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Nanostructured Lipid Carriers: A Novel Targeted Drug Delivery System

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Abstract

Nanostructured lipid carriers are second generation nanoparticulate systems that have a solid nature at ambient temperature. Nanostructured lipid carriers are modified solid lipid nanoparticles that retain the characteristics of the SLN, improve the stability and loading capacity and prevent drug leakage. NLC have provoked the incessant impulsion for the development of safe and valuable drug delivery systems due to their exceptional physicochemical and biochemical characteristics, their binary system which contain both solid lipid and liquid lipid that aims to produce less ordered lipidic core. Lipid nano formulations make dispersions of fairly water-soluble drugs and can decrease the characteristic restrictions of slow dissolution of fairly water-soluble drugs like BCS class II and simplify the formation of solubilized phases from which drug absorption occurs easily. NLC can increase the drug distribution to the targeted organ, change the pharmacokinetic characteristics of drug carriers to enhance the therapeutic effect and decrease the adverse side effects.

Keywords: Nanolipid carriers; Solid lipid Nanoparticals; Controlled release; Bioavailability enhancer; Colloidal drug carrier.

Introduction:

Nanoparticles are colloidal particles with sizes of approximately 1.0 -1000 nm. These nanoparticles can be divided into nanocarriers and nanodrugs. Nanocarriers refer to materials prepared by the dissolution or dispersion of drugs with a variety of nanoparticles, which may be classified as either nanospheres or nano capsules. The excipient for the preparation of nanoparticles may be divided into polymers and lipid materials. The former is referred to as PNP, which include polymer nano capsules & nano spheres as well as polymeric micelles. The latter is called the lipid nanoparticle and includes nanoliposomes and polymers, Nanodrug involves the direct application of micronization and ultrafine powder technologies to the processing of drugs into nanoparticles [1]. NLC, as a new DDS approved in the late 1990s. This DS involves the modification of SLN and mixture of solid and liquid lipids prepared by heating and cooling crystallization [2].

Lipid based DDS is a known, established, commercially viable approach to manufacture pharmaceuticals for different dosage forms [3]. Lipid formulations like NLC's need a variety of the products to be incorporated in formulations. Mainly the bioavailability and Solubility of the insoluble drugs are the two main criteria which can be enhanced with the formulations like NLC's. First many

pharmaceutical companies have developed a well-established industrial process for the manufacturing of large-scale batches of NLC's, but still all major kind of parameters like choice of lipid, surfactants other essential excipients and methods of preparation varies which causes change in parameters like particle shape and size, phase transition, solubility, bioavailability of drug etc. The Lipid nanoparticles show remarkable properties which are required and very essential for their therapeutic action. The exceptional properties of nanoparticles (NP) like surface to mass ratio are additional colloidal particles and their capability to bind and to carry compounds which makes a NP too smart to use as a medicinal product [4].

Lipid nano formulations make dispersions of fairly water-soluble drugs and can decrease the characteristic restrictions of slow and imperfect dissolution of fairly water-soluble drugs like Biopharmaceutics Classification System (BCS) II. It also simplifies the formation of solubilized phases from which drug absorption occurs easily. In any, another vehicle mediated delivery system release from the system are important in relation to the formulations like an emulsion, liposome the degree
and mode of drug movement of the delivery system in-vivo [5]

A lipid matrix is available inside the newly made NLC's having a very special nanostructure which was developed by Muller [6]. This special type of NLC's nanostructure also helps to increase bioavailability, drug loading and solubility of the drug in different conditions and environments [7]. There are multiple techniques and methods by which this kind of NLC's can be prepared or formulated like high-pressure homogenization. As per the literature, about 30-80 percent of the product yield can
be obtained by these methods after adjusting the different conditions and environments [8].

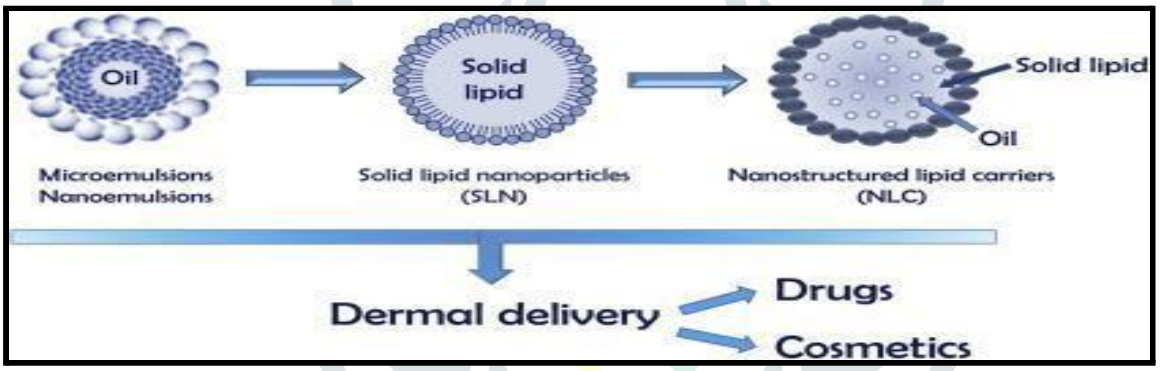

Figure 1: SLN, NLC

The first time NLC's introduced was in the 1990s as another carrier system [9]. The solid lipid carrier systems like solid lipid nanoparticles (SLN)which are available in nanomeric range, were presented as a substitute to liposomes. But there are multiple limitations related with SLN, such as incomplete drug loading ability and drug expulsion through storage, all these limitations can be minimized or waived off by newer solid lipids DDS like NLC's. There are new and modified types of NLC's available which have a meticulous nanostructure. These meticulous nanostructures are responsible and also help to improve the stability of the formulations as well as increase the bioavailability, drug loading [10]. The different problems which are associated with the SLN for many drugs, like low payload, drug expulsion during storage and SLN's dispersions due to the high-water content
in it are also minimizes by NLC [11].

why lipid nanoparticles

- Better control over release kinetics of encapsulated compound

  1. Engineering via size and lipid composition.

  2. Melting can serve as a trigger.

- Enhanced bioavailability of entrapped drugs.

- Chemical protection of labile incorporated compounds.
- Much easier to manufacture than bio-polymeric nanoparticles.
- No special solvents required.
- Wider range of base materials (lipids).
- Conventional emulsion manufacturing methods applicable.
- Raw materials are essentially the same as in emulsions.
- Very high long-term stability.
- Application versatility

1.Can be subjected to commercial sterilization procedures.

2. Can be freeze-dried to produce powdered formulation [12].

Advantages of NLCs over conventional formulations

- The small size and relatively narrow size distribution of NLC allows site-specific drug delivery.
- Controlled and Sustained release of active drugs can be achieved.
- The incorporated drug is protected from biochemical degradation.
- High drug payload.
- Incorporation of lipophilic and hydrophilic drugs feasible ● Can be sterilized by autoclave or gamma radiation.
- Can be lyophilized and spray dried.
- Do not generate any toxic metabolites.
- Relatively cheap and stable.
- Easy for industrial scale production by hot dispersion technique.
- Surface modification can be easily performed [13,14].

Potential Problem Associated with SLN

The review by Mehnert highlights these aspects:

- Pay-load for a number of drugs too low
- Drug expulsion during storage
- High water content of SLN dispersions

The drawbacks associated with SLN can be minimized by NLC. Generally, the NLCs are considered to be the second generation of lipid nanoparticles. Compared to SLN, NLC shows a higher loading capacity for active compounds by creating a less ordered solid lipid matrix, i.e., by blending a liquid lipid with the solid lipid, a higher particle drug loading can be achieved by blending a liquid lipid with the solid lipid. Therefore, the NLC has an increased drug loading capacity in comparison to SLN and the possibility of drug expulsion during storage is less. [15]

Concept of NLC

The three types of NLC can be summarized:

1. **Imperfect type of NLC:**

   In this type, solid and liquid lipids are blended with small amounts of liquid lipids. The difference within the structures of the lipids results in highly disordered, imperfect lipid matrix structure thereby offering space for drug molecules.

2. Multiple type of NLC:

Drugs are often incorporated within the solid, but at increased solubility in the oily parts of the lipid matrix.

3. Amorphous type of NLC:

In this type, lipids are mixed in such a way that forestalls them from crystallizing. The lipid matrix is in an amorphous state. The NLCs of this kind are supported the very fact that for variety of medicines, the solubility in oils is higher than their solubility in solid lipids [16]

Components of NLCs

Generally, NLCs are composed of lipid (s) (solid furthermore as liquid), surfactant(s), organic solvent and other agents like counter-ions and surface modifiers.

Lipids

Lipid is the primary component of nanostructure lipid carriers. Drug loading capacity, prolonged action and stability of the formulations are governed by lipids. Solid lipids are used [17]. The lipids which are physiologically acceptable, biodegradable, non-toxic and generally-recognized-as-safe (GRAS) status are preferred for preparation of lipid nanoparticles [18]. Choice of suitable lipids is important before their use in preparation of nanoparticulate carriers. The various characteristics of nanocarriers are affected by the type and structure of lipids. Practically, solubility or evident partition coefficient of drugs within the lipid has been suggested because the simplest fitting criteria for choosing an appropriate lipid. The drug loading and encapsulation efficiency of the drug molecule is affected by solubility of the drug in lipid; therefore, it is interpreted [18]. On account of upper viscosity of dispersed particles, because of higher melting lipids, the standard particle size of nano dispersion increases. Shape of lipid crystals, lipid hydrophilicity, variation in composition are additional lipid related parameters which can influence the standard of NLC.[19].

While selecting suitable type and amount of surfactant for NLC formulation, required HLB plays an important role [22]. rHLB of lipids and lipid matrix is measured to calculate the amount of emulsifiers to be added in formulation. The rHLB value for lipid is nothing but the HLB value of emulsifier which is necessary for appropriate emulsification. This also helps in achieving a stable nano system and small particle size of NLCs [24]. A right combination of emulsifiers with least concentration can be employed for formulation by determining HLB. rHLB for lipids and lipid matrix is calculated experimentally. It is measured by dispersing in blends of surfactant with different HLB values. The mixture is put through high pressure homogenization and analyzed for least particle size [22,24,25].

Other ingredients

Organic salts and ionic polymers may be used as counter-ions in formulation of nano structure carriers. These are used to overcome the challenge of encapsulating water-soluble drug molecules. Surface-modifiers are another category of excipients employed in formulation of NLC. These are employed to minimize their phagocytic uptake by the macrophages in the reticuloendothelial system (RES). Lipid particles are coated with hydrophilic polymers like PEG, poloxamines or poloxamers in order to increase the residence time of drug molecules in systemic circulation. Surface modification can offer other advantages like enhanced physical stability and biocompatibility, drug targeting, increased transport across epithelium [18,26].

Methods of preparation of NLCs

High-pressure homogenization technique

This technique is reliable for the commercial-scale production of NLCs. High pressure used in homogenization techniques makes it possible to avoid use of organic solvents in preparations thereby rendering them eco-friendly. Additionally high-pressure homogenization is easy to scale up and an attractive technique which is being used in the manufacturing of pharmaceuticals and cosmetics for topical application [27]. Hot homogenisation is performed at high temperature and cold homogenization is done below room temperature. In both approaches, before high pressure homogenization, the drug is dissolved or dispersed in the molten state. High pressure (100–2000 bar) moves the fluid in the narrow gap in the homogenizer.

Hot homogenization

In this technique, homogenization is conducted at high temperature. The solid lipids are melted at a temperature above 5-10°C of their melting point. A dispersion is obtained by incorporating liquid lipid and drug to be encapsulated. The mixture is then dispersed in aqueous solution of surfactant (s) which is heated to the same temperature by a high shear mixing device and results in the formation of pre-emulsion. The pre-emulsion is then introduced in a high-pressure homogenizer at controlled temperature.3 to 5 cycles at 500-1500 bar are generally sufficient for homogenization. The lipid recrystallizes and leads to formation of nanoparticles as nano emulsion is gradually cooled down. Use of high temperature during the process may result in the degradation of heat sensitive ingredients. Another problem which may cause reduction in emulsifying capacity of surfactants due to high temperature as surfactants have cloud points lower than 85°C. This may induce instability to nanocarriers [30,31,27-29].

Cold homogenisation

In this approach, lipid melt-containing drug is rapidly cooled so as to solidify using liquid nitrogen or dry ice, then it is milled and ground before being dispersed in the cold surfactant phase and then it is subsequently homogenized at room temperature. Pressure used in the cold process is higher i.e., 5-10 cycles of 1500 bar. This approach reduces the thermal exposure of drugs and is well suited for

thermolabile drugs. Another benefit of this technique involves improved drug entrapment efficiency and uniform distribution of drugs within the lipid. However, it results in nanoparticles of more variable sizes [30,28,32].

Solvent-emulsification evaporation method

In this approach, the lipids i.e., Solid lipid + liquid lipid along with drug are dissolved in a water immiscible organic solvent like cyclohexane, chloroform. The obtained mixture is then dispersed into aqueous solution of emulsifiers thereby producing an o/w emulsion. Evaporation under reduced pressure is then employed in order to remove solvent from the emulsion. Evaporation results in the dispersion of nanoparticles in the aqueous phase (by lipid precipitation in the aqueous medium). This method avoids any thermal stress, but usage of organic solvent is a disadvantage. Particle size can vary from 30-100 nm
according to the solid lipid and surfactant [31,32]

Solvent-emulsification diffusion method

In this technique, solvent and water are mutually saturated in order to maintain initial thermodynamic equilibrium. Afterwards, the lipids and drugs are dissolved in the water-saturated solvent. Solvent containing drug and lipids are emulsified in a solvent-saturated aqueous emulsifier solution by a homogenizer so as to form an o/w emulsion. The lipid nanoparticles then precipitate after dilution with excess water (ratio: 1:5–1:10) due to diffusion of the organic solvent from the emulsion droplets to the continuous phase. Solvent diffusion is more innovative and most of the solvent employed show a better safety profile compared to volatile solvents [33].

Microemulsion method

In this technique, the solid lipid is melted, then liquid lipid is added and the drug is solubilized within the subsequent mixture. A combination of emulsifier, co-emulsifier and water is heated at the same temperature. Both the lipid and the aqueous phase are mixed in appropriate ratios and stirred to produce thermodynamically stable oil in water hot microemulsion. The resulting hot microemulsion is quickly dispersed into an excess of chilled water (0-4°C) with vigorous stirring. The dilution results in the breakdown of microemulsion into a nanoemulsion with ultrafine particles. The size of the nanoparticles depends on the droplet size of microemulsion and temperature difference between microemulsion and ice water. [28,35,36].

Double emulsion technique

This method is mainly used for the production of hydrophilic drugs loaded with lipid nanoparticles. This technique overcomes the problem associated with incorporating water soluble moiety in aqueous phase from oily phase [37]. In this method, the drug is initially dissolved in aqueous solvent (inner aqueous phase) and then is dispersed in the lipid phase to produce primary emulsion (w/o). Both lipid and the aqueous phase are maintained at the same temperature. Thereafter, primary emulsion is dispersed into a large volume of surfactant aqueous solution followed by sonication to form a double emulsion (w/o/w). The
lipid nanoparticles are then purified by ultrafiltration or solvent evaporation [32,38]

Solvent Injection technique

It is a new approach to manufacture lipid nanoparticles. In this technique, lipids are solubilized in water-miscible solvent (e.g., acetone, methanol, ethanol, isopropyl alcohol) or water-soluble solvent mixture and then rapidly injected into aqueous surfactant solution under continuous stirring. Resultant dispersion is then filtered in order to eliminate excess lipid [39]. The particle size of nanocarriers depends on diffusion rate of the organic solvent through the lipid-solvent interface. This method offers the advantage of easy handling, efficiency, versatility, no employment of technical equipment (e.g., high-pressure homogenizer) and use of approved organic solvents [39].

High shear homogenization and ultrasonication

These dispersing techniques use devices to prepare nanocarriers. Solid and liquid lipids are melted and dispersed in an aqueous surfactant solution under high shear homogenization or ultrasonication. This results in formation of nanodispersion [41,42]. The shear forces necessary for the nano-emulsification are generated by ultrasonic cavitation. This produces violently imploding vacuum bubbles and breaks up particles down to the nanometer scale [44]. Probe-type ultrasonication produces desired effects like homogenization, dispersion, deagglomeration, milling and emulsification [44]. The type and concentration of lipid and surfactant, their ratio, time of sonication or agitation speed are some of the parameters that should be optimized to obtain a reproducible method resulting in small size nanocarriers. Low dispersion quality is a disadvantage of high shear homogenization and ultrasonication methods are low dispersion quality. Another problem associated with the ultrasonication is metal contamination from the equipment [43].

Phase inversion technique

It is a novel, cost effective approach that involves the phase inversion from o/w to w/o emulsion. It involves two steps. -

Step 1: It involves mixing of all the ingredients (lipid, surfactant and water).. The mixture is stirred and temperature is increased at a rate of 4°C to reach up to 85°C from room temperature. Three temperature cycles (85–60–85-60-85°C) are applied to the system to reach the phase inversion zone.

Step 2: Due to dilution with cold water, it results in an irreversible shock introduced to break the system. This rapid addition of cold water causes formation of nanocapsules. Particle aggregation is avoided by applying a slow magnetic stirrer for 5 minutes. Low energy

involvement enables the formation of stable transparent dispersions (smaller than 25 nm), which can be used for encapsulation of various bioactive compounds [46,48].

Microfluidization method

The technique involves use of a high shear fluid device known as microfluidizer. In this process, the liquid is forced at the speed of 400 m/s through microchannels to an impingement area at high operating pressures. Cavitation and the accompanying shear and impact are responsible for the efficient particle size reduction within the "interaction chamber". The technique can be utilized on laboratory as well as production scale [47].

Evaluation

In order to ensure the performance, product quality and stability appropriate techniques are required for characterizing the physicochemical properties of NLCS. Various evaluation parameters like particle morphology, interfacial properties, drug entrapment efficiency, crystallinity studies etc enlighten the workability of NLCs as a drug delivery system.

Particle size measurement

Particle size of NLC is determined by photon correlation spectroscopy (PCS) by using Zetasizer. Photon correlation spectroscopy is based on the measurement of the fluctuations in scattered light which arise from Brownian motion [49]. It provides the average particle size and polydispersity of the system as a measure of the particle size distribution. It characterizes particles of a few nanometers to about 3 microns. Laser diffractometer (LD) has the capacity to characterize a wide range from the nanometer to the micrometer range particles. This evaluation is based on the diffraction pattern showing particle shape and size [57, 58].

Zeta potential

Zeta potential (ZP) is an important factor for evaluation of the stability of nano dispersion. The ZP determination is based on particle electrophoretic mobility in aqueous medium [54,55]. Zeta Potential determines the surface charge and gives the information about long term stability. At higher ZP the particle aggregation is less likely due to electric repulsion while dispersions with lower values tend to coagulate or flocculate, possibly leading to less stability [59].

Generally, the zeta potential of dispersion should be either less than -30 mV or greater than +30 mV for electrostatic stabilization of NLC [60]. It can be done by Laser Doppler electrophoresis, using a Malvern ZetaSizer Nano ZS. By applying an electric field across the sample, particles with a zeta potential will migrate toward the electrode of opposite charge. The velocity is determined using the technique of Laser Doppler anemometry, also known as Laser Doppler velocimetry [61,62].

The Zeta potential is influenced by factors like electrical conductivity, pH, and the nature of the reagents [62].

NLC morphology

Surface morphology of NLC can be determined by transmission and scanning electron microscopy (TEM, SEM), atomic force microscopy (AFM) and PCS. These techniques are tried and true for dimensional and structural characterization of NLCs [67,68].

Negative staining, freeze-fracture and vitrification by plunge freezing are different methods of sample preparation for TEM which can provide different information about the colloidal particles. The sample is placed on a gold or copper grid with an outlined mesh size grid and stained with a heavy metal salt solution which provides high contrast in the electron microscope. After drying, the sample is viewed in the electron microscope. Nanoparticles appear bright against the darker background of the stain [63,64]. Sometimes use of surfactant in SEM imaging leads to artifacts due to formation of smooth camouflaging coating on particle surfaces [65,66].

Entrapment efficiency

It has influence on the release characteristics and determines the amount of drug loaded in NLC. Entrapment efficiency can be defined as the ratio between the weight of entrapped drug and the total weight of drug added to the dispersion. The amount of drug encapsulated per unit weight of the NLC is determined by ultrafiltration-centrifugation method. A known dispersion of NLCs is prepared and centrifugation is performed in a centrifuge tube which is mounted with an ultrafilter. After appropriate dilution the amount of free drug in supernatant is determined by spectrophotometer [51].

The entrapment efficiency in the NLCs is calculated by using the following equation.

$$\text{Entrapment efficiency (\%)} = Wa - Ws / \text{Wa} \times 100\ \%$$

where, *Wa* is the initial weight of drug, Ws is amount of drug in supernatant solution

Crystallinity and polymorphism

The characterization of the crystallinity of the NLC components is crucial as the lipid matrix as well as the loaded drug can undergo a polymorphic transitional change resulting in drug leakage during storage [71]. The status of crystallinity of a particle also influences the encapsulation efficiency & release rates [72]. An increase in thermodynamic stability and lipid packing density, while a decrease in drug incorporation rate is observed in the following order [54]: Supercooled melt > alpha modification > beta' modification > beta modification

DSC measurements reveal the status of lipid, melting and crystallization behaviour of solid lipids in nanostructures [74,75]. DSC analysis is carried out on pure drugs, pure lipids and nanoparticles. DSC characterization can illuminate NLC structure through the mixing behaviour of solid lipids with liquid lipids [76]. The breakdown and fusion of the crystal lattice by heating or cooling the sample furnishes exclusive information of polymorphism, crystal ordering, eutectic mixtures, glass transition processes and drug lipid interactions [76, 77]. Recrystallization index (RI) is a parameter to perform comparative study of the crystallinity between the developed formulations. It can be calculated from the following formula:

$$RI = \Delta H_{NLC}\ \Delta H_{bulk} \times \text{Concentration of lipid} \times 100$$

where ΔH_{NLC} =Melting enthalpy of 1 g NLC suspension,

ΔH_{bulk} = Melting enthalpy of 1 g bulk lipid,

ΔH is given in J/g and the concentration is given by the percentage of lipid phase [78].

Gonullu et al determined Crystallization index (CI %) to determine the crystalline state of the drug in formulations.

$$CI = Ms \times 100$$

where, Ms = Melting enthalpy (J g^{-1}) of lipid nanoparticles,

Mp = Melting enthalpy (J g^{-1}) of pure solid lipid, and

Υ =solid lipid concentration (%) in nanoparticle dispersion [74].

An increase in the amount of liquid lipid lowers the crystallinity and increases the defects in highly ordered structure of NLCs. The principle behind performing DSC rests on different enthalpy and melting points for different lipid changes. NLCs having a smaller size, therefore a higher surface area and employing more surfactants showed a decline in enthalpy and melting point of lipids.

XRD analysis is another useful technique to reveal polymorphic structural changes of compounds in XRD, the monochromatic beam of X-ray is diffracted at angles as per the type and arrangement of the atoms and space between the planes in the crystals [71]. Lipids have the ability to aggregate in a variety of ways giving rise to different polymorphic forms. This can be in the form of micelles, lamellar phase, tubular arrangement or cubic phases. Wide angle and small angle X-ray scattering techniques (WAXS, SAXS) are utilized to explore the layer arrangements, crystal structure, phase & polymorphic behaviour of lipid and drug molecules. It also gives an idea regarding length of the short and long spacing of lipid lattice46 and localization of drug in it [79].

Surface tension measurement

An increase in concentration of emulsifiers lowers the surface tension of the system due to the emulsification process. Surface tension of the lipid nanoparticles is often measured by the Wilhelmy plate method. Another method for detecting surface tension of the nanoparticulate systems is the measurement of the contact angle [68].

Kibron instrument is a high precision, easy to use torsion balance instrument to measure surface tension of NLC .

Conclusion

The NLC's are the carrier systems with suitable perspectives to be marketed very successfully. The NLC's are the new generation of formulations which offer much more flexibility in drug loading, modulation of release and improved performance in producing final dosage forms such as injectable, creams, tablets, capsules etc. Because of the great consistency of NLC dispersions, they can be used as numerous formulations. This special type of NLC's nanostructure also helps to increase bioavailability, drug loading and solubility of the drug in different conditions and environments and these carriers can increase the drug distribution to the target organ, change the pharmacokinetic characteristics of drug carriers to enhance the therapeutic effect, and reduce adverse side effects.

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