Nano lipid Particles - A New Generation of Solid Lipid Drug Carriers in Drug Delivery

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ABSTRACT

Development of delivery systems for new drug for its safe and effective delivery it into the body is an integral part of drug research program. This is particularly true due to increased incidence of adverse pharmacokinetic behavior of new drugs developed recently. Alternative delivery systems have been researched extensively not only to modulate their kinetic behavior in the body but also to achieve target drug delivery. Among them lipid based colloidal system have high potentiality for success. To overcome stability problems and to achieve high drug loading capacity in lipid nanoparticles, nano-lipid carriers composed of solid and liquid lipids have gained much importance in recent years due to their resistance to lipid polymorphism and structural imperfection in comparison to solid lipid particles. Selection of appropriate lipid composition and their influence on physico-chemical and bio-distribution characters has given ample scope for designing nano-lipid particulates for target delivery to various organs in various pathological conditions. This review covers various salient information on nano-lipid particulate technology including stability, production along with numerous examples of research reports on the subject.

Keywords: Solid lipid nanoparticles, Nano-lipid particulates, Target drug delivery, High pressure homogenization, lipid polymorphism.

Abbreviations: Solid lipid nanoparticle (SLN); Nano-lipid carrier (NLC); Nano-lipid particle (NLP); High pressure homogenization (HPH); Supercritical fluid (SCF).

1. Introduction

Breakthrough in drug research is not only about development of new drugs but also development of new technologies for its safe and effective delivery into the body. In fact it is more challenging to develop delivery systems for new drug substances and recently introduced blockbuster drugs which suffer from poor water solubility and insufficient bioavailability. Thus, there is an expanding need to develop a suitable and versatile pharmaceutical carrier that overcomes these problems. The carrier should be free of toxicity, have controlled release characteristics; have an adequate drug loading capability and the possibility of targeting to the diseased site. The system should also provide physical and chemical stability for the incorporated drug during production, storage and in the body after administration. However, It is equally important that the system developed should be affordable and commercially feasible [1-3].

Colloidal systems have been researched extensively as a viable means to deliver drugs with most of the qualities needed for a carrier. However, shortcomings are often encountered even withcolloidal systems like liposomes, micro and nanoemulsions, nanocapsules, nanosponges, polymeric nanoparticles etc. Restricted physical and chemical steadiness throughout storage and rapid degradation *in-vivo* after dosage are the

common parameters of concern with all these nano systems [4-6] in addition to certain specific issues like; need of large-scale output methods, fast drug release from its carrier system, stability difficulties, remnant organic solvent residues used in the output method and toxicity from the residual monomer in polymer used [7,8]. Any one of these issues disqualifies the carrier material for use in a pharmaceutical delivery system.

Solid lipid nanoparticles (SLNs) have been presented as an alternate carrier system to emulsions, liposomes and polymeric nanoparticles. These nanoparticles are formulated from bio-compatible solid lipids that have wide spread availability and economic feasibility. However, they suffer from certain physical transitions that are encountered during manufacturing or storage, particularly, when subjected to fluctuating temperature conditions. Particle crystallization with the generation of higher energy polymorphs followed by transformation into low energy, more organised stable state (β) modification during storage could often lead to drug expulsion [9] from the matrix.

NLC are developed recently to overcome the drawbacks affiliated with SLN. These are prepared by blending liquid lipids with solid lipids in contrast to SLNs which are prepared using solid lipids only. Hence NLCs are claimed to be the second generation lipid nanoparticles with most of the qualities desired by a carrier system even with drugs having low water solubility and poor bio-availability. NLCs show advantage over SLNs in having higher drug loading capability particularly in formulating lipid particles containing hardworking compounds by conceiving a less organized solid lipid matrix, In contrast to SLNs, NLCs have an expanded drug stacking capacity and the likelihood of drug expulsion during storage is very less [2,10-13]. NLCs also have lower water content and therefore less inclined to exhibit unpredictable gelation [14-16]. Chemical stability of the incorporated drugs is an additional benefit disclosed by NLCs in comparison to the other colloidal carrier systems. This property is exploited very well in producing lipid particulate systems on a large scale even when using hard technology like high-pressure homogenization and the process can be modified to yield lipid particle dispersions with solid contents from 30–80% [2,12,15-17].

2. Structure of Lipid Nanoparticles

Lipid nanoparticles (LNP) are colloidal particles composed of a lipid matrix that is solid at room and body temperature. LNP have been tested for a number of administration routes including parenteral [18-21], oral and peroral [22-25], ocular [26-29], topical [30] and rectal [31,32]. Drugs loaded in LNP are also reported to show additional performance *invivo* like improved bioavailability, targeting capacity [33-35] and enhanced cytotoxicity against MDR cancer cells [36,37]. Structural difference between SLNs and NLCs is illustrated in figure 1. SLNs are orderly in nature while NLCs are disorderly. NLCs are composed of solid lipids and liquid lipids (oils) blended cohesively. The structural differences in the lipids and the crystallization process lead to a highly disordered, imperfect lipid matrix structure for NLCs. This structure offers sufficient space to load drug molecules and amorphous clusters of drugs in the lipid matrix. In general, drug solubility is higher in liquid lipids than in solid lipids. Depending on the percentage of the liquid lipid in the matrix, three possible structures are observed (**Figure 1**). Type I: Highly imperfect matrix; type II: Multiple O/F/W type and type III: Non crystalline amorphous NLC.

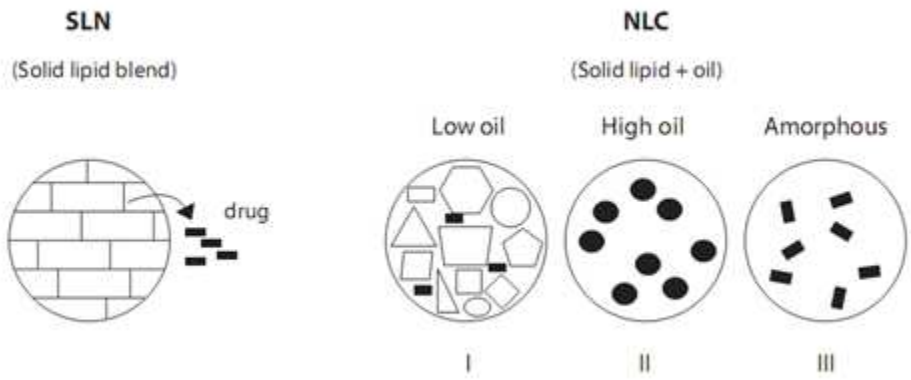

Figure 1: Different types of NLCs compared with SLN

Low oil composition leads to type I structure while high oil concentration forms Type II due to a miscibility gap between the two lipids (solid lipid plus oil). It may also leads to the precipitation of tiny oily nano compartments (**Figure 1-II**) during cooling. Type III is amorphous solid due to critical composition of lipids which eventually prevents them from crystallizing [38].

3. Composition

Lipid composition is critical in the preparation of LNPs with the desired characteristics including the drug-loading, stability and sustained-release characteristics. LNPs generally consist of solid lipid(s), surfactant(s), cosurfactants (if required) and incorporated active ingredients. The lipids used include fatty acids fatty esters, fatty alcohol, glycerides and mixtures of glycerol esters. Commonly used lipids are listed in **Table 1**. Waxes such as Cutina CP (cetyl palmitate), solid paraffin and beeswaxes are also sometimes used for LNP preparation. Synthetic lipids, such as para-acyl-calix [39] arene, are also developed recently to prepare LNP [40].

Table 1 Lipids used in the preparation of LNPs

Class of lipid	General structure	Examples (n value)
Fatty acids	$H_3C\text{-}[CH_2]n\text{-}COOH$	Dodecanoic acid (n = 10) Myristic acid (n = 12) Palmitic acid (n = 14) Stearic acid (n = 16)
Triglycerides	$H_2C\text{-}O\text{-}CO\text{-}[CH_2]n\text{-}CH_3$ | $HC\text{-}O\text{-}CO\text{-}[CH_2]n\text{-}CH_3$ | $H_2C\text{-}O\text{-}CO\text{-}[CH_2]n\text{-}CH_3$	Caprylate triglycerides (n = 6) Caprate triglycerides (n = 8) Trilaurin (n = 10) Tripalmitin (n = 14) Tristearin (n = 16) Tribehenin (n = 20)
Monoglycerides	$H_2C\text{-}O\text{-}CO\text{-}[CH_2]n\text{-}CH_3$ | $HC\text{-}OH$ | $H_2C\text{-}OH$	Glyceryl monostearate (n = 16) Glyceryl behenate (mono; n = 20)

Hydroxy monoglycerides	H_2C-O-CO-[CH_2]n-CH-[CH_2]m CH_3 | | HC-OH OH | H_2C-OH	Glyceryl hydroxystearate (n = 10; m = 5)
Diglycerides	H_2C-O-CO-[CH_2]n-CH_3 | HC-O-CO-[CH_2]n-CH_3 | H_2C-OH	Glyceryl behenate (di-n = 20)
Fatty esters	O || $H_{31}C_{15}$-C-O-$C_{16}H_{33}$	O Cetyl palmitate

1.1. *Drug loading capacity*

Loading capacity for the same drug differs depending on its apparent partition with lipid phases and HLB of the lipid mix which depends on the length of hydrocarbon chain of the fatty acids. A fatty ester is more hydrophobic than the fatty acid of the same chain length. Triglycerides are more hydrophobic than mono- and diglycerides due to substitution of all three hydrophilic hydroxyl groups. In addition, polymorphism of lipids also affects the properties of LNP due to the existence of multiple crystalline forms of solid lipids with varied crystalline lattice. Imperfect crystalline lattice of polymorphic lipids show defects in the LNP lattices. These defects help to accommodate more drug molecules and hence show high drug loading. However, any polymorphic transition of lipid molecules from the metastable form into the stable form could make the lattice more orderly leading to the disappearance of some of the defects in the lattices. As a result, drug expulsion from the lipid core to the particle surface may occur which naturally leads to high initial burst release and drug leakage during storage. To avoid this problem, lipid oils, for example, Miglyol 812 (caprylic/capric triglycerides) or oleic acid, or a binary mixture of physically incompatible solid lipids are incorporated into solid lipids to disrupt the crystallinity of the solid lipid matrix. The resulting modified form of LNP is often called as nanostructured lipid carrier.Positively charged lipids are used to prepare cationic LNP formulations particularly for gene delivery [41], due to their positive surface charge which may enhance the *invivo* transfection efficiency of genes. Some of the cationic lipids used in LNPs include stearylamine, N,N-di-(β-stearoylethyl)-N,N-dimethylammonium chloride, benzalkonium chloride, cetyltrimethylammonium bromide (CTAB), dimethyldioctadecylammonium bromide (DDAB), N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTAP) and cetylpyridinium chloride (CPC). However, cytotoxicity is a matter of concern in selecting cationic lipids. Under this circumstance, two-tailed cationic lipids are preferred over one-tailed cationic lipids (i.e., CPC and CTAB). Surfactants are used to stabilise LNP by dispersing the melt lipid in aqueous phase and to stabilise LNP on cooling. The surfactants commonly used are listed in **Table 2.** Although ionic surfactants such as sodium dodecyl sulfate form LNP with narrow size distribution and better stability, many of them are not recommended due to their undesirable toxicity.

Table 2. Commonly used surfactants in LNP

Ionic surfactants	Nonionic surfactants	Amphoteric surfactants	Cosurfactants
Sodium cholate, sodium cocoamphoacetate, sodium dodecyl sulfate, sodium glycocholate, sodium oleate, sodium taurocholate, sodium taurodeoxycholate	Brij-78, poloxamer 188, poloxamer 407, poloxamine 908, polyglycerol methylglucose distearate (Tego Care 450), Solutol HS15, Span 85, Tween-80, tyloxapol, Tween-20, trehalose	Egg phosphatidyl-choline, egg lecithin (Lipoid E80), soy phos-phatidylcholine (SP), (Epikuron 200, 95% SP), (Lipoid S100), (Lipoid S75, 68% SP), (Lipoid S75, 68% SP), (Phospholipon 90G, 90%)	Butanol, butyric acid

Table 3 Commonly used charge modifiers (counterions) in NLP

Organic salts	Ionic polymers
Monodecylphosphate Monohexadecylphosphate Monooctylphosphate Sodium hexadecylphosphate	Dextran sulfate sodium salt Hydrolysed and polymerised epoxidised soybean oil

Selection of surfactants for LNP preparation are based on parameters like the route of administration, particle size of NLPs desired, HLB value, and toxicity. Surfactants with HLB values in the range of 8–18 are suitable for the preparation of LNP through oil-in-water dispersion medium. Nonionic surfactants are preferred over ionic surfactants for oral and parenteral preparations due to their lower toxicity and biocompatibility. Encapsulation of water-soluble drugs in LNP is a challenge due to the lipophilicity of solid lipids. However, charge neutralization by incorporating counterions (**Table 3**) such as polymers and esters (organic salts) could solve the problem and may improve hydrophilic drug loading. In addition to lipids and surfactants, other agents like poloxamers, poloxamines or polyethylene glycol (PEG) are used for surface-modification of NLP for long circulation. This technique is valuable to minimise clearance of NLP by phagocytosis [42] due to high surface hydrophobicity of the particles.

4. Preparation of LNPs

Several techniques were reported for the production of LNPs including high-shear homogenization [43], high pressure homogenization (HPH), emulsification and solvent evaporation [44,45], emulsification and solvent diffusion [46,47], dilution of microemulsions [48,49], Super critical technique etc. Of all the afore-mentioned techniques, HPH is the most practical, reliable and industrially feasible technique [50].

4.1. *High pressure homogenization technique*

In this method, the lipid phase is melted and the drug is dissolved in it. The melt is then dispersed in an aqueous surfactant solution heated at the same temperature, usinghigh speed stirring. The pre-emulsion obtained is then homogenized to form hot o/w emulsion. This emulsion is cooled down to room temperature to solidify the dispersed lipid phase in to nano-particles. In large scale manufacturing of LNPs, the liquid to be homogenized is pumped with high pressure (1000–25,000 psi) through a narrow gap (in the range of few microns) in the high-pressure homogenizer. In the process, the fluid accelerates on a very short distance to very high velocity (>1000 km/h) thus experience high-shear stress and cavitations forces that disrupt the particles down to the sub-micron range. The lipid concentrations up to 40% have been homogenized to lipid nano-dispersions [51]. HPH method can be subdivided into two types, i.e., hot homogenization and cold homogenization technique for the production of LNPs [52, 53]. In hot homogenization technique, a pre-emulsion of the drug-loaded lipid melt and the aqueous emulsifier phase is homogenized by processing at higher temperatures, which results in lower particle sizes due to the decreased viscosity of the inner phase [54]. The nanoemulsions formed upon cooling to room temperature yield solid particles. In cold homogenization technique, the drugs are dispersed or solubilised in the molten lipid and the lipid melt is cooled using dry ice or liquid nitrogen. The solid matrix is then milled into micro-particles by means of ball or mortar milling to obtain particles of 50–100 µm size. These micro-particles were further dispersed in chilled aqueous emulsifier solution and subjected to HPH.

Cold homogenization has advantages of processing thermo labile drugs and also facilitating the entrapment of hydrophilic drugs [2]. However, it suffers from the disadvantage of formation of a fraction of micro-particles.

NLC dispersions with high lipid content (up to 80%) are highly viscous, gel-like or pasty. To prepare such concentrated dispersions, a multistep production method is adopted. In the first step, a 50% SLN dispersion is prepared by high-pressure homogenization. To this, lipid is added in an incremental manner with high speed stirring to increase the solid content step wise until a lipid content of 80% is formed. Large-scale production of NLC is easily possible by using commercial high-pressure homogenizers. There are no sterility issues during production as high pressure homogenizer is a concealed instrument capable of sterilizing and can be fixed in an on-line sterile assembly.

4.2. *Emulsion solvent diffusion method*

This method employs the use of water miscible solvents such as acetone or methanol along with the non-polar solvents. Due to the solubility of polar organic solvents in water, they spontaneously diffuse into the aqueous phase, and an interfacial turbulence is created between two phases leading to the precipitation of droplets in to nanoparticles. As the above-described techniques utilize organic solvents, regulatory issues arise if traces of organic solvents are not removed to the accepted level in the final formulation. Hence other alternative techniques such as salting out technique [55,56], emulsification solvent diffusion technique [57] are more popular. The salting out method is based on the separation of a water-miscible solvent (acetone) phase from aqueous solutions promoted by the addition of electrolytes like sodium chloride or sodium sulphate. The emulsion diffusion method is a slight modification of the salting-out technique. It differs mainly because the organic solvent is only partially miscible with water, and it is previously saturated with water, in order to reach an initial thermodynamic equilibrium between water and the organic phase. After

addition of water, solvent diffusion is observed, and a nanoparticle suspension is formed.

4.3. *Supercritical fluid (SCF) Technology*

SCFs have presently become attractive alternatives because they involve the use of environmental friendly solvents like super critical carbon dioxide or nitrogen and the method can be used to process the particles with high purity and very low traces of organic solvents [58, 59]. The SCF precipitation techniques are divided into two major types: rapid expansion of supercritical solution (RESS) for drugs soluble in the SCF (generally, CO2), and supercritical anti-solvent (SAS) process for drugs insoluble in the fluid.In the RESS method, the solute is solubilised in a SCF and the solution is expanded through a nozzle causing sudden decrease in solubility followed by particle precipitation [60, 61]. The solvent power of SCF dramatically decreases resulting in the rapid precipitation of solute. In this technique, each particle is surrounded by same kind of particles in the expansion zone, and hence often results in larger particles due to aggregation. Hence SAS method is generally recommended for the production of nanoparticles. In the SAS method [62], the solute is dissolved in organic solvent and the solution is charged into the SCF in the precipitation vessel. At high pressures, enough anti-solvent enters into the liquid phase causing precipitation in to nanoparticles. After precipitation when the final operation pressure is reached, the anti-solvent flows through the vessel so as to strip the residual solvent. The vessel is depressurized when the solvent content has been reduced to the desired level and the solid product is collected. A modified version of the afore-mentioned technique known as gas anti-solvent technique [59] is recently developed to prepare sub-micron particles.

5. Drug loading

Lipid nanoparticles and microparticles made from blends of solid lipids can experience the problem of drug expulsion, especially when nanoparticles are prepared from highly purified lipids, for example, tristearin [9]. The formation of highly ordered βi or β modifications, particularly during storage, leaves little space for drug molecules, and the expulsion of drugs leads to drug crystals in suspensions and solid dosage forms. Suitable composition and preparation techniques are adapted to prepare particles that have a controlled nanostructure that offers enough space to accommodate the drug. However, techniques of preparation differ depending on the solubility of the drug to be entrapped.

5.1. *Poorly Water-soluble Compounds in LNPs*

Several poorly water-soluble anti-cancer drugs have been successfully encapsulated in LNPs. In general, poorly water-soluble compounds partition well in the lipid phase and can be efficiently encapsulated by LNP without much problems. However, these agents carry other problems such as poor stability and risk of precipitation. In fact, each group of drugs has its specific problems that need to be addressed individually. Seriously diminished practical value of camptothecin due to its poor water solubility was solved by loading the drug in LNP to the extent of 99.6% encapsulation efficiency [63]. Similarly, the water solubility of SN-38 was improved by preparing its LNP formulation [64] containing 1.0 mg/ml of the drug. Paclitaxel-loaded LNP were developed in an attempt to eliminate Cremophor EL (reported to cause serious hypersensitivity reactions and nephrotoxicity in human subjects) in its present nonaqueous micellar formulation [65-67] using surfactants or stabilisers such as Pluronic F68, Brij-78 and phosphatidyl-choline. Etoposide, a poorly water-soluble drug, was successfully encapsulated in LNP composed of tripalmitin to achieve an encapsulation

efficiency of 98.96% and a loading capacity of 4% [68]. The anti-neoplastic activity of camptothecin is vulnerable to hydrolysis in an aqueous environment, particularly at basic pH due to hydrolytic lactone ring cleavage of the drug molecule leading to the loss of therapeutic inactivity. This was overcomed by preparing LNP that provides lipid environment to prevent hydrolytic degradation where the drug mostly remained in its active lactone form [63] until it was released. Similarly, the lactone ring stability of irinotecan and SN-38 was also improved when delivered as LNP.

Conventional paclitaxel formulation faces the problem of precipitation when diluted to 0.6–1.2 mg/ml prior to IV administration for clinical use. Studies have reported that the LNP formulation of paclitaxel did not exhibit this phenomenon even when it was diluted to nanomolar range [65]. Cavalli and co-workers [67] showed that their lyophilized LNP formulation containing the unaltered paclitaxel was autoclavable without a significant reduction in the amount of the incorporated drug and was found stable for over 18 months after lyophilisation. Another chemically unstable drug, all-trans retinoic acid (ATRA), was stabilized in LNP during storage [69]. More than 90% of the encapsulated ATRA remained intact after 1 month of storage at 4 °C versus less than 60% when the drug was stored in the form of methanol solution or Tween-80 solution under the same conditions.

5.2 *Water-soluble Ionic Salts in LNPs*

Incorporation of water-soluble anticancer pharmaceuticals into NLC is comparatively difficult but apparently vital. Water-soluble drugs generally pose problems of poor loading in LNP due to neutralization of their ionic charges with a counter ion. This problem can simply be avoided by using the free bases of these agents. However, the use of free base compounds in many cases will lead to solubility problems as faced by lipophilic drugs, such as slow release and possibility of low bio-availability. Thus, it is generally preferred to use the water-soluble salt of an anti-cancer drug for LNP formulation using any of the following strategies:***Ion pair approach:*** Ion pair molecules generally show increased lipophilicity and hence get incorporated in to lipid nanoparticles. Gasco and co-workers used decyl phosphate or hexadecyl phosphate to form ion pairs with water-soluble drugs (e.g., doxorubicin hydrochloride, idarubicin hydrochloride) to enhance their loading into LNP prepared using stearic acid and egg lecithin. The increase in lipophilicity was to the extent of 1000-fold in the case of doxorubicin hydrochloride and 300-fold in the case of idarubicin hydrochloride. However, because of the high lipophilicity, these LNP formulations would release drugs slowly and thus could pose risk of resistance development due to chronic exposure to sublethal concentrations of cytotoxic drugs in cancer cells [34, 35, 70-72].

Polymeric coating of LNP: In this approach, polymers, such as dextran sulfate, may be used as counterions to prepare LNP of positively charged drugs like doxorubicin hydrochloride. Wong and co-workers [73] reported the encapsulation of doxorubicin hydrochloride up to 70% in LNP in the presence of dextran sulfate versus approximately 40% in its absence.

5.3 *Water-soluble Nonionic Drug Molecules in LNPs*

Small water soluble nonionic hydrophilic molecules are difficult to formulate using above strategies of designing LNP and needs specialized techniques. A nonionic anti-viral water-soluble drug 3′-azido-3′-deoxythymidine was conjugated with palmitate to increase lipophilicity for encapsulating in LNP [74]. Similarly, drugs like 5-Fluorouracil (5-FU), mitomycin C and cisplatin also need modification for LNP encapsulation. Wang and co-workers [75] reduced the water solubility of 5-FU by

conjugating two octanoyl groups to the 5-FU molecule to obtain 3′,5′-dioctanoyl-5-fl uoro-2′-deoxyuridine. This lipophilic drug derivative was loaded into LNP with an encapsulation efficiency at over 90%.

6. Characterization & *invitro* drug release

Nano lipid particles (NLPs) are claimed to be different from SLNs and micro emulsions in various physical properties in addition to shape and size of the lipid particles. In addition, interaction between the entrapped drugs in the lipid particles with the aqueous outer phase needs demonstration to claim protection of the loaded drug in an NLP suspension. Many direct and indirect methods used to investigate these characteristics are briefly described below;

6.1. *Physical characterization*

Particle size analysis

Particle size is the prominent feature of nanoparticles and the fastest and routine methods of size analysis are photon correlation spectroscopy (PCS) and laser diffractometry. The former method is useful for determination of smaller particles [76] while the latter method is useful for the determination of larger particles. PCS determines the hydrodynamic diameter of the nanoparticles via Brownian motion. The electron microscopy methods also allow the exact particle determination but require the coating of a conductive material such as gold. The gold coating usually results in the estimation of particle sizes slightly more than the normal. The method is also limited to dry sample, which are stable under the conditions of coating. Transmission electron microscopy (TEM) with or without staining is a relatively easier method of particle size determination.

Particle shape analysis

Scanning electron microscopy (SCM) is one of the powerful tools to investigate the surface morphology of nanoparticles. In addition, Cryo-scanning electron microscopy studies are also used for morphological studies particularly to determine the extent of sphericity and surface characteristics [77]. Recently, new types of high-resolution microscopes such as atomic force microscope, laser force microscope and scanning tunnelling microscope are available to study structural details of nanoparticle surface [78-81].

Structure of NLPs

Information about the nanoparticle structure and crystallinity may be obtained by X-ray diffraction and thermo-analytical methods such as differential scanning calorimetry, differential thermal analysis, thermo-gravimetry and thermal optical analysis [82]. The charge on nanoparticle surfaces is mainly determined by electrophoretic mobility, laser Doppler anemometry and amplitude-weighted phase structuration. Mobility of the components and the molecular environment of model drugs were measured by Jores et.al, 2003 [83] to investige the structure and performance of NLCs using photon correlation spectroscopy, wide-angle x-ray scattering, and differential scanning calorimetry. Proton nuclear magnetic resonance spectroscopy and electron spin resonance experiments were performed to investigate the mobility of the components and the molecular environment of model drugs. Proton nuclear magnetic resonance spectra clearly demonstrated that NLC nanoparticles differ from nanoemulsions and from SLNs by forming a liquid compartment that is in strong interaction to the solid lipid. The electron spin resonance model drug was found to be accommodated either on the particle surface with close water contact (SLN) or additionally in the oil (NLC).

Density of packaging in NLPs

NLPs or NLCs are claimed to have disordered structures with lose packing pattern than orderly structure of SLNs. This property is the basis on which high drug loading characteristics of NLCs are hypothesized. Jores et.al (2005) measured packing pattern of the NLP by Raman

spectroscopy and by densitometry [86]. Density measurements can be performed by the helium compression pycnometry and by density gradient centrifugation. A comparison of these two methods may offer information about the internal structure of nanoparticles.

Interaction of entrapped lipophilic drug with the outer aqueous phase

The hydrophobic fluorescent marker nile red (NR) was used as model drug, and by fluorometric spectroscopy, the molecular environment (polarity) was elucidated because of solvatochromism of NR. Fluorometric spectroscopy clearly demonstrates that NLC nanoparticles offer two nanocompartments of different polarity to accommodate NR. Nevertheless, in both compartments, NR experiences less protection from the outer water phase than in a nanoemulsion. In conventional SLN, lipid crystallization leads to the expulsion of the lipophilic NR from the solid lipid [76].

cases a controlled release. Selection of proper lipid mix modulates drug release through *in-vivo* degradation and provides optimum drug diffusion through lipid matrix. Drug partitioning between lipid mix and the aqueous bio-fluid is another parameter that decides the release rate in-vivo. However, transformation of dis-ordered state of lipid structure in NLCs to a comparatively ordered state due to bio-environment could also probably trigger the release of the loaded drug [84].

7. Long-Term Stability

Long-term storage of SLN dispersions leads to aggregation and cake formation [85]. Individual particles diffuse in the dispersion medium gaining kinetic energy. With increase in kinetic energy, particle collides each other causing perikinetic flocculation (**Figure 2A**).

In highly concentrated NLC dispersion, the particles pattern a 'pearl-like network' which

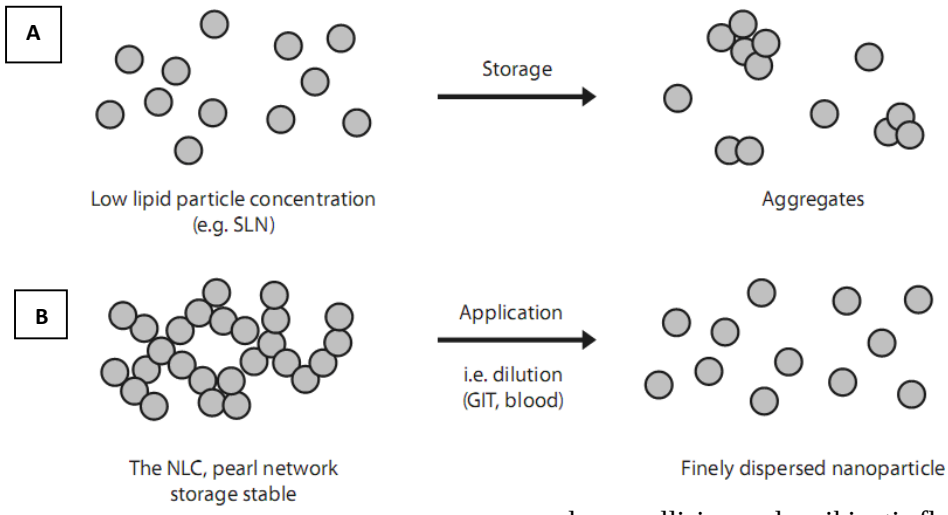

Figure 2: Aggregation process in low concentrated dispersion (A) and pearl like network in NLC dispersion with stabilizing effect (B).

reduces collision and perikinetic flocculation. On dilution in vivo, with body fluids (gastrointestinal fluids, for example), this pearl like mesh is decimated yielding single, non-aggregated particles (**Figure 2B**). Addition of surfactant at critical concentration with off course lower lipid content helps in the formation of lipid dispersion [85].

6.2. ***In-vitro Drug Release Characteristics***

Drug release from lipid particles takes place by diffusion and lipid degradation in the body. In some cases it might be desirable to have a quick release while in most of the

8. *In-vivo* Evaluation of LNPs

It has long been reported that the efficiency of anti-neoplastic drugs can be increased by targeting and maintaining their concentration at the site of action for a sufficient length of time [86]. In comparison to healthy tissue, neoplastic tissues show a rapidly expanding tumor vasculature often with a discontinuous endothelium, facilitating leakage of colloidal particles of critical dimension to the tumor tissues. It also shows a compromised clearance via lymphatics, the so-called 'enhanced permeability and retention (EPR) effect. In the case of intravenous (IV) injection, highest amount of nanoparticles will get accumulated within the cancer cells due to hyper-permeability and hindered lymphatic clearance [87-89]. However, even if located in the neoplastic region, the efficacy of anti-cancer drugs can be affected by the development of multidrug resistance
(MDR). Colloidal delivery systems were able to overcome the problem of MDR to some extent [90].

LNP loaded with antitumor agents should be evaluated for their anti-tumour activities, stabilities and/or biodistribution in cultured cell lines and animal models. Properties of LNP that are desirable for anti-tumour drug delivery include;

- Versatility, which allows encapsulation of cytotoxic agents of diverse physicochemical properties;
- Improved anti-cancer drug stability;
- Improved in vitro cytotoxicity against cancer cells;
- Ability to load more than one drug in one carrier system;
- Activity against cancer cells that are normally refractory to chemotherapy;
- Enhanced drug efficacy in animal models; and
- Improved pharmacokinetics and *in- vivo* drug distribution.

However, most studies that included animal models focused on drug biodistribution. *In vivo* anti-cancer studies generally include pharmacokinetics and biodistribution analyses and anti-cancer studies on tumour-induced mice models.

In principle, unmodified nanoparticulates and vesicular systems get rapidly cleared from the systemic circulation by the reticuloendothelial system (RES). LNP can be coated with stealth agents, such as PEG, to minimise the clearance by RES to achieve extended systemic circulation time. Using stearic acid-PEG2000, long-circulating LNP formulations of doxorubicin and paclitaxel have been formulated [34, 69].

As an alternative to pegylation, surface modification of the nanoparticles by polysaccharide coating has been attempted. Biomaterials such as dextran [91], heparin [92], hyaluronic acid [93], chitosan [94, 95], etc. were used as polysaccharides for surface coating of nanoparticles. On the other hand, the polysaccharides possess various advantages including tissue targeting. The polysaccharide-coated nanoparticles also showed low macrophage uptake (96) and increased the half-life of the incorporated drugs (97). Dextran-coated nanoparticles allowed tissue-specific targeting, such as for lymph nodes and brain tumours (98).

The influence of route of administration on the pharmacokinetics and drug biodistribution of the etoposide-loaded LNP was studied by Reddy and co-workers [68]. Improvements in tumoral drug accumulation were observed when the etoposide formulations were intraperitoneally or subcutaneously (SC) injected in comparison to IV injection. Many studies also report the accumulation of LNP-delivered drugs in the brain. The ability of LNP to carry drugs across the P-gp-rich BBB is consistent with the previously described findings in P-gp-overexpressing cancer cell lines. This P-gp bypassing feature of LNP may be useful for cancer chemotherapy if properly exploited.

Basically, cytotoxic drug therapy is aimed at destroying as many cancer cells as possible and at preventing their proliferation. Therefore, it is

important to ensure that the drug administered as an LNP formulation should be as cytotoxic to cancer cells as that of free drug at the same dose level. However, most of the LNP formulations are reported to demonstrate cancer cytotoxicity comparable or even superior to the corresponding free drugs. Miglietta and co-workers [66] demonstrated improved cytotoxicity, which was retained for 72 hours, with LNP incorporating doxorubicin or paclitaxel. Studies on the combination therapy of anti-cancer agents loaded in LNP showed synergistic activity, thus giving scope for overall dose reduction in the course of therapy. The anti-adhesive effect of cholesteryl butyrate LNP was studied by Dianzani and co-workers [99] by coincubating cholesteryl butyrate LNP with human polymorphonuclear (PMN) cells and human umbilical vein endothelial cells (HUVEC). The results showed that cholesteryl butyrate LNP were in all cases more active than that of sodium butyrate.

MDR phenotype in cancer cells presents a significant obstacle to anti-tumour therapy at a cellular level. MDR was demonstrated when tumour cells that have been exposed to one cytotoxic agent develop cross-resistance to a broad range of structurally and functionally unrelated compounds [100,101]. Typically, hydrophobic and amphipathic anti-cancer drugs, for example, vinca alkaloids (vincristine, vinblastine), taxanes (paclitaxel, docetaxel), epipodophyllotoxins (etoposide, teniposide), anthracyclines (doxorubicin, daunorubicin, epirubicin),

topotecan and mitomycin C [102, 103], are most frequently associated with P-gp efflux associated MDR [104, 105]. Out of many approaches are worked out to reduce P-gp expression to ultimately reduce MDR, use of particulate delivery systems including LNP has been shown to be very effective. Wong and co-workers [36, 37] reported enhanced doxorubicin uptake and retention in MDR breast cancer cells using polymer-lipid hybrid nanoparticles containing a nonionic block copolymer (Pluronic F68).

10. Other Applications of LNPs

10.1. NLCs in oral delivery

Oral administration of NLCs is definitely a very interesting due to following reasons;

(i) Lipids are resistant to acidic conditions of the stomach and hence could protect encapsulated drug from degradation.

(ii) NLCs are generally made up of biocompatible lipids that can easily handled by the body enzyme system.

(iii) Improve drug absorption particularly for lipophillic drugs that inherently have low bio-availability. Striking example of oral lipid drug delivery is the case with cyclosporine in improving it's bio-availability when administered as NLCs and microemulsion.

(iv) Lipid–drug conjugate (LDC) nanoparticles provide high-loading capacities for hydrophilic drugs.

(v) NLCs can also be incorporated into traditional dosage forms such as tablets and pellets using the NLC dispersion as granulation fluid or wetting liquid for the pellet mass.

(vi) NLCs produced in oil or polyethylene glycol (PEG) 400 can be filled directly into soft gelatin capsules also.

10.2. NLC in topical delivery

The second easy-to-realize area is topical application. All the lipids and surfactants used in traditional pharmaceutical creams can be employed, thus leaving little regulatory hurdles. Because of the high consistency of NLC dispersions, they can be used as topical dosage forms without further processing. Potential advantages of lipid nano particles in the topical delivery are listed below

(i) Protection from labile drugs against chemical degradation: Example, tocopherol, retinol etc.

Typical release characteristics (Initial limited burst release followed by Controlled release) are suitable for dermal application. Initial burst helps in improving penetration while sustained release would provide medication to the skin appendages over a long period of time. [104,105]

(ii) Inherent adhesive character of lipids used in NLPs would form an ultrathin film on the skin and show occlusive effect which promotes penetration of the loaded drug in to the upper part of the epidermis by improving skin hydration. [106].
(iii) Lipid particles loaded with inorganic sunscreen materials like titanium dioxide thus decreasing the penetration of such sun screen agents. This would avoid systemic toxicity of titanium dioxide
(iv) Regulatory consideration for lipid particle entrapped cream formulation is not a big issue as quantification methods for LNPs are well known.
(v) Colored ingredients in a cream base possess organo-leptic problem and so lacks in consumer acceptance. LNP base materials being white can effectively conceal the colored ingredients and gives acceptable products with white sheen appearance.

11. Summary

Drug delivery research has gained equal importance as drug discovery programme due to unfavorable pharmacokinetic properties of majority of drugs designed off late by through put screening. Most of these bio-actives have high hydrophobicity and are practically water insoluble. In addition, problems of low bio-availability results in high dosing leading to dose related toxicity particularly with anti-cancer drugs. Hydrophillic drugs have other problems of chemical instability and rapid clearance from the body after administration. Adaptation of Colloid chemistry nanotechnology in medicine has brought hopes in solving these crucial problems. In addition, targeting capabilities of particulate delivery systems has shown new path in overcoming not only unwanted side effects and toxicity but also problems of drug resistance. Large number of Polymers was worked out in the last few decades for designing particulate delivery systems but very few of them have got regulatory clearance. Off late, lipids were considered suitable as carrier materials for nano-particle drug delivery due to its bio-compatibility, ease of preparation and capability to accommodate both lipophillic and hydrophilic drugs. Among many lipid delivery devises lipid nano particles (LNPs) have gained much importance due to in-vitro and in-vivo stability, controlled drug release and suitability for passive and active targeting. LNPs are heavily worked out for anticancer drug delivery with targeting capability and also to solve problems of drug resistance. In addition, LNPs are also considered suitable for brain delivery, ophthalmic delivery, colon delivery and topical delivery. They have also find their place in cosmetics and transdermal medication.

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