiency is highly affected by this[110]. Drug solubility in both solid and liquid lipids must be high so that the hydrophobic drug will remain dissolved in the lipid core of the NLCs. Moreover, drug loading is highly affected by the solubility of the drug in the lipids[61]. Drug loading demonstrates the maximum amount of the drug that can remain dissolved and lodged in the lipid matrix until it reaches the desirable site of action. During the pre-developmental phase, screening of lipids is significantly crucial in order to scientifically justify the use of specific lipids and surfactants. Most of the published literature have overlooked this key point and materials were randomly selected based on lipid types or surfactant types.

Upon optimising NLCs formulation, there are several aspects that need further exploration like solid to lipid ratio, surfactant and drug concentration, as well as the total lipid concentration, as their selected amounts/concentrations affect the particle size, polydispersity index (also referred to as size distribution), zeta potential and entrapment efficiency (which lead to a successfully prepared formulation). In lipid phase, solid to liquid lipid ratio normally varies between 70:30 and 99.9:0.1% w/w[111]. Various combinations and concentrations of solid and liquid lipids can result in a less/more ordered lipid matrix, giving less/more space to the active ingredient respectively[112]. This applies to NLCs in comparison to SLNs; where the actual space that the drug will occupy within the lipid matrix is dependent on the solubility of the drug in the matrix[110].

The structural differences and amount of liquid lipid used during the development of the NLCs affect drug incorporation into the lipid matrix as well as drug stability[113]. There are limited studies that showed the effect of the liquid and solid lipids on the stability and performance of the NLCs[114]. A few of the most common solid lipids that have been previously used for NLCs are stearic acid, glyceryl monostearate, Glyceryl dibehenate (COMPRITOL®888 ATO), Glyceryl palmitostearate (Precirol®ATO5), Tristearin (Dynasan®118), and liquid lipids are oleic acid, olive oil, Propylene glycol monocaprylate (Capryol™90), and medium chain triglycerides (Miglyol 812)[110,112,115].

Effects on Particle Size

The first and far most pivotal aspect examined during the development of any lipid-based drug delivery system is their particle size, as this is essential for targeting particular site during transport of an active ingredient. Polydispersity index is also measured as part of the size distribution of the formulation sample and it is important to show the presence or absence of agglomerates in the sample, which might affect drug distribution, drug-dose consistency and the desired pharmacological effect[116].

Type of solid lipid and structure

After comparing various studies (Table 3), the use of various solid lipids affected the particle size of the resulting NLCs. NLCs that used glyceryl monostearate as a solid lipid exhibited extremely low particle size varying from 33 nm to 179 nm[71,77,84,87,95,97,117-119]. This is important, especially for targeting and treating lung diseases. Smaller particles avoid particle deposition in the upper respiratory tract via inertial impaction or sedimentation (where particles are able to manoeuvre their pathway due to their small size and low density), and offer higher deposition in the peripheral regions of the lungs via Brownian diffusion[120], hence targeting and interacting with the cancer cells[121]. Stearic acid as a solid lipid also showed small particle size with a variation from 84 nm to a maximum of 179 nm[64,122,123]. Another solid lipid which has been employed in several studies and proved to produce NLCs with particle size varying from 108 to 400 nm is Precirol®ATO 5[62,63,67,96,98,124,125]. COMPRITOL®888 ATO is also a solid lipid which has been used in many NLC formulations and showed a particle size of 129 nm to 323 nm[56,66,68,85,93,94,126]. Dynasan®118 has not been used significantly as a solid lipid in NLCs formulation; however, a research conducted by Duong et al. demonstrated particle size of circa 266 nm in NLCs formulation[127].

The structures of the aforementioned solid lipids are presented in Table 4. Using Precirol®ATO 5, COMPRITOL®888 ATO or Dynasan®118 as a solid lipid resulted in NLCs with bigger particle size and this could be attributed to the more complex structure when compared to glyceryl monostearate and stearic acid. In addition, higher molecular weight of solid lipids potentially may be another factor, which in turn could end up with more complex linkages between the molecules that could result to aggregation and in turn could result in larger particle size[128]. It is noteworthy that these solid lipids still provided NLCs with particle size in the nano-sized range, with a maximum particle size of 400 nm. Therefore, solid lipids selection is dependent on the target particle size as well as desirable site of action.

Type of liquid lipid and structure

Upon comparing numerous studies (Table 3), the use of various liquid lipids affected the particle size of the resulting NLCs. NLCs that used Capmul MCM as liquid lipid had the lowest particle size; formulations of this

lipid showed a particle size of 33 to 165 nm[77,87]. The following liquid lipid, based on increasing particle size, was oleic acid, which has been used in various formulations targeting site, such as the liver[84], skin[71] and lung[67,97,118,126] and exhibited a particle size in the range of 50 to 197 nm[67,71,75,84,93,95,97,118,122,123,126]. This is followed by Soybean oil resulting in NLCs with particle size of 92 to 151 nm[83,117]. Capryol 90 was employed in various formulations; this liquid lipid provided NLCs with varying particle sizes from 115 to 185 nm[58,86,98,119]. Miglyol 812 has been widely incorporated into the production of NLCs and various studies showed that it resulted in NLCs with particle size between 157 and 279 nm[56,63,66,68,94]. In contrast, Lauroglycol 90 has been used to formulate NLCs with particle size of circa 323 nm[85].

The structures of the above-mentioned liquid lipids are different from each other (Table 5). In addition, a number of studies have been conducted employing NLCs as a delivery system using various liquid and solid lipids as well as their combination, as can be seen in Table 3. There is still no clear correlation or trend between the structure and the molecular weight of the liquid lipid and the particle size of the developed NLCs. However, it is suggested that lodging of drugs in the vesicles may potentially affect the size, due to their befitting phenomenon or stearic fit as well as solubility in lipids. For example, solid lipid (Precirol®ATO 5) and combination of liquid lipids (Squalene and Soya phosphatidylcholine) as well as surfactants (Tween 80 and Dioleoyl-3-trimethylammonium propane) demonstrated NLCs particle sizes of 110 nm[125] and 400 nm[124], and employed Doxorubicin hydrochloride and Prostaglandin E2/siRNA, respectively. Therefore, drug solubility and stearic fit may potentially affect the particle size of NLCs formulation.

Ratio of Solid lipid to liquid lipid

The ratio of solid lipid compared to the liquid lipid has visible effect on the particle size of NLCs after comparing various studies (Table 3). A study conducted by Kelidari et al. investigated and analysed the effect of different solid to liquid lipid concentrations (90:10, 80:20 and 70:30) on particle size and demonstrated that the higher the solid lipid concentration, the larger the particle size (i.e. 288, 240 and 146 nm, respectively)[122]. This has been confirmed as well from Emami et al. who concluded that by increasing the liquid lipid concentration, the particle size decreased[75]. This could be attributed to the fact that more solid lipid could affect the melting process and may create agglomerates during the NLCs production. Additionally, during solidification process of solid lipids in NLCs preparation, higher concentration of solid lipid may tend to fuse or make aggregates, which may be unable to break and so emerge as big particles, with wider size distribution. These results were further confirmed by another study, where various ratios of solid to liquid lipid showed larger particle size of NLCs with higher concentration of solid lipid in the formulation[31]. However, on the contrary, Kaur et al. demonstrated that there should be an optimum ratio between solid to liquid lipids, as there is no trend that could relate the solid to liquid lipid ratio to the particle size and polydispersity index[97]. This has been confirmed by Zhang et al., where various solid to liquid lipid concentrations (i.e. 9:1, 8:2, 7:3 and 6:4) were investigated[117]. Upon analysis, an optimized formulation was found to be with a solid to liquid lipid concentration ratio of 8:2. Formulations with various ratios showed similar particle size, i.e. approximately 100 nm. However, a significant difference was seen in the polydispersity index, demonstrating wider particle size distribution. It was further suggested that

a concentration of solid lipid of more than 80% is high when compared to the liquid lipid, offering not enough liquid lipid to formulate NLCs, and as a result, different shapes of particles with large particle sizes and high polydispersity indices were found. On the other hand, a solid lipid concentration less than 80% was considered too low to form the NLCs in combination with the liquid lipid. Therefore, the liquid lipid would separate as spare lipid droplets which would be responsible for large particle size and their wider distribution[97]. Formulations with wider size distribution may significantly affect drug loading, release profile of drug, and bioavailability and efficacy; therefore, particle size and polydispersity index play essential role in formulation optimisation and achieving optimum effect.

Effects on Entrapment Efficiency (EE)

One of the other vital factors during development and optimisation stage of drug delivery system is attaining high EE. Liposomes as a drug delivery system are associated with a disadvantage of drug leakage, where the drug escapes from the vesicles and therefore they end up with lower EE[8,143]. NLCs, a next generation particles system has been developed in order to increase the low EE and drug loading when compared to the counterpart delivery systems[144]. EE is highly affected by the drug solubility in the lipid matrix and surfactant further helps to keep the drugs within and minimize their escape by making a protective external surfactant layer.

Type of solid lipid and structure

Upon comparing solid lipids in NLCs formulation (Table 3), the highest EE (i.e. 99.98%) was observed by Bang et al. who used Precirol®ATO 5 as a solid lipid, investigating anticancer effect of paclitaxel-loaded NLCs[119]. COMPRITOL®888 ATO as a solid lipid demonstrated promising EE, varying from 81.90 to 98.3%[56,66,68,85,93,94,126]. Whereas glyceryl monostearate in many studies showed a general trend of lower EE in NLCs formulation ranging from 48.34 to 87.00%[71,77,84,95,97,117,118], only one study had a high EE of 95.07%[58]. The use of stearic acid and Dynasan®118 as solid lipids for NLCs formulation is very limited, however EEs of 69.95%[123] and 90.60%[122] for stearic acid and 90.90%[127] for Dynasan®118 were found. There is no direct correlation between the type of solid lipid, their molecular weight and structure and the corresponding EE. It is suggested that the molecular weight of the drug used in each case in combination with each solid lipid plays a significant role for the EE.

Types of liquid lipids and their structures

Incorporation of liquid lipids in NLCs formulation (Table 3), significantly higher EE (i.e. 99%) was observed employing Capryol 90 as a liquid lipid[119]. Similarly, incorporating Capryol 90 in NLCs demonstrated higher EE as well[58,98]. In contrast, it showed an extremely low EE of 51.00%, which further increased to 99.45% post

formation of a gel consisting of the same valdecoxib-loaded NLCs[86], which may be related to the gel structures closely adhere or adsorb the drug on to the surface of the NLCs. Higher drug entrapment and lowed drug leakage from the bilayers of the NLCs may be related to more ordered gel structure[17,145], and the flexible core further improves drug accommodation within the particles of the NLCs. Oleic acid was observed to have varying EE from 48.34 to 98.78%[67,71,75,84,93,95,97,118,122,123,126]; this may be related to the kink in the structure of this lipid as well as the stearic fit of drug lodging themselves in NLCs particle (Table 5). A number of various studies where Miglyol 812 has been used as a liquid lipid showed a promising EE from 89.30 to 98.30%[56,63,66,68,94]. Soybean oil also followed a trend of higher EE in NLCs, with EE of 97.11%[83] and 88.60%[117]. Similarly, Capmul MCM also displayed higher EE of 90.86%[62] but it showed a slightly lower EE of 70.42%[87] and 74.78[77] in other studies, which are related to the use of a different drug with higher molecular weight (where drug molecules due to their structure occupy more space and make it competitive for higher drug accommodation). The study with the highest entrapment efficiency among these three studies used the drug with the lowest molecular weight allowing more drug to be entrapped within the core, i.e. curcumin with molecular weight of 368.38 g/mol[62]. The other two studies used docetaxel[87] and raloxifene[77] with molecular weights of 861.90 and 510.04 g/mol, respectively. Last, Lauroglycol 90 showed high EE for acyclovir in NLCs, i.e. 90.54%[85]. It is hard to identify a clear correlation between the type of liquid lipid and the EE of the drug in the NLCs; however, it is more drug dependant and especially based on the structure and molecular weight. Furthermore, literature is not clear regarding whether an initial screening or solubility studies has been performed in each study for the lipids that have been used ahead of the NLCs formulation, however this is a significant step and should be followed ahead of any formulation development of NLCs since the drug in combination with the lipids seems to alter the physicochemical properties of the resulting NLCs.

Solid to liquid lipid ratio

The ratio of solid lipid compared to the liquid lipid seems to have an effect on the EE of the developed NLCs. A research conducted by Kelidari et al.[122] examined the effect of different solid to liquid lipid concentrations (90:10, 80:20 and 70:30) in the EE, and the results showed that the higher the solid lipid concentration, the lower the EE (i.e. 84.70 to 90.60%, respectively). This can be explained by the fact that the addition of the liquid lipid adds more flexibility to the core of the NLCs, and therefore allows more drug to be entrapped within the lipid matrix[114]. Besides, the main purpose originally for the development of NLCs was to enhance the drug loading of the SLNs within their solid lipid core[13]. On the other hand, no clear association between the ratio of the solid to liquid lipid and the EE was explored by other researchers. Bang et al.[119] investigated various formulations with solid lipid amounts varying from 70-280 mg; however, all expressed an EE higher than 99%, proving that the solubility of the drug within the lipid matrix actually affects the EE. Similarly, a perfect correlation between solid to liquid lipid ratio and the EE was not identified due to the presence of other core variables like surfactant and drug molecule/structure of the NLCs formulation. Based on the different outcomes of various studies, there should be an optimized solid to liquid ratio for every unique formulation as concluded by Kaur et al[97].

Effects of lipid and drug on Charge and Stability

Zeta potential is used to measure the charge of the particles and is of significant importance in terms of identifying formulation stability. An absolute value of 30 mV is required, where the electrostatic repulsion between the particles keeps them away and separate from each other and hence improves formulation stability[123,146]. Reports regarding the type of solid and liquid lipids and the resulting charge of the formulation are conflicting and therefore there is not a proven correlation yet between the type of lipid and the charge (Table 3).

The effects of the different solid to liquid lipid concentrations (9:1 to 7:3) on the charge were observed by Kelidari et al., where higher solid lipid concentration demonstrated lower absolute value of zeta potential (-17.3, -22.1 and -35.1 mV, respectively)[122]. This can be explained by the fact that the liquid lipid used in the specific study (i.e. oleic acid) added additional negative charge to the formulation[114,147]. However, there are a few studies where no clear association was observed between the solid to lipid ratio and the charge. It is only the drug concentration that seemed to affect the charge in one study in reverse proportion, zeta potential decreased when the drug concentration increased[31].

Conclusion

The aim of this study was to review the literature to explore the physicochemical properties of NLC formulations based on the solid and liquid lipids, as well as their ratio and structure. More specifically, the effects the type of the solid and liquid lipid, their structure and their ratio have on NLC formulation were explored. A noticeable trend regarding the use of specific solid lipids and their effect on the particle size of the NLCs was observed. The particle size of the NLCs increased as the molecular weight of the solid lipid increased, making the structure more complex. However, no apparent correlation was found between the molecular weight and the complexity of the structure of specific liquid lipids and the resulting particle size of the NLCs. The ratio of solid to lipid amount seemed to have a proportional effect on the particle size of the NLCs; it seemed that, as the solid amount increases, the particle size increases. Few studies demonstrated an optimized formulation, achieving desired particle size or entrapment; however, their optimization is not applicable to all formulations. This could be explained by the actual accommodation space that the lipid matrix creates for the drug. Another key aspect during the development of the NLCs is the EE. Generally, it is accepted that the solubility of the drug in both the liquid and solid lipid highly affects the EE of the drug in the final formulation. Lastly, the stability of the formulation is defined by their charge. There was no conclusive evidence demonstrating the effects of different types of lipids and their ratio on the zeta potential; only one study identified a proportional correlation between increasing liquid lipid and increasing zeta potential and another study showed an association between the zeta potential and the drug concentration rather than the type and ratio of lipids used. However, and as a general observation, the higher the value, the better the stability.

Conflicts of interest

The authors declare no conflict of interest.

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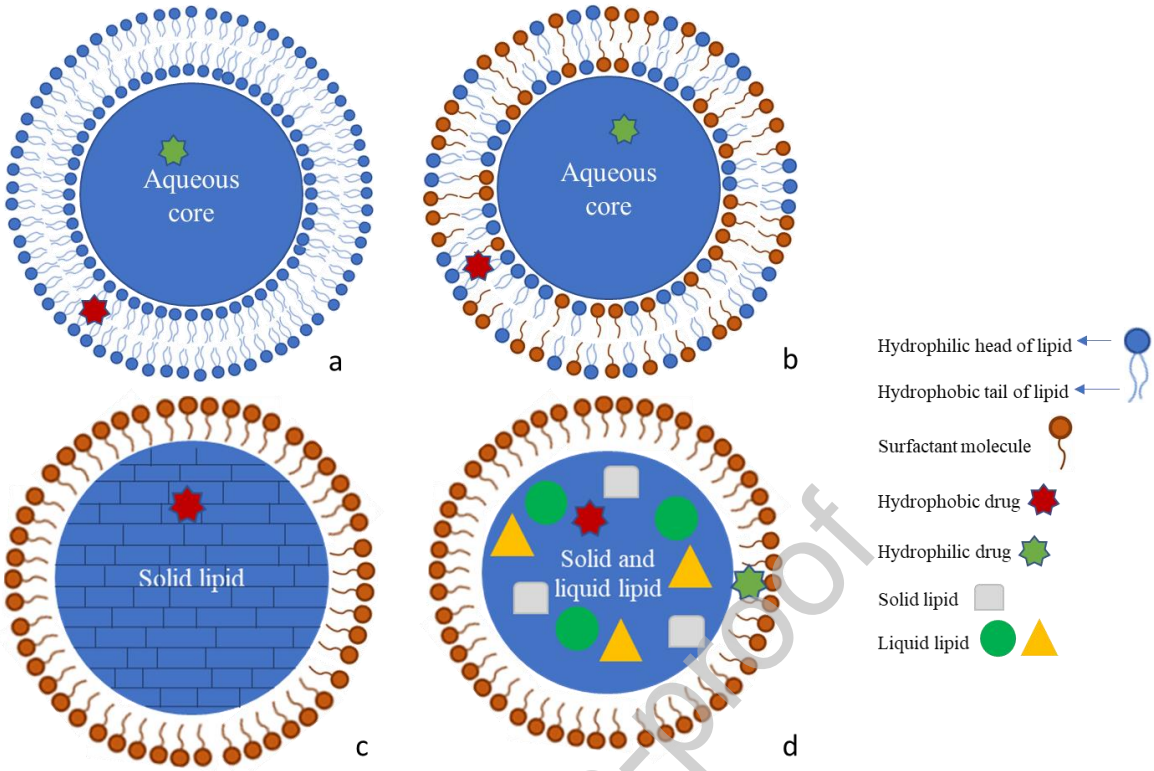


Figure 1. Structure of (a) liposomes, (b) transfersomes, (c) SLNs and (d) NLCs. The main difference between liposomes and transfersomes is the addition of the surfactant to the transfersomes, making them more flexible as drug delivery systems. Due to stability issues, SLNs were developed where the solid lipid offered a more structured core. For drug loading purposes and improved stability, NLCs were developed as they offered a less structured core, offering more flexibility and more space to accommodate the drug.

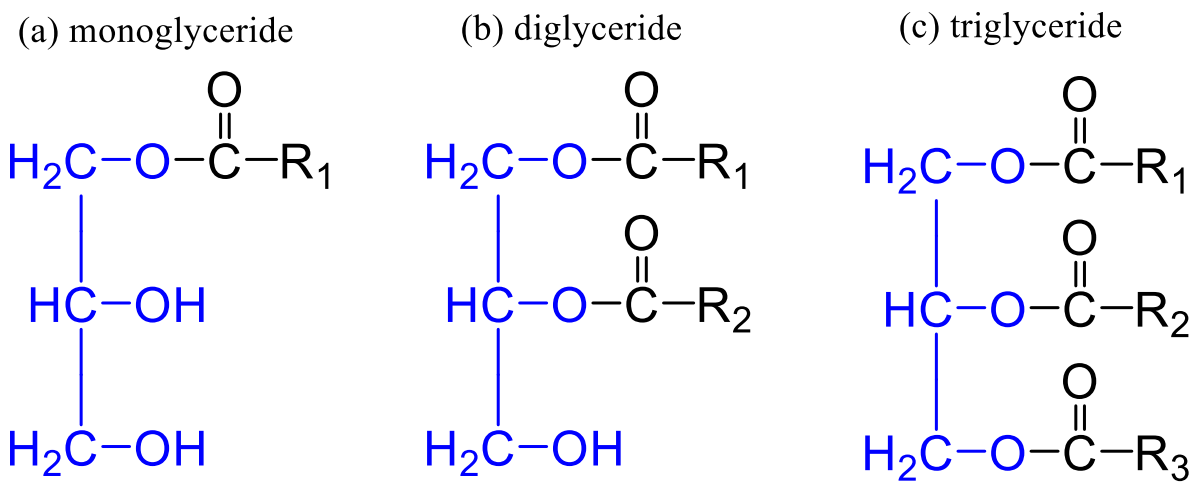


Figure 2. Glycerolipids classes based on glycerol substitution, where one, two and three fatty acid molecules substitute the glycerol molecule in monoglycerides, diglycerides and triglycerides, respectively. The blue colour represents the glycerol molecule of the structure.

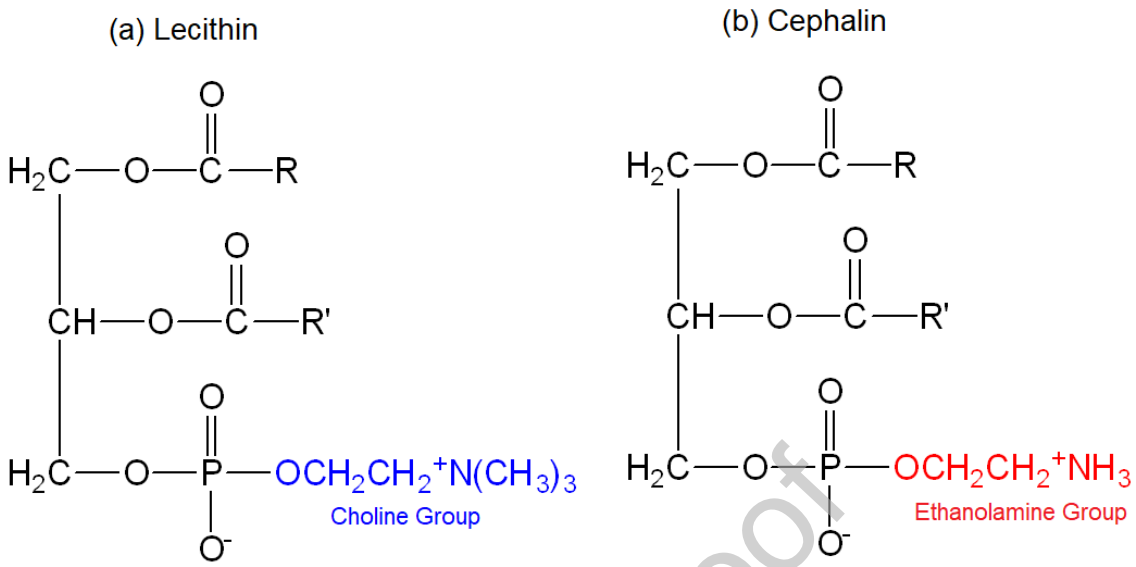


Figure 3. Glycerophospholipids classes based on amino alcohol group esterification, there might be a choline or an ethanolamine group in lecithins and cephalines, respectively. The blue and red colours represent the choline and ethanolamine group respectively.

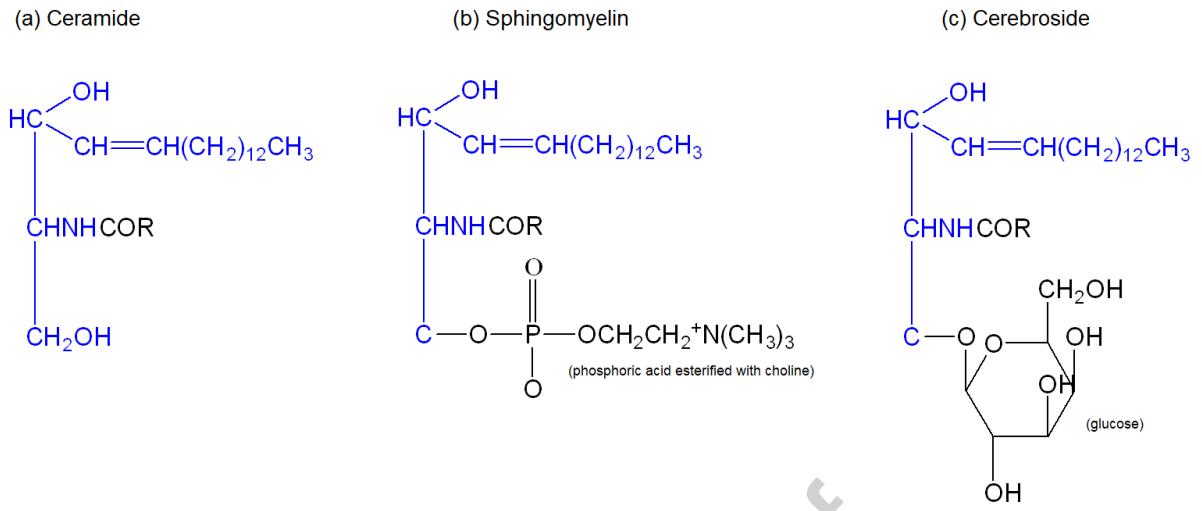


Figure 4. Sphingolipids classes based on a substitution group. Ceramides consist of a fatty acid and sphingosine. Sphingomyelin occurs when the 1-hydroxyl group of the long chain of ceramide is esterified with choline or ethanolamine. Cerebrosides occur when there is a glycoside linkage at the 1-hydroxyl group of the long chain of ceramide. The blue colour represents the sphingosine molecule.

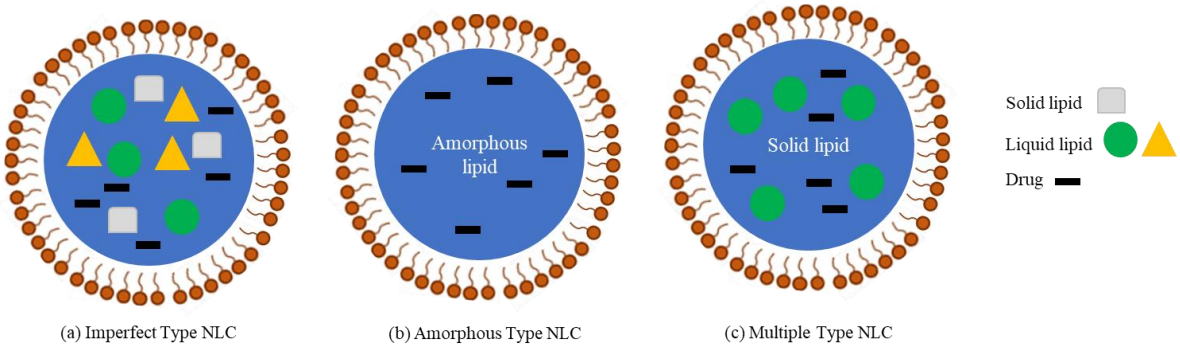


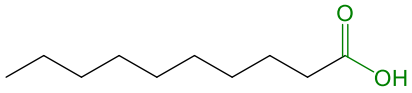
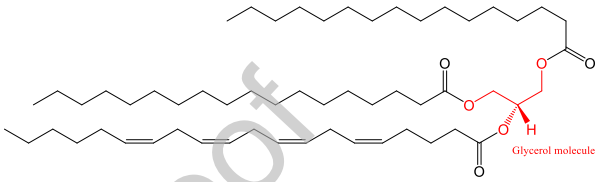
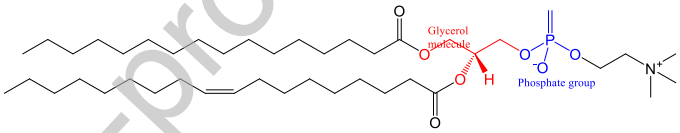
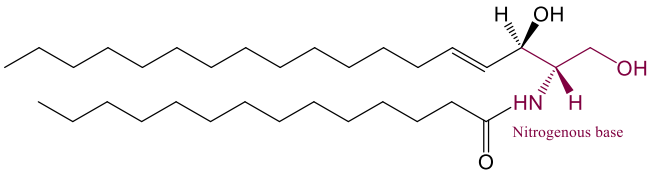
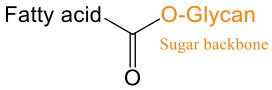
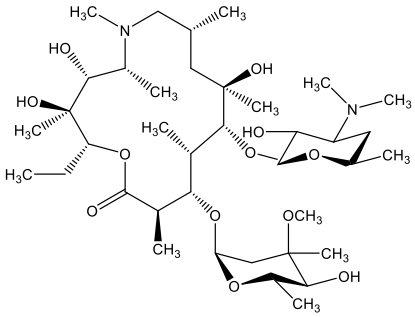
Figure 5. Different types of NLCs: (a) Imperfect type, which mainly consists of fatty acids, (b) Amorphous type, which consists of a specific type of lipid, and (c) Multiple type, where various liquid lipid compartments are distributed into the solid matrix of the core.

Table 1. Main lipid-based delivery systems, their compositions, advantages and disadvantages. These systems have been developed for increased bioavailability and stability. Transfersomes were developed by adding surfactant to the liposomes formulation in order to add flexibility to the particles. NLCs were developed by adding liquid lipid to the SLNs formulation in order to add flexibility to the core.

Drug Delivery System	Composition	Advantages	Disadvantages	References
Liposomes	Phospholipid(s) neutral/charged, with/without cholesterol	• Size varies from 25 nm to 2,500 nm • Increased efficacy • Increased stability (via encapsulation) and reduced toxicity of the drug which is encapsulated; reduced dosage which in turn results in decreased allergic and immunological reactions • Non-toxic, biodegradable • Increased biocompatibility • Flexible to attach to site-specific ligands for targeting • Decreased exposure of sensitive tissues to drugs that can be extremely toxic • Able to trap both hydrophobic and hydrophilic drugs	• Decreased solubility • Decreased half-life • Phospholipid can undergo oxidation • Increased chances of drug leakage • Cost of production is high • Hydrophilic drugs have low encapsulation	14-19
Transfersomes	Phospholipid(s) neutral/charged, with/without cholesterol and surfactant	• Size varies from 10-210 nm • Flexible, highly deformable, significant for skin penetration as they can squeeze through skin pores • Can accommodate drugs with various solubilities since they consist of hydrophobic and hydrophilic moieties, but mostly hydrophilic • Increased entrapment efficiency • Protect the drug from degradation, especially for peptides and proteins • Offer sustained release • Can be used for topical and systemic	• Might undergo oxidation, which makes them unstable • Cost of production is high • High dose of drug is not recommended	20-25

		administration of drugs, for example can be used for skin therapies		
Solid Lipid Nanoparticles (SLNs)	Solid lipid and surfactant	• Size varies from 40 to 1000 nm • Increased stability compared to liposomes • Prolonged and sustained release of targeted drug delivery while minimising the undesirable side effects of the drug • Safer than other polymeric carriers as organic solvents are not used during their manufacture • Can carry both hydrophobic and hydrophilic drugs	• Gelation tendency when low viscosity SLN dispersion transforms into a viscous gel due to shear forces • Low incorporation rates • Drug degradation during SLNs formation because of the high-pressure homogenisation technique • Low drug loading	26-30
Nanostructured Lipid Carriers (NLCs)	Solid lipid, liquid lipid and surfactant	• Size varies from 10 to 500 nm • Safer since organic solvents are not used during their manufacture • Protect sensitive drugs from acidic environment • Can encapsulate both hydrophobic and hydrophilic drugs and can deliver both at the same time, if required • Easy to scale up • Higher drug loading than SLNs • Decreased drug leakage • Better stability	• Could have cytotoxic effects depending on the concentration and the nature of lipid matrix • The use of few surfactants might make them irritants and sensitizers • There are not many studies conducted using NLCs compared to other lipid-based delivery systems	28,31-34

Table 2. Lipids were categorized into simple and complex, based on the number of products formation upon hydrolysis; where simple consist of two groups and complex consist of over two groups. Simple lipids include fatty acyls and glycerolipids, whereas complex lipids include glycerophospholipids, sphingolipids, saccharolipids, polyketides, sterol lipids and prenol lipids. The coloured groups represent the functional group for each lipid category.

Lipid Category		Synthesis	Example
Simple	Fatty acyls	Their synthesis involves a chain elongation of an acetyl-CoA with malonyl (or methylmalonyl)-CoA groups	
	Glycerolipids	Consist of mono-, di- and tri-substituted glycerol molecules	
Complex	Glycerophospholipids	Same as glycerolipids, but include an additional phosphate or phosphonate group which is esterified to one of the hydroxyl groups of glycerol	
	Sphingolipids	Their core structure is a long-chain nitrogenous base	
	Saccharolipids	There is a direct linkage between the fatty acyl group and a sugar backbone	
	Polyketides	They form a unique group consisting of microbial, animal and plant sources	

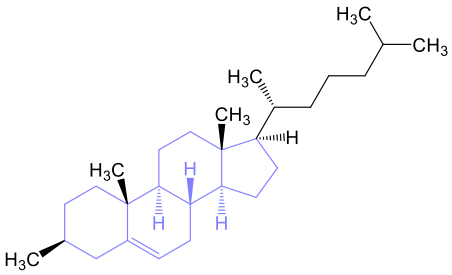
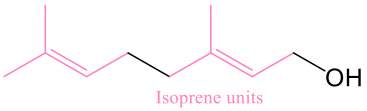
Sterol lipids	They consist of four fused carbon rings with a variety of groups attached on the edges	
Prenol lipids	Share the same pathway with sterol lipids but have obvious difference in their final function and structure, as they consist of isoprene units	

Table 3. Summary of studies that used NLCs: information about the lipid phase, including the type of solid and liquid lipid used as well as their amount and ratios, the aqueous phase, the drug and the method used. This table provides the experimental results which include the particle size, the polydispersity index (PDI), the zeta potential and the entrapment efficiency (EE%).

Lipid Phase			Aqueous phase	Drug	Method	Use	Particle size (nm)	PDI	Zeta potential (mV)	EE (%)	References
Solid Lipid	**Liquid Lipid**	**Ratio Solid: Liquid (%)**									
Cholesterol	Oleic acid (OA)	70:30	Poloxamer188	Paclitaxel	Emulsion solvent diffusion, evaporation method and ultrasonication	Colorectal cancer	182	0.100	-12.9	53.00	75
Compritol ®ATO 888	Miglyol 812	40:60	mPEG-Hyd-DSPE, lecithin, and Tween® 80	Doxorubicin hydrochloride & β-elemine	Hot homogenisation and ultrasonication	Lung cancer (pulmonary delivery)	190	<0.200	Between −31 and −41	89.3 (DOX)	94
Compritol ®ATO 888	Oleic acid	70:30	Poloxamer 188, Soya Lecithin and sodium taurocholate	Glipizide	Solvent diffusion method	Type II diabetes mellitus (oral delivery)	197	0.212	-30.3	82.50	93
Compritol ®ATO 888	Miglyol 812	56:44	Soybean Lecithin/Brij 78	Docetaxel	Hot high pressure honogenisation	Lung cancer (pulmonary delivery)	157	n/a	-43.6	98.30	66
Compritol ®ATO 888	Lauroglycol® 90	58:42	Tween® 40	Acyclovir	Hot microemulsion technique	Ocular delivery	323	n/a	-25.5	90.54	85
Compritol ®ATO 888	Miglyol 812	70:30	Sodium taurocholate	Celecoxib	Hot melt homogenisation	Lung cancer (pulmonary delivery)	217	0.200	-25.3	95.60	68

Compritol ®ATO 888	Oleic acid & soybean phosphatidylcholine	48:48:4	N-[1-(2,3-dioleyloxy)propyl]-N,N,Ntrimethyl-ammonium chloride (DOTMA)	Paclitaxel and Doxorubicin	Melted ultrasonic dispersion method	Lung cancer (pulmonary delivery)	129	0.180	26.6	81.9 (PTX) & 83.7 (DOX)	126
COMPRITOL®888 ATO and Precirol® ATO 5 (50:50)	Miglyol 812	n/a	Tween 80 and Poloxamer188	Tobramycin	Hot melt homogenisation	Cystic fibrosis (pulmonary delivery)	279	0.371	-22.3	94.03	56
Glyceryl Dilaurate	Capryol 90	50:50	Cremophor RH 40 with solubilizers: Transcutol and Solutol HS 15	Valdecoxib	Warm microemulsion	inflammation (topical delivery)	157	0.582	n/a	51.00	86
Glyceryl monostearate	Capryol 90	75:25	Tween 80 & Poloxamer 188	Paclitaxel	Hot melt emulsification and sonication	Anticancer drug – not site specific	115	0.284	-15.0	99.98	119
Glyceryl monostearate	Capmul MCM C8	85:15	PVA	Raloxifene	Solvent diffusion method	Osteoporosis (oral delivery)	33	n/a	-12.8	74.78	77
Glyceryl monostearate (GMS)	Oleic acid, soya lecithin and PEG:SA	29:29:29:13	Tween 80 & 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP)	Doxorubicin base (DOX)	Solvent diffusion method	Lung cancer (pulmonary delivery)	86	0.112	8.7	86.70	95
Glyceryl monostearate (GMS)	Oleic acid	90:10	Cremophor RH-40	Azelaic acid	Melt emulsification and ultrasonication method	Acne (topical delivery)	50	0.355	-14.3	83.40	71
Glyceryl monostearate	Oleic acid	60:40	Tween 20	Paclitaxel	Emulsification and ultrasonication	Lung cancer (pulmonary deliver	179	0.158	-15.2	85.60	97

					method	y)					
Glyceryl monostearate	Labrasol	60:40	Pluronic F-127	Terbinafine hydrochloride	High pressure homogenisation	Fungal infection (topical delivery)	128	0.211	n/a	80.24	92
Glyceryl monostearate	Soybean oil	80:20	Pluronic F68	10-Hydroxycamptothecin (HCPT)	Melt emulsification & high-pressure homogenisation	Lung cancer (pulmonary delivery)	92	0.114	-32.5	88.60	117
Glyceryl monostearate	Oleic acid & soya lecithin	33.3:33.3:33.3	DNA,DOTMA & Tween 80	Paclitaxel / Transferrin	Microemulsion technique	Lung cancer (pulmonary delivery)	79	n/a	25.0	87.00	118
Glycerin monostearate	Oleic acid	60:40	Poloxamer 188	Oleanolic acid and gentiopicrin	Film-ultrasonic method	Hepatic injury	111	0.287	-23.8	48.34	84
Lauric acid	Capryol-90	70:30	Cremophor RH40	Rosuvastatin (RSVS) (Respitose SV010 as cryoprotectant)	Melt-emulsification and ultrasonication method	COPD (pulmonary delivery)	164	0.268	-29.4	95.07	58
lecithin	Soybean oil	50:50	F68 and tween 80	Dexamethasone acetate	A film dispersion-ultrasonication method	Hepatitis and prevention of liver fibrosis	151	0.215	-38.7	97.11	83
M lipid	Capmul MCM	25:75	Tween 80	Docetaxel	Microemulsion (ME) template	Anticancer drug – not site specific	165	0.258	-3.9	70.42	87
Precirol® ATO 5	Squalene & SPC	49:49:2	Tween-80 & DOTAP	Prostaglandin E2 / siRNA	Modified melted ultrasonic dispersion	Idiopathic pulmonary fibrosis (pulmonary deliver	400	n/a	Close to 0	n/a	124

						y)				
Precirol® ATO 5	Olive oil & lipoid S100	50:25:25	pEGFP-N1, Tween-80 and Dimethyldioctadecylammonium bromide (DDAB)	pEGFP / transferrin	Melted sonication method	Lung cancer (pulmonary delivery)	157	0.120	15.9	82.00	96
Precirol ATO 5	Oleic acid	90:10	Eumulgin SLM 20	Itraconazole	Hot high pressure honogenisation	Lung fungal infections (pulmonary delivery)	108	0.247	-32.7	98.78	67
Precirol® ATO 5	Miglyol 812	n/a	Polysorbate 80 and Poloxamer 188	Sodium colistimethate (D-mannitol as cryoprotectant)	Hot melt homogenisation	Cystic fibrosis (pulmonary delivery)	255	0.339	-26.1	94.79	63
Precirol® ATO 5	Capryol-90	70:30	DL-Pyrrolidonecarboxylic acid salt of L-cocyl arginine ethyl ester (CAE)	Montelukast (sodium) (mannitol as cryoprotectant)	Melt-emulsification-ultrasonication	Pulmonary and systemic disorders (pulmonary delivery)	185	0.286	37.7	95.86	98
Precirol® ATO 5	Squalene & SPC	49:49:2	Tween-80 and DOTAP	Doxorubicin hydrochlorate (DOX·HCl)	Melted ultrasonic dispersion method	Lung cancer (pulmonary delivery)	110	0.400	60.3	n/a	125
Precirol OTO5	Capmul MCM	n/a	Tween 80	Curcumin	Hot high pressure homogenisation	Brain cancer (brain delivery)	147	0.189	-21.4	90.86	62
Stearic acid	Oleic acid	70:30	Sodium dodecyl sulfate	Clobetasol propionate	Solvent diffusion method	Drug is used for skin treatment; however, study does	179	0.240	-56.5	69.95	123

						not mention use of NLCs					
Stearic acid	Crodamol ® GTC	70:30	Tween® 80 and Span®85	n/a	Hot high pressure homogenisation	Not specified	84	0.540	-15.2	n/a	64
Stearic acid	Oleic acid	70:30	Span 80 and Tween 80	Spironolactone	Ultrasonication	Not specified	146	0.225	-35.1	90.60	122
Tristearin	Phosal®53 MCT	60:40	Tween®80	Ondansetron hydrochloride	Cold high pressure homogenisation	Treat nausea and vomiting caused by chemotherapy (nasal delivery)	266	0.280	-16.4	90.90	127

Table 4. Solid lipids chemical structures, their melting points and their molecular weights.

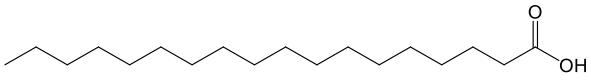
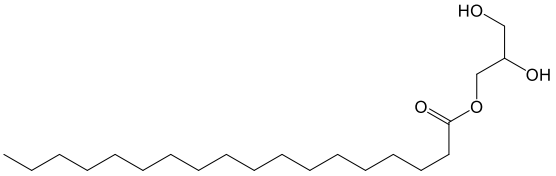
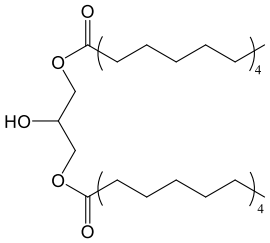
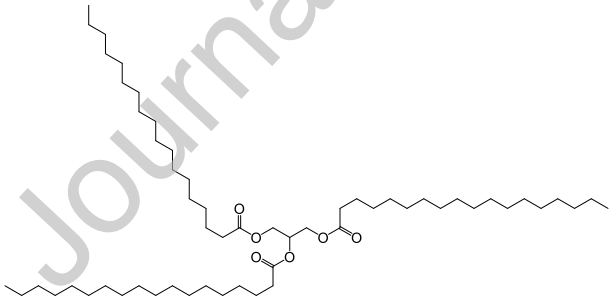
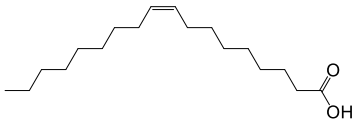
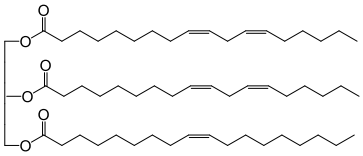
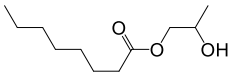
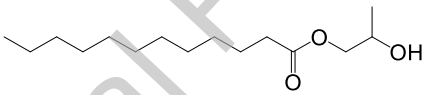
Name	Structure	Melting Point (°C)	Molecular Weight (g/mol)	References
Stearic Acid		70	284.50	51,129
Glyceryl Monostearate		50-55	358.60	130,131
Glyceryl dibehenate (COMPRITOL® 888 ATO)		69-74	432.70	131,132
Glyceryl palmitostearate (Precirol®ATO 5)		61	625.02	133,134
Tristearin (Dynasan®118)		72-75	891.48	135

Table 5. Liquid lipids and their chemical structures and molecular weights.

Name	Structure	Molecular Weight (g/mol)	References
Capmul MCM	Monoglyceride (45–75%), Diglyceride (20–50%), Triglyceride (< 10%) of Caprylic acid (C8, 50–90%) and Capric acid (C10, 10–50%)	218.29	136,137
Oleic acid		282.46	138
Soybean oil		238.19	139
Propylene glycol caprylate (Capryol™90)		202.29	140
Medium chain triglycerides – MCT (Miglyol 812)	**55% triglycerides of C8 and 45% triglycerides of C10 fatty acids**	n/a	141
Lauroglycol 90		258.40	142