The Pharma Innovation Journal 2018; 7(7): 779-789

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(7): 779-789

www.thepharmajournal.com
Received: 14-05-2018
Accepted: 16-06-2018

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Lipid nano particulate drug delivery: An overview of the emerging trend

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Abstract
Among the variety of nanoparticles being at present examined by pharmaceutical researchers, lipid nanoparticles have led the pack in view of clear preferences of higher level of biocompatibility and flexibility. Lipid nanoparticles, including solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), lipid-drug conjugates (LDC) and polymer lipid hybrid nanoparticles (PLN), are colloidal carriers with a lipid framework that is solid at body temperature. The execution of lipid nanoparticle plans is extraordinarily affected by their arrangement and structure. The most part made out of lipids, surfactants and co-surfactants are formed the lipid nanoparticles. The lipid materials utilized as a part of the generation of lipid nanoparticles are generally strong at room temperature. Being all around endured in physiological conditions, lipid nanoparticles are ordinarily biocompatible. Fluid lipids, or oils, are particularly utilized for generation of NLCs. Much of the time, lipid nanoparticles are created as scatterings and surface-customized with surfactants to enhance scattering steadiness. Polymers are regularly used to frame polymer-lipid centres in the generation of PLNs. These colloidal carriers have pulled in expanding enthusiasm for their utilization in restorative and corrective applications. Lipid nanoparticles are frequently utilized as managed discharge frameworks, with the structure of the lipid nanoparticles directing their discharge properties. Lipid nano details can be custom-made to meet an extensive variety of item necessities managed by illness condition, course of organization and contemplations of cost, item dependability, harmfulness, adequacy and dosage diminishment. The demonstrated security and viability of lipid-based transporters make them alluring possibility for the plan of pharmaceuticals.

Keywords: Nanoparticles, nanostructured lipid carrier, components, method of preparations, analysis of NPs

Introduction
Industry estimates suggest that approximately 40% of lipophilic drug candidates fail due to solubility and formulation stability issues, which has been solved by various novel and advanced lipophilic drug delivery technologies [1]. The lipids employed to prepare lipid nanoparticles are usually physiological lipids (biocompatible and biodegradable) so, that drugs can be delivered at the required site of action with controlled release with low acute and chronic toxicity [2]. Nanotechnology is being applied extensively to provide targeted drug therapy, diagnostics, tissue regeneration, cell culture, biosensors and other tools in the field of molecular biology. To overcome the drawbacks associated to the traditional colloidal systems such as emulsions, liposomes and polymeric nanoparticles, various nanotechnology platforms like NLC, fullerenes, nanotubes, quantum dots, nanopores, dendrimers, liposomes, magnetic nanoprobes and radio controlled nanoparticles are being developed. Figure 1 depicts the difference in types of nanoparticles.

Structure of Solid Lipid Nanoparticles
SLNs have three different morphologies, based on the location of the incorporated drug molecule they are classified as below;
• Homogenous matrix (Solid Solution) model
• Drug-enriched shell model
• Drug-enriched core model

These structures have been described based on the results observed by Müller and co-workers [3].

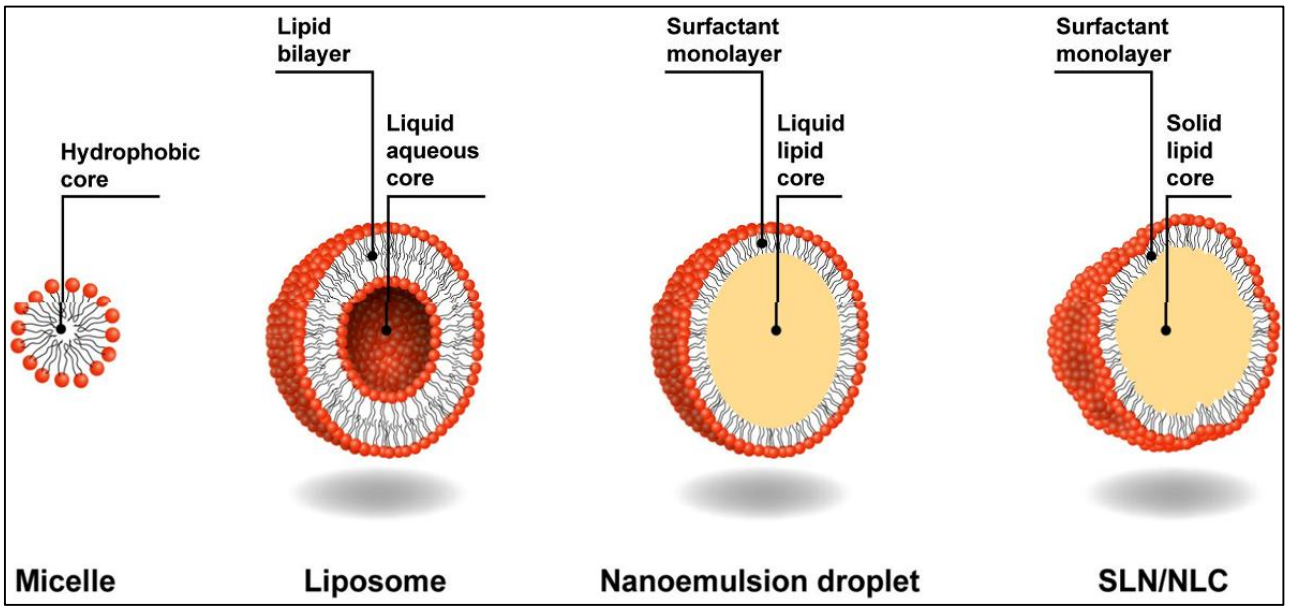

Fig 1: Comparison between micelles, liposomes, nanoemulsions and solid lipid nanoparticles. (Micelles with hydrophobic core which is formed by the tails of the surfactant molecules. Liposomes with aqueous core surrounded by a double phospholipid layer. Nanoemulsions droplets with hydrophobic liquid core composed of the oil that is dispersed in the water and stabilized by a surfactant monolayer. SLN and NLC with hydrophobic core of solidified lipid; often the solidification/crystallization of the lipid results in non-spherical shape of the particles).

Homogeneous Matrix Model (Solid Solution)

A solid solution model, also referred to as the homogenous matrix model, is obtained when the drug is homogenously dispersed within the lipid matrix in molecules or amorphous clusters. This model is usually described for lipid nanoparticles prepared by a cold homogenization technique, or when highly lipophilic drugs are incorporated such that a hot homogenization technique can be employed without the use of surfactants or drug-solubilizing molecules. When a cold homogenization technique is employed, the solubilized drug is dispersed in the bulk lipid. When subjected to high pressure homogenization, mechanical agitation leads to the formation of lipid nanoparticles with a homogenous matrix. A similar result is obtained when the lipid droplets produced by a hot homogenization technique are rapidly cooled; droplets tend to crystallize and there is no phase separation between the drug and the lipid. Such models are suitable for incorporation of drugs that exhibit prolonged release from particles [4]. An example of such a model is a prednisolone-loaded SLN system that exhibits slow release of prednisolone, usually from 1 day to 6 weeks [5].

Drug-Enriched Shell Model

A schematic of the drug-enriched shell model is depicted in Figure 2. A drug enriched shell is a lipid core enclosed by a drug-enriched outer shell. Such a structure is obtained when hot liquid droplets cool rapidly to form lipid nanoparticles as a result of phase separation. The drug-enriched shell morphology can be explained by a lipid precipitation mechanism that occurs during production and by repartitioning of the drug that occurs during the cooling stage. After hot homogenization, each droplet is a mixture of melted lipid and drug. Rapid cooling accelerates lipid precipitation at the core with a concomitant increase in drug concentration in the outer liquid lipid. Complete cooling leads to precipitation of a drug-enriched shell. This structural model is suitable for incorporation of drugs that are released as a burst. Such a rapid release is highly desirable for dermatological SLN formulations that require increased drug penetration, in addition to the occlusive effect of the SLN [4]. The controlled release of clotrimazole from a topical SLN formulation was due to its drug-enriched shell structure [6]. The solubility of the drug in the surfactant-water mixture at elevated temperatures is another factor that can influence precipitation of drug in the shell. During the hot homogenization process, drug partially moves out of the lipid core due to its increased solubility in the surfactant solution. However, solubility of the drug in the surfactant solution decreases as the dispersion is cooled. This leads to drug enrichment in the shell, in cases where lipid core solidification has already started [4].

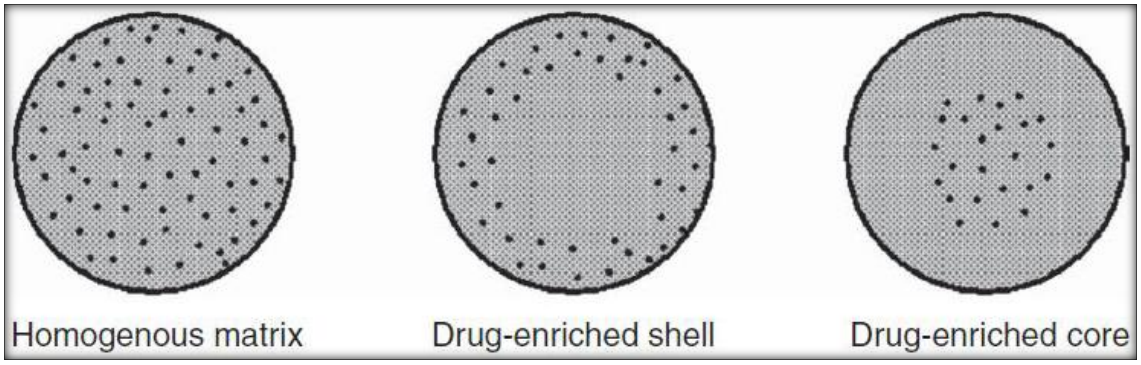

Fig 2: Types of SLN: (I) Homogeneous Matrix Model (II) Drug-Enriched Model and (III) Drug-Enriched Core Model [4]

Drug-Enriched Core Model

A drug-enriched core model is obtained when the re-crystallization mechanism is the opposite of that described for the drug-enriched shell model. Figure 3 shows a schematic representation of a drug-enriched core model. This morphology is obtained when the drug has a tendency to crystallize prior to the lipid. The drug is solubilized in the lipid melt close to its saturation solubility. Subsequent cooling of the lipid emulsion causes super-saturation of the drug in the lipid melt; this leads to the drug re-crystallizing prior to lipid re-crystallization. Additional cooling leads to lipid re-crystallization that forms a membrane around the already crystallized drug-enriched core. This structural model is suitable for drugs that require prolonged release over a period of time, governed by Fick's law of diffusion [3].

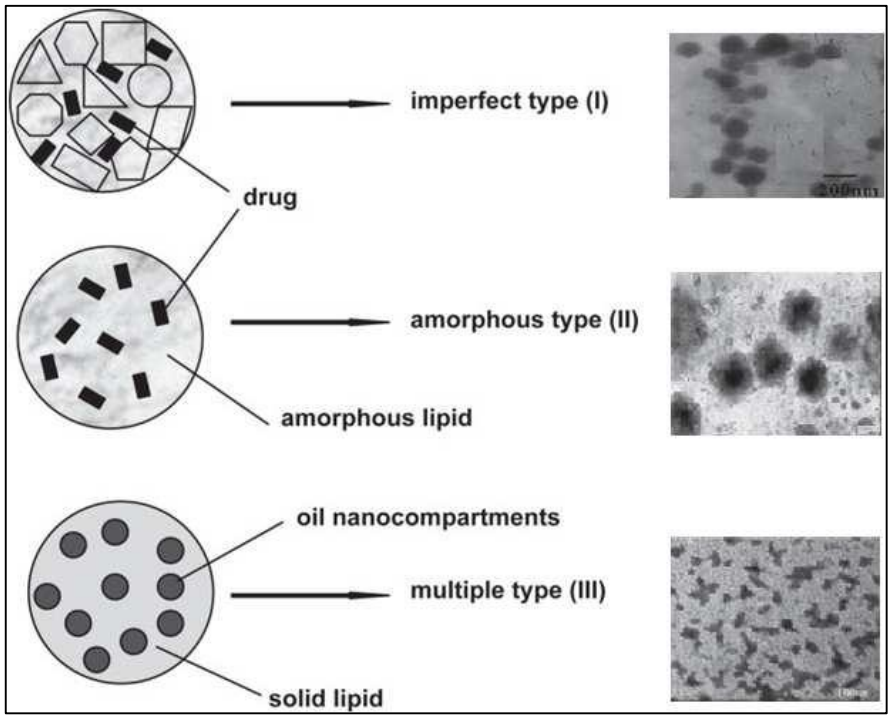

Fig 3: Types of NLC: (I) Imperfect Type, (II) Amorphous Type and (III) Multiple Type [54]

Structure of Nanostructured Lipid Carriers

Like SLNs, NLCs have been proposed to possess three different morphologies, based on the location of incorporated drug molecules [7-9];

- NLC type I or "imperfect crystal" type
- NLC type II or "amorphous" type
- NLC type III or "multiple" type

NLC Type I or "Imperfect Crystal" Type

Imperfect crystal type NLCs has an imperfectly structured solid matrix. Such imperfections can be increased by using glycerides composed of different fatty acids. Good drug accommodation can be achieved by increasing the number of imperfections. In order to achieve "maximum imperfections", rather than using solid lipids only, the imperfect type of NLC is prepared by mixing spatially different lipids, resulting in imperfections in the crystal lattice. The disordered crystal accommodates more drug molecules, either in molecular form or as amorphous clusters. Using a mixture of glycerides with varying fatty acid chains forms a solid matrix with variable distances. Addition of a small amount of liquid lipid further increases drug-loading [10].

NLC Type II or "Amorphous" Type

The phenomenon of crystallization often leads to drug expulsion. To minimise this, NLCs can also be prepared by carefully mixing solid lipids with special lipids such as Hydroxy octacosanyl hydroxyl stearate, isopropyl palmitate or MCT. Solid, but non-crystalline lipid nanoparticles are formed. The lipid core congeals in an amorphous nature. This type of NLCs, called "amorphous" type NLC, and minimizes drug expulsion by maintaining the polymorphicity of the lipid matrix.

NLC Type III or "Multiple" Type

The third type of NLC is the oil-in-lipid-in-water type. The solubility of lipophilic drugs in liquid lipids (oils) is higher than that in solid lipids. This principle can be used to develop the "multiple" type NLC. In this type of NLC, higher amounts of oil are blended in solid lipids. At low concentrations, oil molecules are easily dispersed into the lipid matrix. Addition of oil in excess of its solubility leads to phase separation producing tiny oily nano-compartments surrounded by the solid lipid matrix. Such models allow controlled drug release and the lipid matrix prevents drug leakage [5]. Lipophilic drugs can be solubilised in the oils and multiple types of NLCs are formed during the cooling process of a hot homogenization process.

Composition of Lipid Nanoparticles

Lipid nanoparticles are typically composed of solid lipid(s), surfactant(s), co-surfactant (optional) and active ingredients (typically drugs). The lipids used in the production of lipid nanoparticles are physiological lipids. Based on their structural diversity, lipids used in the production are broadly categorized into fatty acids, fatty esters, fatty alcohols, triglycerides or partial glycerides. A few researchers have also reported the use of waxes in the preparation of lipid nanoparticles [11]. Lipid nanoparticles are surface-tailored with surfactants, which stabilize the colloidal system. They are

sometimes used in combination with a co-surfactant, if necessary.

Lipids

The lipid, itself, is the main ingredient of lipid nanoparticles that influence their drug loading capacity, their stability and the sustained release behaviour of the formulations. Lipid nanoparticle dispersions based on a variety of lipid materials including fatty acids, glycerides and waxes have been investigated [12, 21]. Most of these lipids, with the notable exception of cetyl palmitate, are approved as generally-recognised-as-safe (GRAS) and are physiologically well-tolerated. Selection of appropriate lipids is essential prior to their use in preparation of lipid nanoparticle dispersions. The main factors needs to be considered for the selection of appropriate lipids are as follows;

1) The solubility of the drug in lipid matrices

It is critical because it invariably influences the drug encapsulation efficiency and loading capacities, and subsequently the usefulness of the lipid nanoparticles in drug delivery [22]. The solubility of drug can be easily quantified using UV-Visible spectroscopy or chromatographic techniques [23, 25].

2) The partitioning of drug between the lipid/oil and aqueous phases

This can also be predicted using mathematical equations. Such predictions are based on drug-lipid and drug-water interactions. Lipid nanoparticles with high drug loading can be prepared if the drug has high solubility in lipid or a high partition coefficient. Since the drug has different solubility in different lipid matrices, its apparent partition coefficients in those lipids also differ. This consequently leads to different loading capacities in different lipid matrices for the same drug. The complexity thus makes predictive models difficult; however they remain very useful as screening and prediction tools. Even though there are no specific guidelines, empirical values, such as the solubility of drug in the lipid have been proposed as suitable criteria for selection of an appropriate lipid [26].

3) Lipid polymorphism

Lipid polymorphism is another factor that influences the properties of a lipid nanoparticle system. The occurrence of multiple crystalline forms in solid lipids is particularly useful as they provide structural defects in which drug molecules can be accommodated. The perfect crystalline lattice, however, is more thermodynamically stable than the others. For example, the β-forms of triglycerides are more stable than the α-forms and β′-forms [27]. Thermodynamically less stable or metastable forms eventually tend to transform to a more stable form. Such transitions pose a significant challenge in development of SLNs since drug molecules are accommodated in the crystal defects of the solid lipids. Their disappearance with time thus creates an obvious issue to drug loading. This results in drug expulsion during storage or burst release after administration.

4) Tendency to form perfect crystalline lattice structures

Another factor that influences the selection of an appropriate lipid is thus its tendency to form perfect crystalline lattice structures or, at least, the rate at which meta-stable to stable transitions take place. No definitive guidelines exist for the choice of lipids based on these properties. Generally, crystallisations in lipids with longer chains of fatty acids are slower than those with shorter fatty acid chains [28]. Wax-based lipid nanoparticles are physically more stable, however they exhibit significant drug expulsion due to their more crystalline nature [11].

To avoid such problems with lipid crystallinity and polymorphism, a binary mixture of two spatially different solid lipid matrices, i.e. a solid lipid and a liquid lipid (or oil) was used to prepare lipid nanoparticle dispersions, now known as "nanostructured lipid carriers (NLC)" [5, 6, 10]. Cationic lipids utilised in lipid nanoparticle preparation have been reported for use in gene delivery. The positive charge on the particle surface due to the use of a cationic lipid may enhance transfection efficiencies. Two-tailed (or branched) cationic lipids are preferred over one-tailed cationic lipids due to the cytotoxicity of the latter [29, 30].

Both solid and liquid lipids are included in NLCs for constructing the inner cores [5]. The solid oils those are cost effective, non-irritating, and capable of being sterilized before application. Vitamin E (α-tocopherol) and other tocols have been investigated as materials for nanoemulsions [31]. Tocols can serve as a choice of oils for NLCs because of their stability, ease of production on a large scale and good solubility in lipophilic drugs. Lipids commonly used for NLCs include glyceryl behenate (Compritol® 888 ATO), glyceryl palmitostearate (Precirol® ATO 5), fatty acids (e.g. stearic acid), triglycerides (e.g. tristearin), steroids (e.g. cholesterol), and waxes (e.g. cetyl palmitate). These lipids are in a solid state at room temperature. They would melt at higher temperatures (e.g. > 80 °C) during the preparation process. Liquid oils typically used for NLCs consist of digestible oils from natural sources. The medium chain triglycerides, such as Miglyol® 812, are often utilized as the constituents of liquid lipids because of their similar structures to Compritol® [5].

Other oily components such as paraffin oil, 2-octyl dodecanol, propylene glycol dicaprylocaprate (Labrafac®), isopropyl myristate and squalene are included as well. Alternatively, the fatty acids, such as oleic acid, linoleic acid, and decanoic acid, are included in NLCs for their value as having oily components and as being penetration enhancers of topical delivery. In general, these lipids are already approved by European and American regulatory authorities for clinical applications and for their "generally recognized as safe" (GRAS) status. There is a need for novel and biocompatible natural oils from plants are also currently popular. Averina *et al*. have used Siberian pine seed oil and fish oil from Baikal Lake as the liquid oils since they show acceptable physical and chemical stability to NLCs [32, 33].

Surfactants

Surfactants (also known as surface-active agents or emulsifiers) form the other critical component of the lipid nanoparticle formulation. Surfactants are amphipathic molecules that possess a hydrophilic moiety (polar) and a lipophilic moiety (non-polar), which together form the typical head and the tail of surfactants. At low concentrations, surfactants adsorb onto the surface of a system or interface. They reduce the surface or interfacial free energy and consequently reduce the surface or interfacial tension between the two phases [34]. The relative and effective proportions of these two moieties are reflected in their hydrophilic lipophilic balance (HLB) value. Surfactants used in the preparation of

lipid nanoparticle preparations play two quite distinct and important roles;

1) Surfactants disperse the lipid melt in the aqueous phase during the production process
2) Surfactants stabilize the lipid nanoparticles in dispersions after cooling.

Surfactants can be broadly categorized into three classes based on their charge: ionic, non-ionic and amphoteric. In all cases, the surfactants are surface tension lowering, which aids in the dispersion process required to form the product (first role). Ionic surfactants are traditionally thought to infer electrostatic stability, whilst non-ionic surfactants are traditionally thought to infer steric repulsion stability.

In reality, the situation is much more complex and many non-ionic surfactants used are too small to infer genuine steric stability, but probably result in stability through the Gibbs-Marangoni effect [35]. Members from the Pluronic® and Tween® families are the most commonly used non-ionic surfactants. As discussed, most of these surfactants contain a hydrophilic moiety (ethylene oxide) and a hydrophobic moiety (hydrocarbon chain). Phospholipids and phosphatidylcholines are the common amphoteric surfactants employed in lipid nanoparticle preparation. These surfactants have both negatively and positively charged functional groups. They exhibit features of a cationic and an anionic surfactant at low and high pH conditions, respectively. Selection of surfactants for nanoparticle preparation depends on a number of factors, including;

- Intended route of administration
- HLB value of surfactant
- Effect on lipid modification and particle size
- Role in in-vivo degradation of the lipid

Non-ionic surfactants are preferred for oral and parenteral preparations as they are less toxic and exhibit less irritation than ionic surfactants [36]. Amongst the ionic surfactants, cationic surfactants are more toxic than anionic or amphoteric surfactants. Therefore, the surfactants arranged in the decreasing order of toxicity are: Cationic > Anionic > Non-ionic > Amphoteric. Non-ionic surfactants effectively inhibit the in-vivo degradation of lipid matrix. The poly (ethylene oxide) (PEO) chains on the non-ionic surfactants hinder the anchoring of the lipase/co-lipase complex that is responsible for lipid degradation. Adjusting the density of PEO chains on lipid nanoparticle surfaces can modify its in vivo degradation rate. Olbrich *et al*. Studied the effects of surfactants on in vivo lipid degradation [37, 38]. They suggested that Poloxamer 407 and sodium cholate have the most and least lipid degradation inhibitory effect amongst a selection of tested surfactants.

The emulsifiers have been used to stabilize the lipid dispersions. Most of the investigations employ hydrophilic emulsifiers such as Pluronic F68 (poloxamer 188), polysorbates (Tween), polyvinyl alcohol, and sodium deoxycholate [39-41]. Lipophilic or amphiphilic emulsifiers such as Span 80 and lecithin are employed for fabrication of NLCs if necessary. It has been found that the combination of emulsifiers can prevent particle aggregation more efficiently [42].

Polyethylene glycol (PEG), sometimes added in NLCs, resides on the nanoparticulate shell to prevent uptake by the reticuloendothelial system (RES) and to prolong the circulation time of drugs. Another prerequisite for NLCs' stability is the ability for preservation. The preservatives can impair the physical stability of lipid dispersions. Obeidat *et al.* demonstrate that Hydrolite® 5 is proved suitable for the preservation of coenzyme Q10- loaded NLCs [43].

Other Agents

Apart from lipids and surfactants, lipid nanoparticle formulations can also contain a number of other ingredients including counter-ions and surface modifiers. The lipid nanoparticles engineered for encapsulation of cationic, water-soluble drugs may contain counter-ions such as organic anions or anionic polymers [44-46]. Tailoring of the lipid nanoparticle surface with surface-modifiers such as hydrophilic polymers may reduce their uptake by the reticuloendothelial system (RES). The so-called "stealth" or long-circulating carriers stay longer in the systemic circulation and increase the residence of drug in blood [47-48]. These "stealth" SLNs have been widely studied for delivery and targeting of anti-cancer cells as they are effectively and selectively taken up by tumour cells [49-51]. Table 1 lists some of the counter-ions and surface-modifiers used in lipid nanoparticle preparation.

Table 1: The Excipients For Composing Lipidic Nanpoparticulate Drug Delivery Systems

Ingredients	Materials
Solid lipids	Tristearin, stearic acid, cetyl palmitate, cholesterol, Precirol® ATO 5, Compritol® 888 ATO, Dynasan®116, Dynasan® 118, Softisan® 154, Cutina® CP, Imwitor® 900 P, Geleol®, Gelot® 64, Emulcire® 61.
Liquid lipids (Used in Nanostructured Lipid Carrier)	Medium chain triglycerides, paraffin oil, 2-octyl dodecanol, oleic acid, squalene, isopropyl myristate,vitamin E, Miglyol® 812, Transcutol® HP, Labrafil Lipofile® WL 1349, Labrafac® PG, Lauroglycol® FCC, Capryol® 90.
Hydrophilic emulsifier	Pluronic® F68 (poloxamer 188), Pluronic® F127 (poloxamer 407), Tween 20, Tween 40, Tween 80, polyvinyl alcohol, Solutol® HS15, trehalose, sodium deoxycholate, sodium glycocholate, sodium oleate, polyglycerol methyl glucose distearate.
Lipophilic emulsifiers	Myverol® 18-04K, Span 20, Span 40, Span 60
Amphiphilic emulsifiers	Egg lecithin, soya lecithin, phosphatidylcholines, phosphatidylethanolamines, Gelucire® 50/13

Preparation Procedures of Lipid Nanoparticulate Drug Delivery Systems

There numerous strategies for the preparation of lipid nanoparticulate DDS. The technique utilized is to be based on the type of drug particularly its physicochemical properties of the lipid matrix, route of administration and so forth.

High Pressure Homogenization (HPH) Technique:

HPH has been used as a dependable and capable method of choice for the large scale production of NLCs, lipid drug conjugate, SLNs, and parenteral emulsions. In High Pressure homogenization system lipid are pushed with high pressure (100-200bars) through narrow gaps of few micron ranges. So shear pressure and cavitation are the powers which make the

disruption of particles to submicron run. Typically the lipid contents are in the range of 5-10%. As opposed to other preparation method High Pressure Homogenization does not indicate scaling up problem. Basically there are two methodologies for preparation of NLCs by high pressure homogenization, hot and cool homogenization techniques [52]. For both the methods drug is dissolved in the lipid being melted at around 5-10º C over the melting point.

Hot Homogenization Technique

In this method the drug along with liquid lipid is dispersed under steady stirring by a high shear device in the aqueous surfactant solution of same temperature. The pre-emulsion obtained is homogenized by utilizing a piston gap homogeniser and the hot nanoemulsion is chilled off to room temperature where the lipid recrystallises and prompts to formation of nanoparticles [53].

Cold Homogenisation Technique

Cold homogenisation is done with the solid lipid containing drug. Cold homogenisation has been developed to overcome the issues of the hot homogenisation procedure, for example, temperature mediated accelerated degradation of the drug payload, partitioning and subsequently loss of drug into the aqueous phase during homogenisation. The initial step of both the cold and hot homogenisation techniques is the same. In the consequent advance, the melt containing drug is cooled quickly utilizing ice or liquid nitrogen for circulation of in the lipid matrix as appeared in the figure 4. Cold homogenization technique minimises the thermal exposure of the sample [54].

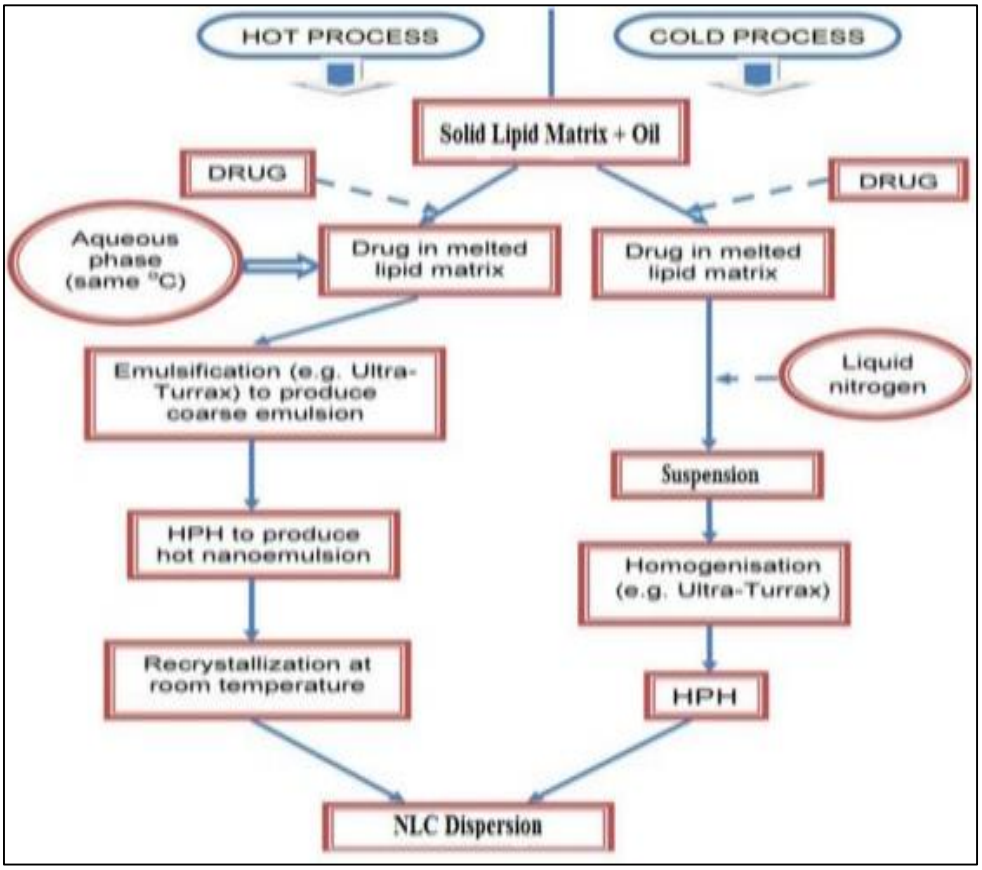

Fig 4: Schematic overview of the hot and cold homogenisation technique

Microemulsion technique

The lipids (fatty acids or glycosides e.g., Stearic acid) are melted and drug is added in the molten liquid lipid. A blend of water, co-surfactant(s) and the surfactant is heated to the same temperature as the lipids and added under gentle mixing to the lipid melt. A transparent, thermodynamically stable framework is formed when the mixes are blended in the right proportions for microemulsion development. Thus microemulsion is the basis for the development of nanoparticles of a requisite size. This microemulsion is then dispersed in a cold aqueous medium under gentle mechanical mixing of hot microemulsion with water in a proportion in the range 1:25 - 1:50. And the dispersion in cold aqueous medium prompts to quick recrystallisation of the oil droplets [55].

Solvent emulsification-evaporation technique

In solvent emulsification-evaporation method, the hydrophobic drug and lipophilic material were dissolved in a water immiscible organic solvent (e.g., cyclohexane, dichloromethane, toluene, chloroform) and afterward that is emulsified in an aqueous stage using high speed homogenizer. To enhance the efficiency of fine emulsification, the coarse emulsion was instantly passed through the micro fluidizer. From that point, the natural dissolvable was evaporated by mechanical mixing at room temperature and reduced pressure (e.g. rotary evaporator) leaving lipid precipitates of SLNs. Here the mean particle size depends upon the concentration of lipid in organic phase. Small particle size could be acquired with low lipid load (5%) related to organic solvent. The advantage of this technique is the absence of any thermal stress, which makes it appropriate for the thermolabile drugs. A reasonable drawback is the utilization of organic solvent which may interact with the drug molecule and may limit the solubility of the lipid in the organic solvents [56].

Solvent emulsification-diffusion technique

In solvent emulsification-diffusion method, the solvent used (e.g. benzyl alcohol, butyl lactate, ethyl acetate, methyl acetate, isopropyl acetate) must be partially miscible with water and this technique can be done either in aqueous phase or in oil. At first, both the solvent and water were mutually saturated keeping in mind the end goal to guarantee the underlying thermodynamic balance of both liquid. When heating is required to solubilize the lipid, the saturation step was performed at that temperature. At that point the lipid and drug were broken down in water saturated solvent and this organic phase (internal phase) was emulsified with solvent saturated aqueous solution containing stabilizer (dispersed medium) utilizing mechanical stirrer.

After the development of o/w emulsion, water (dilution medium) in run of the mill proportion ranges from 1:5 to 1:10, were added to the system so as to permit solvent diffusion into the continuous phase, hence forming accumulation of the lipid in the nanoparticles. Here the both the phases were keep up at elevated temperature and the diffusion step was performed either at room temperature or at the temperature under which the lipid was dissolved. All through the procedure steady stirring was kept up. At long last, the diffused dissolvable was elevated by vacuum distillation or lyophilization [57].

Phase inversion temperature (PIT) method

Phase inversion of O/W to W/O emulsions and the other way around prompted by temperature change is a long known technique to produce micro emulsions stabilized with non-ionic surfactants [58]. The system depends on the adjustment in the properties of polyoxyethylated surfactants at various temperatures. The hydrophilic lipophillic balance (HLB) estimation of surfactants characterized by Griffin is substantial at 25 °C. At this temperature the hydrophilic parts of the SAC atoms are hydrated to a specific degree. An increase in the temperature causes lack of hydration of the ethoxy gatherings. Accordingly, the lipophilicity of the atoms of the SAC ascends with comparing diminish in HLB esteem. At one point the partiality of the SAC to the fluid and lipid stage is equivalent - this temperature is characterized as the stage reversal temperature. This particulate state is portrayed by low surface pressure and nearness of complex structures in the framework. In the event that the temperature is additionally expanded the SAC's fondness to the lipid phase ends up noticeably sufficiently higher to balance out emulsions of w/o compose.

Melting dispersion method

In melting technique, drug and solid lipid are melted in an organic solvent called as oil phase, and all the water phase is additionally heated to an indistinguishable temperature from oil phase. In this way, the oil stage is added to a little volume of water stage and the subsequent emulsion is stirred at high speed for couple of hours. At long last, it is cooled down to room temperature to yield nanoparticles [58].

High Shear Homogenization or Ultrasonication Technique

Ultrasonication is based of cavitations. In initial step, the drug was added to previously melt solid lipid. In second step, the heated aqueous phase (heated to same temperature) was added to the melted lipid and emulsified by probe sonication or by utilizing high speed stirrer or aqueous phase added to lipid phase drop by drop followed by magnetic stirring. The acquired pre-emulsion was ultrasonicated using probe sonicator with water bath (at 0 ºC). With a specific end goal to counteract recrystalization amid the process, the creation temperature kept no less than 5 ºC over the lipid melting point. The acquired item was filtered through a 0.45µm film with a specific end goal to remove impurities carried amid ultrasonication [59].

Solvent injection (or solvent displacement) technique

In this method a solvent that distributes very rapidly in water (DMSO, ethanol) is utilized [60]. In the first place the lipid is broken down in the solvent and afterward it is immediately infused into an aqueous solution of surfactants through an injection needle. The solvent migrates quickly in the water and lipid particles precipitate in the aqueous solution. Particle size depends upon the velocity of distribution process. Higher velocity brings about smaller particles. The more lipophilic solvents give bigger particles which may turn into a problem. The strategy offers advantages, for example, low temperatures, low shear pressure, easy handling and fast production process without actually advanced hardware (e.g. high-weight homogeniser). However, the fundamental impediment is the utilization of organic solvents.

Double emulsion technique

In double emulsion technique the drug (mainly hydrophilic drugs) is dissolved in aqueous solution, and further emulsified in melted lipid. The essential emulsion is stabilised by including stabilizer that is dispersed in aqueous phase containing hydrophilic emulsifier, which is trailed by stirring and filtration. Double emulsion system avoids the need to melt the lipid for the preparation of peptide-loaded lipid nanoparticles and the surface of the nanoparticles could be changed with a specific end goal to sterically stabilise out them by methods for the incorporation of lipid-PEG derivatives [61].

Physicochemical Characterization of Lipid Nanoparticulate Drug Delivery Systems

The physicochemical portrayal for NLCs is basic to affirm quality control and stability. Both physical and substance properties can be resolved for NLCs. Microscopic and Macroscopic strategies are utilized as a part of advancement of colloidal system. Different systems like molecule estimate examination, zeta-potential, transmission electron microscopy, differential checking calorimetry (DSC), X-Ray scrambling, captivated light microscopy, laser diffraction (LD), field-stream fractionation (FFF) were performed to research the structure, versatility and sub-atomic condition of the mixes. These strategies likewise uncover the physical and concoction strength of definition, surface charge has a tendency to decide the particles will flocculate or not.

Particle Size

The particle size is important parameter in process control and quality assurance because physical stability of vesicle dispersion depends on particle size and as particle size decreases, surface area characteristics increases as a function of total volume, photon correlation spectroscopy (PCS) based on laser light diffraction provides an appropriate method for investigation and can be applied for particles ranging below 200 nm and up to 1µm 86. For particles below 200nm Rayleigh's theory holds that the scattering intensity to be proportional to the sixth potency of the particle diameter.

Both, Fraunhofer's and Rayleigh's theories, are only approximations of Mie's theory which claims that the scattering intensity depends on the scattering angle, the absorption and the size of the particles as well as the refractive indices of both the particles and the dispersion medium.

Zeta potential (ZP)
Zeta potential is the electric potential of a particle in a suspension. It is a parameter which is very useful for the assessment of the physical stability of colloidal dispersions. In suspensions the surfaces of particles develop a charge due to ionization of surface groups or adsorption of ions. This charge depends on both the surface chemistry of the particles and the media around these particles. The surface charge generates a potential around the particle, which is at the highest near the surface and decays with distance into the medium. The zeta potential can be measured by determining the velocity of the particles in an electrical field (electrophoresis measurement).

Scanning electron microscopy (SEM)
This technique can be used to investigate the shape of the particles prepared and to assess the particle size of these particles. Aqueous NLC dispersions can be applied and spread on a sample holder (thin carbon film). The samples will be placed inside of the vacuum column of the microscope and the air was pumped out of the chamber. An electron gun placed at the top of the column emits a beam of high energy primary electrons. The beam of the electrons passes through the lenses which concentrates the electrons to a fine spot and scan across the specimen row by row. As the focused electron beam hits a spot on the sample, secondary electrons are emitted by the specimen through ionization. A detector counts these secondary electrons. The electrons are collected by a laterally placed collector and these signals are sent to an amplifier.

Differential scanning calorimetry (DSC)
DSC is usually used to get information about both the physical and the energetic properties of a compound or formulation. DSC measures the heat loss or gain as a result of physical or chemical changes within a sample as a function of the temperature. DSC and powder is performed for the determination of the degree of crystallinity of the particle dispersion. The rate of crystallinity using DSC is estimated by comparison of the melting enthalpy/g of the bulk material with the melting enthalpy/g of the dispersion.

Nuclear magnetic resonance (NMR)
NMR can be used to determine both the size and the qualitative nature of nanoparticles. The selectivity afforded by chemical shift complements the sensitivity to molecular mobility to provide information on the physicochemical status of components within the nanoparticle. The mobility of the solid and liquid lipids is related to the width at half amplitude of the signals [62]. Broad signals and small amplitudes are characteristics of molecules with restricted mobility and strong interactions. The higher line width of NLCs compared to the physical mixture of the materials added in NLCs indicates the interaction of liquid oil with the solid lipid. Immobilization of the nanoparticles of NLCs is stronger compared to SLNs with totally crystallized cores.

Atomic Force Microscopy (AFM)
AFM is optimal for measuring morphological and surface features that are extremely small. AFM does not use photons or electrons but a very small sharp tipped probe located at the free end of a cantilever driven by inter-atomic repulsive or attractive forces between the tip and surface of the specimen [63]. Although electron microscopy is still frequently used, the AFM technique offers substantial benefits: real quantitative data acquisition in three dimensions, minimal sample preparation times, flexibility in ambient operating conditions, and effective magnifications at the nano levels [64].

X-ray Scattering
With X-ray scattering experiments characteristic interferences the generated from an ordered microstucture. A typical interference pattern arises due to specific repeat distances of the associated interlayer spacing d. According to Bragg's equation, d can be calculated as;
$d = n/\lambda\ 2 \sin \theta$
Where, λ is the wavelength of the X- ray being used, n is an integer and nominates the order of the interference and θ is the angle under which the interference occurs.

Transmission Electron Microscopy
It is a technique where colloidal samples could be visualized at high resolution. Sufficient contrast can be given to a thin film of the frozen sample by use of osmium tetra- oxide. This allows the sample to be viewed directly in the TEM (at temperature -196 ºC). The adjustment of the temperature to -196 ºC leads to a very poor pressure, so that the amination of the sample is possible by preservation of microstructure despite the high vacuum.

Drug Release
The controlled or sustained release of the drugs from NLCs can result in the prolonged half-life and retarded enzymatic attack in systematic circulation. The drug release behaviour from NLCs is dependent upon the production temperature, emulsifier composition, and oil percentage incorporated in the lipid matrix [65]. The drug amount in the outer shell of the nanoparticles and on the particulate surface is released in a burst manner, while the drug incorporated into the particulate core is released in a prolonged way. Sustained release of the drugs can be explained considering both drug partitioning between the lipid matrix and water, as well as the barrier function of the interfacial membrane [66, 67]. The dialysis method and the utilization of the Franz cell are the modes for measuring in vitro drug release from nanoparticles. The interpretation of in vitro drug release profiles should consider the specific environment in the in vivo status. Enzymatic degradation of lipid nanoparticles may be influenced to a relevant extent by the composition of the particles.

Factors affecting the Drug release
The release study must be performed to compare the capacity of different samples to retain the drug incorporated for a longer time and release it slowly from the lipid matrix of the nanoparticles. Many factors that could affect the release profile of the drug from the NLC system. The effect of the particle size, the lipid matrix, the surfactant, the drug concentration in the lipid matrix and the drug type can be studied.

Particle size
The particle size of a colloidal system (e.g. NLC) is a crucial factor for the release of the material(s) incorporated inside the particles.

Lipid matrix
Different lipid matrices lead to different release profiles. The lipids have different crystals order and crystallization modification, different melting points and different hydrophilic lipophilic balance (HLB) values, e.g. Apifil HLB = 9.4, Compritol 888 HLB = 2. This makes the affinity of the drug to be entrapped within the lipid matrix different from one lipid to another.

Surfactant
Surfactants as they are used to stabilize the particles in the dispersion media (or emulsify the oil in water) may the structure of the lipid nanoparticles. This happens because of the interaction between the emulsifying agent molecules and the lipid molecules. Depending on the HLB of the surfactant and the molecular weight of the surfactant molecules, the affinity of the surfactant to the lipid differs. Having the surfactant molecules embedded in the lipid matrix might dramatically affect the crystallization of the lipid, and leave spaces in the lipid lattice. These spaces will give rise to higher loading capacity of drug, incorporation in imperfections inside the particle matrix and eventually a slower release profile. Moreover, the ability of the surfactant to stabilize the oil droplets (in the lipid melted state during homogenization) and form smaller NLCs gives the surfactant also a role through the size of the formed lipid particles. The physicochemical properties of the NLCs are essentially influenced by the type of surfactant used.

Drug loading
Drug loading might affect the release profile. It depends on the affinity of the drug to mix with the lipid and be enclosed in the matrix.

Drug type
The drug type affects the release profile because with the different compositions of drugs there are different affinities to the lipid matrix. NLCs have unique characteristics that can enhance the performance of a variety of incorporated drug forms.

Conclusion
The physicochemical characteristics and stability of lipid nanoparticles are dependent on the composition of the lipid nanoparticle formulations. The lipid nature of these carrier systems is one of the major features that have attracted the interest of many researchers. Based on the organisation of lipids and drugs in the particles, a wide variety of structural models have been described for SLNs and NLCs. The drug release from lipid nanoparticles is a compromise between the composition and the structural model obtained for each formulation.
In the 20th century, Paul Ehrlich envisioned his magic bullet concept; the idea that drugs reaches the right site in the body, at the right time, at right concentration. The aim has been to develop therapeutic nanotechnology undertaking, particularly for targeted drug therapy. The smart NLCs as the new generation offer much more flexibility in drug loading, modulation of release and improved performance in producing final dosage forms such as creams, tablets, capsules and injectables. The effort to develop alternative routes and to treat other diseases with NLCs should be continued to extend their applications. Permeation via the gastrointestinal tract and BBB may be a future trend. The combination of two therapeutically active agents to be included in a single nano-system is another consideration for future development.

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