IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Lipid of Nanoparticles

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Abstract:

Lipid-containing drug delivery systems like NLC are well-established methods for preparing pharmaceuticals for all major kind of drug delivery systems of nanoscience. Lipid formulations require a variety of product-related requirements and problems associated with NLC, which is discussed thoroughly in this paper. There are multiple DDS currently available which leads to enhance the solubility of the drugs in different medium as well as also increase the bioavailability of the drugs in different conditions and environments. NLC's are a novel type of DDS which are stable in different environment and which have capabilities to form concentrated dispersions. In this chapter, we discussed different process variables, steps involved in the manufacturing of NLC and responses with their outcome. NLC's 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

Key words :- NLC's, therapeutic effect, side effect, DDS,

INTRODUCTION :-

Nano-particles are colloidal particles with sizes of approximately 10–1000 nm. These particles may be divided into nano-carriers and nanodrugs. Nanocarriers refer to materials prepared by the disolution or dispersion of drugs with a variety of nanoparticles, which may be classified as either nanospheres or nano capsules (1). The material of the preparation of nanoparticles may be divided into polymers and lipid materials. The former is referred to as PNP, which includes polymer nano capsules and nanospheres as well as polymeric micelles. The latter is called the lipid nanoparticle, and include nanoliposomes and NLCs. LPN is a new type of carrier developed in recent years and is a combination of liposomes and polymers. Nanodrugs involve the direct application of micronation and ultrafine powder technologies to the processing of drugs into nanoparticles (2).

Nanostructure lipid carriers (NLC) are the new generation of lipid nanoparticles, attracting major attention as novel colloidal drug carriers for topical use. NLC were developed to overcome the limitations associated with the SLN. SLN consist of solid lipids, while NLC consist of a mixture of specially blended solid lipid (long chain) with liquid lipid (short chain),preferably in a ratio of 70:30 up to a ratio of 99.9:0.1. The resulting matrix of the lipid particleshows a melting point depression compared to the original solid lipid; however, the matrix remains solid at body temperature. Commonly observed disadvantages of SLN include limited drug-loading capacity, drug expulsion during storage, and relatively high-water content in the dispersions (70–99.9%). As compared to SLN, NLC have a higher drug-loading capacity for a number of active compounds, and avoid or minimize potential expulsion of active compounds during storage(3) . For a number of drugs, the solubility of liquid lipid is higher than that of solid lipid, which enhances drug-loading. NLC possess numerous features that are advantageous for the topical route of application. These carriers are composed of physiological and

biodegradable lipid, exhibiting low systemic toxicity and low cytotoxicity. The small size of lipid particles ensures close contact to the stratum corneum and can enhance drug flux through the skin, and due to their solid lipid matrix, a controlled release from these carriers are possible Many types of NPs used as drug carriers are generally, but not necessarily, made of polymers or lipids. A comparison between the two types highly favors the lipid NPs, as they resolved many challenges presented with the polymeric NPs such as cytotoxicity and the lack ofsuitable methods for large-scale production. The first generation of lipid-based NPs was reported in the early 1990s and is known as SLNs mentioned in (Figure 1). They were originally developed as a simulation of oil-in-water nano emulsions where the internal oily phase was substituted by a solid lipid matrix. The development of such system resulted in multiple advantages over the traditional lipid-based formulations, namely liposomes and nano emulsions, such as avoiding the use of non-aqueous solvents, facilitating the upscaling processes, and improving the protection of labile loaded therapeutic agents conferred by using solid lipids (SL) instead of oily phase in emulsions. Nonetheless, SLNs as drug carriers were limited by low drug loading efficiency and increase in the risk of expulsion of the drug from the formulation upon storage due to polymorphic transitions. To overcome those limitations, developed NLC using mixtures SL and liquid lipids (LL) that form an amorphous solid matrix at both body and room temperature. The incorporation of LL in the matrix is the fundamental step, as it significantly enhanced the properties of the formulation as compared to SLNs. LL contribute to the creation of an amorphous lattice. (4)

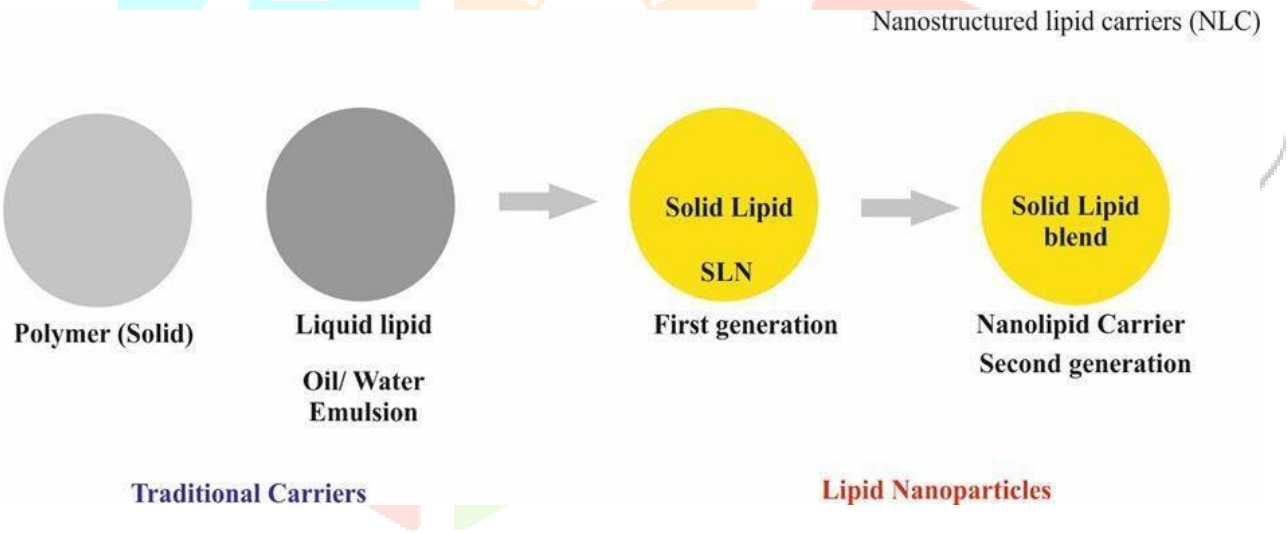


Figure 1: Nanostructured lipid carrier (4).

Furthermore, NLCs were able to broaden the spectrum and overcome many of the limitations associated with conventional lipid-based carriers. For instance, having NLCs in solid state at room temperature enhanced their physical stability which is a major barrier in emulsion-basedformulae. The availability of suitable methods for large scale production of NLCs resolved the expensive technological requirement for mass production of liposomes. In addition, NLCs are biocompatible systems distinguished by a rigid morphology that contributes to their unique properties compared to other lipid-based formulations. (5)

WHY LIPID NANOPARTICLES? (6).

Better control over release kinetics of encapsulated compound.

a. Engineering via size and lipid composition.

b. Melting can serve as trigger

Enhanced bioavailability of entrapped bioactive.

Chemical protection of labile incorporated compound Much easier to manufacture than biopolymeric nanoparticles.

No special solvents required.

Wider range of base materials (lipids).

Conventional emulsion manufacturing methods applicable.Raw materials essential the same as in emulsions.

Very high long-term stability.Application versatility:-

a. Can be subjected to commercial sterilization procedures
b. Can be freeze-dried to produce powdered formulation

Types of NLCs-

The structure of NLS's is very and somehow similar to SLNs, NLCs have three very specific features. These properties are based up on the location the drug is going to be integrated three different methods were adopted for a development and formulation of nanostructureNLCs (7).

- NLC type I also called as imperfect crystal.
- NLC type II also called as amorphous type.
- NLC type III also called as multiple type

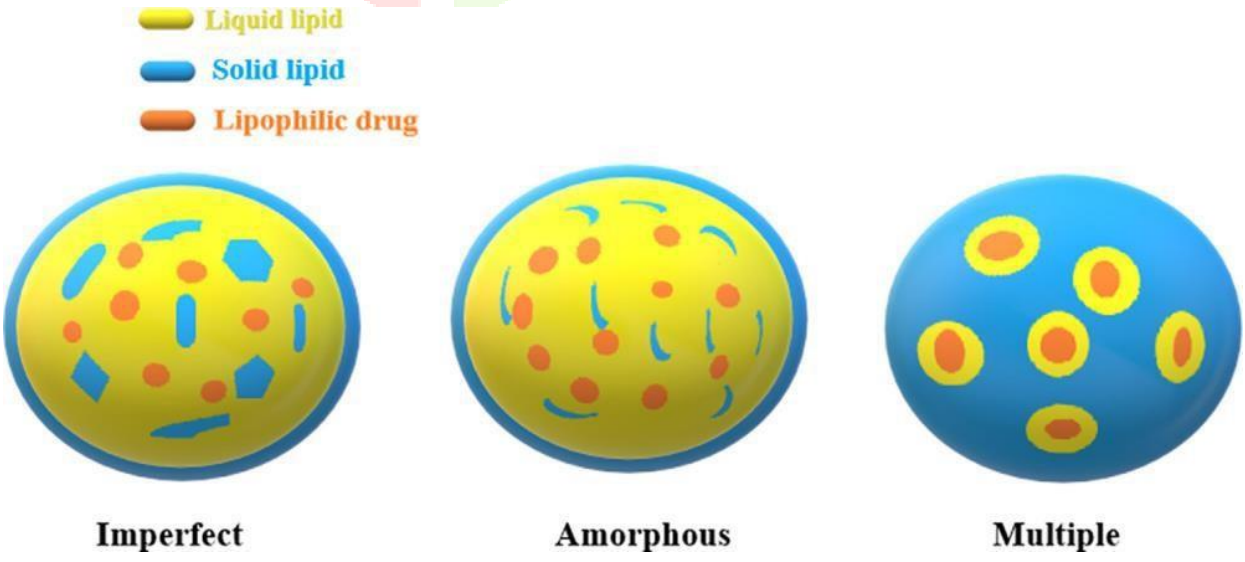
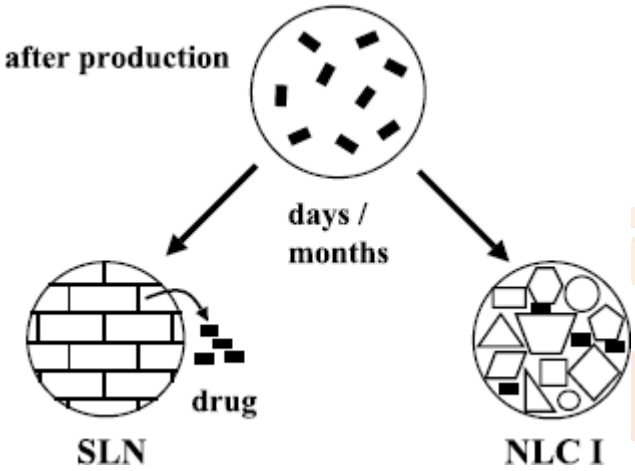


Figure 2-Structures if nanostructural lipids carries (7).

- **NLC type I also called as imperfect crystal**.

NLC type I also called imperfect crystal types have a badly structured solid matrix. The different fatty acids such as glycerides can be used to improve and modify the structure. The total number of imperfections in the structure are responsible and also helpful for the

property of good drug which can be easily increased (4). The type I of NLC's can be prepared by mixing spatially different lipids which can leads to imperfections in the crystal lattice. The drug molecules lodges extra disorderly crystal as molecular form and amorphous clusters. To avoid this adding to a minor quantity of liquid lipid additional leans to increases the drug- loading. The small quality of the glycerides can be used to overcome this situation (4). It was well documented in literature that If there is the change in the structure of the lipids, the problems like cluster of drugs arise and leads to disorderly imperfect lipid matrix and all this occurs is due to crystallization method (4).



3: Crystallization process during storage to perfect crystal in SLN (left) and unchanged remaining NLC I structure with imperfections (6).

- **NLC type II also called as amorphous type**.

In this technique of preparation of NLC's, the lipids are mixed in such a way that crystallizing can be prevented through mixing procedure. In type III method the lipid matrix remains solid but, in an amorphous state. The technique and method of crystallization often leads to drug expulsion. To minimize this, NLCs can also be formulated by carefully mixing of solid lipids with special lipids such as hydroxyl ctacosanyl hydroxyl stearate, isopropyl palmitate or MCT. Solid, but nanocrystalline NLC are formed (4,8). High drug entrapment efficiency, regulated drug release, and little drug leakage are all advantages of the Type II device.(9)

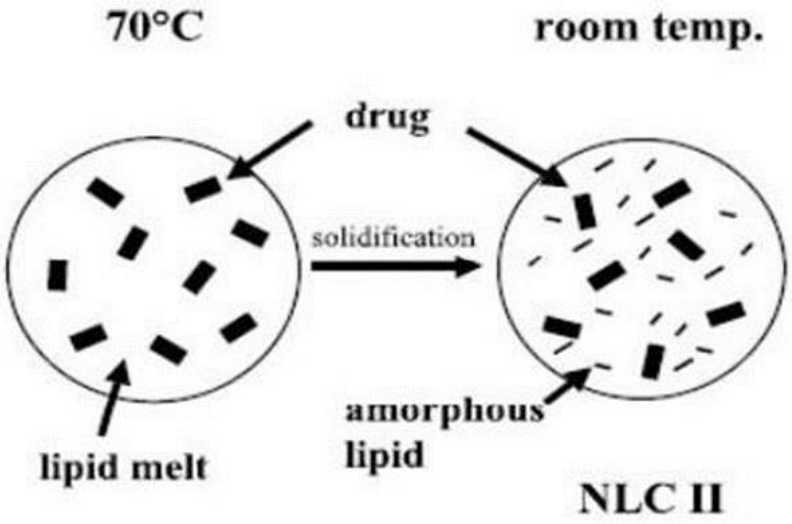


Figure 4: Structureless type II of NLC the lipid solidifies in the solid butamorphous (6).

The oil-in-lipid-in-water type is II type of NLC's also called as multiple type.
In type II NLC's, the solubility of oil is greater as compare to solubility of solid lipids (10). In type II NLC's high amount of oil are mixed with solid lipids due to this oil molecule can effortlessly spread into the lipid matrix at a low concentration of oil (6). If the added oil in
excess quantity than required of its solubility can lead to separation of different phases, finally produces small oily nano compartments which are bounded by the solid lipid matrix (4).

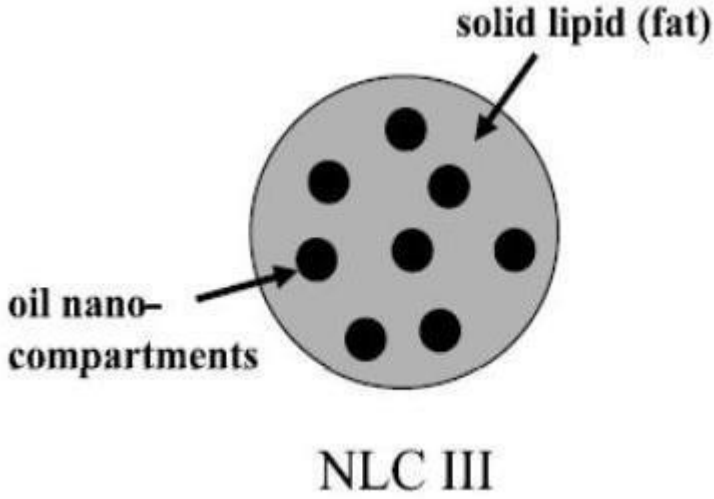


Figure 5: Theoretical proposed structure of multiple type NLC (oil-in-solidfat-in-water) (O/F/W) (6).

This kind of formulation permit controlled drug release and leakage of drug from lipid matrix . In this case, lipophilic drugs can be made soluble in oil first and type II method can be followed with the cooling procedure of a hot homogenization process (4). of Because their highly disordered lipid structures, NLCs can accept pharmaceuticals. Because of their highly disordered lipid structures, NLCs can accept pharmaceuticals.(11)

Characterization of NLCs

Zeta Potential:-

The zeta potential (ZP) indicates the overall charge a particle acquires in a specific medium. Stability of the nano dispersion during storage can be predicted form the ZP value. The ZP indicates the degree of repulsion between close and similarly charged particles in the dispersion. High ZP indicates highly charged particles. Generally, high ZP (negative or positive) prevents aggregation of the particles due to electric repulsion and electrically stabilizes the nanoparticle dispersion. In terms of rate of incorporation, neither SLN nor NLC lipid nanoparticles outperformed typical nanoemulsions.(12). With increasing oil content, the zetapotential value of SLNs and NLCs has increased(13).

Cooling procedure of a hot homogenization process [n the other hand, in case of low ZP, attraction exceeds repulsion and the dispersion coagulates or flocculates. However, this assumption is not applicable for all colloidal dispersion, especially the dispersion which contain steric stabilizers. The ZP value of −30 mV is enough for good stabilization of a nano dispersion .The ZP of the nano dispersions can be determined by PCS (14).

Particle Size:-

Size of particles and zeta potential PCS determined the particle size. (15)Particle size plays a crucial role in the gastrointestinal uptake and their clearance by the reticuloendothelial system. Hence, the precise determination of the particle size is very important. Particle size less than 300 nm are advisable for the intestinal transport(16) . Photon correlation spectroscopy and laser diffraction are the most powerful and widely used techniques for the particle size measurement of lipid nanoparticles. PCS is also known as dynamic light scattering. The fluctuation of the intensity of the scattered light, caused by particles movement, is measured by this technique. PCS is relatively accurate and sensitive method. However, only size range from few nano-meters to about 3µcan be measured by PCS) (17). This size range is enough to characterize lipid nanoparticles. On the other hand, LD can measure bigger particle sizes (>3µ). LD covers a broad size range from the nanometer to the lower millimeter range. This method is based on the dependence of the diffraction angle on the particle radius. Smaller particles lead to more intense scattering at high angles than the larger particles.(18) However, it is always recommended to use both PCS and LD method simultaneously as both methods do not directly measure particle Sizes, rather particle sizes are calculated from their light scattering effects. This is because particles are non-spherical in many instances (14).

Crystallinity and Polymorphism:-

Determination of the crystallinity of the components of SLN/NLC formulations is crucial as the lipid matrix as well as the incorporated drug may undergo a polymorphic transition leading to a possible undesirable drug expulsion during storage . Lipid crystallinity is also strongly correlated with drug incorporation and release rates. Thermodynamic stability and lipid packing density increase, whereas drug in corporation rates decrease in the following order: Super-cooled melt, α-modification, β'-modification, and β-modification. However, lipid crystallization and modification changes might be highly retarded due to the small size of the particles and the presence of emulsifiers. Differential scanning calorimetry (DSC) and X-Ray diffractometry (XRD) are two widely used techniques to determine the crystallinity and polymorphic behavior of the components of the SLNs/NLCs (19). DSC provides information on the melting and crystallization behavior of all solid and liquid constituents of the particles, whereas XRD can identify specific crystalline compounds based on their crystal structure. (20)DSC utilizes the fact that different lipid modifications possess different melting points and melting

enthalpies. In XRD, the mono-chromatic beam of X-ray is diffracted at angles determined by the spacing of the planes in the crystals and the type and arrangement of the atoms, which is recorded by a detector as a pattern. The intensity and position of the diffractions are unique to each type of crystalline material. XRD pattern can predict the manner of arrangement of lipid molecules, phase behavior, and characterize and identify the structure of lipid and drug molecules .However, best results are observed when SLN dispersions are investigated directly as solvent removal may change the modification. Another two techniques, infrared and Raman spectroscopy are also useful to investigate structural properties of lipids. However, they have not been extensively used to characterize SLNs/NLCs (21).

Limitations with lipid nanoparticles (22):

Despite the great potential of NLCs in targeted delivery, they face certainlimitations like:

Cytotoxic effects related to the nature of matrix and concentration, Irritative and sensitizing action of some surfactants,

Application and efficiency in case of protein and peptide drugs and gene delivery systems still need to be better exploited, and Lack of sufficient preclinical and clinical studies with these nanoparticles in case of bone repair.

Due to customer desire for food devoid of synthetic ingredients, natural essential oils are becoming increasingly popular as antimicrobials.(23).

Component of the NLC Lipids:-

The lipid is the primary component of NLC, and it has an impact on their drug loading capacity, stability, and sustained release behavior. Various lipid components, such as fatty acids, glycerides, and waxes, are used to make lipid nanoparticle dispersions. With the significant exception of cetyl palmitate, the majority of these lipids have been approved as generally- recognized-as-safe (GRAS) and are physiologically well-tolerated (24). Prior to using lipids inthe manufacture of lipid nanoparticle dispersions, it is critical to choose the right ones. Although no precise standards exist, empirical data such as medication solubility in lipid have been presented as relevant criteria for selecting an adequate lipid.(25) The lipid is the primary component of NLC, and it has an impact on their drug loading capacity, stability, and sustainedrelease behavior. Various lipid components, such as fatty acids, glycerides, and waxes, are used to make lipid nanoparticle dispersions. With the significant exception of cetyl palmitate, the majority of these lipids have been approved as generally-recognized-as-safe (GRAS) and are physiologically well-tolerated (26). Prior to using lipids in the manufacture of lipid nanoparticle dispersions, it is critical to choose the right ones. Although no precise standards exist, empirical data such as medication solubility in lipid have been presented as relevant criteria for selecting an adequate lipid (4).

Solid lipids:-

A combination of numerous chemical compounds which have a melting pointhigher than 40°C.

These solid lipids are well tolerated.

 Accepted for human use.

 Also in vivo biodegradable.

Examples are beeswax, carnauba wax, dynasan, precifac, stearic acid, ppifil, cutina CP 8 etc(27).

Liquid lipids (oil):-

These liquid lipids are well tolerated and accepted for human use.
Examples are Cetiol V, miglyol, castor oil, oleic acid, davana oil, palm oil, olive oil etc. as shown in Table 1.

Table 1: Lipids used in the preparation of nanostructured lipid carriers (2,8).

Fatty acids	Dodecanoic acid, Myristic acid, Palmitic acid and Stearic acid.
Monoglycerides	Glyceryl monostearate, and Glyceryl behenate.
Diglycerides	Glyceryl palmitostearate and Glyceryl dibehenate
Triglycerides	Caprylate triglyceride, Caprate triglyceride, Glyceryl and tribehenate /Tribehenin
Waxes	Cetyl Palmitate, Carnauba, and wax Beeswax
Liquid lipids	Soya bean oil, Oleic acid, Medium chain triglycerides (MCT)/caprylic- and capric triglycerides, α-tocopherol/Vitamin E, Squalene Hydroxyoctacosanylhydroxystearate and Isopropyl myristate

EMULSIFYING AGENTS-

Surfactants are molecules that adsorb at interfaces and help to lower interfacial tension. When surfactants, also known as surface-active agents, are present in modest concentrations, they improve stability by lowering surfactant rate. Surfactants adsorb onto the surface of a system or interface at low concentrations. Surfactant lowers thesurface tension or interfacial tension.

In the case of NLC formulation, the combination of solid and liquid-lipid mixes will not help much with flawless crystallization. To address this issue, researchers reduced the likelihood of the encapsulated medicine being expelled during storage. Polysorbate 80 added to the mix may have supplied larger interfacial area than polysorbate 20. As a result, NLC's 80 had a smaller average size than NLC's 20. The type of surfactant utilized in the formulation can affect the characteristics of NLCs. The average size and charge of the NLCs were considerably changed by the type of stabilizer, but not the size distribution(28) .

NLCs offer exceptional characteristics and properties that allow for the presentation of a wide range of integrated pharmacological forms. The type of surfactantutilized has a significant impact on the characteristics of NLCs. The surface or interfacial tension between the two phases is reduced, which saves

energy.

Table 2 list the different types and categories of surfactants. Surfactants for NLCs are chosenbased on a variety of parameters, including the route of administration of NLCs and the surfactant's HLB value. Table 2- lists the surfactants and co-surfactants. The effect of surfactant concentration on the size and dispersion of NLC particles. The electrostatic and steric repulsion between particles allows NLC to be stable. Some electrostatic and steric repulsion features are discussed. The extent of the compression of the adsorbed surfactant molecules is determined by the separation distance between the internal aqueous droplets and the external aqueous phase, the thicknesses of the two adsorbed surfactant layers, and
the size of the internal aqueous droplets and the oil globules. Thicker adsorbed layers must be used to reach the thickness of each of the two surfactant layers, which can effectively(28).

Table: 2 List Of Surfactant

Surfactants	
Ionic surfactants	Non-ionic surfactants
Sodium taurodeoxycholate, Sodium oleate, Sodium dodecyl sulphure	Span 20, 80, 85, Tween 20, 80, Tyloxapol, Poloxamer 188 Poloxamer 407, Solutol HS15
Amphoteric surfactants	Co-surfactants
Egg phospholipid (Lipoid E 80, Lipoid E 80 S)Soy Hydrogenated soy phosphatidy lcholine(Lipoid S PC-3, Hydrogenated egg phosphatidylcholine(Lipoid E PC-3) Phospholipon 80 H, Phospholipon 90 H)	Butanol, Butyric acid

Prevent the interior aqueous droplets from coalescing with the exterior aqueous phase. Smaller oil globules and bigger internal aqueous droplets create higher steric repulsion than larger oil globules, indicating that a more stable double emulsion system can be achievedby preparing the system with smaller oil globules and larger internal aqueous droplets.

Polyhydroxy surfactants stabilize systems by creating spatial exclusion, and because of their non-ionic nature, low and zero zeta potential are obtained. It is stated that the ionic strength of the continuous phase and the charge density on the surface of the water and fat influence the stability of nano lipid carriers against aggregation(28).

Surface modifiers

Dipalmitoyl-phosphatidyl-ethanolamine conjugated with polyethylene glycol 2000 (DPPE-PEG2000).

Distearoyl-phosphatidyl-ethanolamine-N-poly (ethylene glycol)2000 (DSPE-PEG2000)

Stearic acid-PEG 2000 (SA-PEG2000).

α-methoxy-PEG 2000-carboxylic acid-α-lipoamino acids (mPEG2000-C-LAA18).
α-methoxy-PEG 5000-carboxylic acid-α-lipoamino acids (mPEG5000-C-LAA18) . Ionic polymers: Dextran sulphate sodium salt.

Methods of preparation-

Various formulation techniques exist for the production of SLNs and NLCs. Among them, high- pressure homogenization (HPH) and microemulsion techniques have demonstrated strong potential for scaling up to industrial production scale . The following sections describe different existing approaches for SLN and NLC formulations. However, in some instances combination of different methods has been utilized to prepare the nanoparticles (14).

Methods of preparation-

Various formulation techniques exist for the production of SLNs and NLCs. Among them, high- pressure homogenization (HPH) and microemulsion techniques have demonstrated strong potential for scaling up to industrial production scale . The following sections describe different existing approaches for SLN and NLC formulations. However, in some instances combination of different methods has been utilized to prepare the nanoparticles (14). The preparation of aqueous NLC was carried out according to the method reported by Muller and co-workers. Accordingly phase which was heated at 10–15degree (29) Methods for preparing NLCs are relatively mature and can be used in mass production and dispersing techniques that do not involve organic solvents. These methods can be divided into high-temperature, high- pressure and low-temperature, high-pressure homogenization protocols. The high- temperature, high-pressure homogenization method is the more commonly adopted and involves the melting of solid lipid materials first before mixing them with liquid lipid and drugs. After mixing, the molten liquid is scattered throughout the aqueous phase, which contains surfactants. The mixture is stirred to form the beginning of an emulsion. Then, by high-speed impact and decompression expansion under an extremely high shear force, fluid droplets are gradually broken into nanoparticles. Generally, high temperatures reduce the viscosity of the mixed liquid, decreasing the particle size but increasing the probability of degrading the drug and the carrier. This method can be successfully used for insoluble drugs and lipophilic ones, but is not entirely suitable for hydrophilic drugs. The advantages are avoidance of organic solvents and large-scale production (2).
Hot high-pressure homogenization.

In this technique, first the lipid(s)is/aremeltedat5–10°C above its/their melting point and thedrug is dissolved or homogeneously dispersed in the melted lipid(s). Then a hot aqueous surfactant solution (pre-heated at the same temperature) is added to the drug–lipid melt and homogeneously dispersed (pre-emulsion) by a high shear mixing device. Subsequently, this hot pre-emulsion is subjected toa high-pressure homogenizer at the

same temperature (30). This homogenization process is repeated till the nano emulsion of desired average particle size is obtained. The obtained nano-emulsion is then cooled down to room temperature. During this cooling down, lipid droplets of the nano emulsion re-crystallize and form lipid nanoparticles with solid matrix (14).

Cold high-pressure homogenization

Similar to hot HPH, the lipid(s) is/are melted at 5–10°C above its/their melting points and the drug is dissolved or homogeneously dispersed in the melted lipid(s) in the cold HPH technique. Then the drug-lipid melt is rapidly cooled down by means of liquid nitrogen or dry ice and subsequently milled to microparticles by means of a ball mill or mortar. These microparticles are suspended in a cold aqueous surfactant solution and then homogenized at or below room temperature forming lipid nanoparticles. This cold HPH techniques suitable for hydrophilic or thermo-labile drugs as this method is expected to avoid temperature-induced drug degradation and drug distribution into aqueous phase during homogenization. However, complete avoidance of drug exposure to high temperature is impossible as the drug needs to dissolve or disperse in the molten lipid and some heat is generated during the homogenization process. Generally, scaling up of a process encounters several problems(31). Nevertheless, usage of the larger scale machines during HPH leads to an even better quality of the product with regard toa smaller particle size and its homogeneity. Additionally, HPH technique is widely used and well-established technique in pharmaceutical and food industry. SLN prepared by HPH can also be produced in non-aqueous dispersion media as long as the dispersion medium does not dissolve the lipid, e.g., liquid polyethylene glycol or oils (e.g., mineral oil) (14).

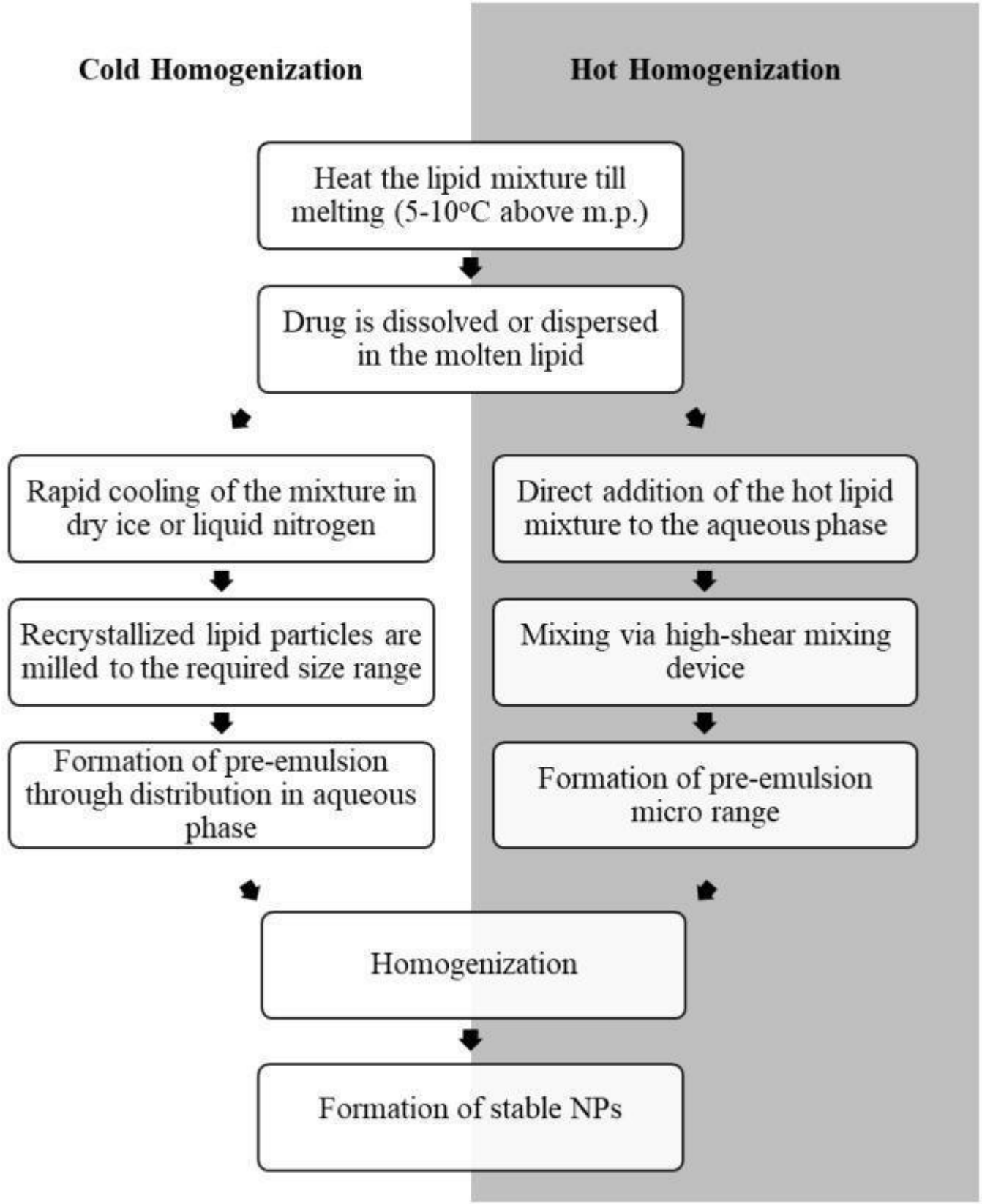


Figure 6- Preparation of NLCs by cold and hot high-shear

Ultrasonication:

Ultrasonication or high speed homogenization is another method for the production of SLNs. The advantage of this method is that the equipment used is commonly available at lab scale. However, this method suffers from problems such as broader size distribution ranging into micrometer range. Potential metal contaminations, physical instability like particle growth uponstorage are other drawbacks associated with this technique (33,34).

Micro-emulsion technique

In this technique, the lipids are melted and drug is incorporated in molten lipid. A mixture of water, co-surfactant(s) and the surfactant is heated to the same temperature as the lipids and added under mild stirring to the lipid melt. A transparent, thermodynamically stable system is formed when the compounds are mixed in the correct ratios for microemulsion formation. Thusthe microemulsion is the basis for the formation of nanoparticles of a requisite size. This microemulsion is then dispersed in a cold aqueous medium under mild mechanical mixing of hot microemulsion with water in a ratio in the range 1:25 – 1:50. Th is dispersion in cold aqueous medium leads to rapid recrystallisation of the oil droplets (5). Surfactants and co- surfactants include lecithin; biliary salts along with alcohols such as butanol. Excipients such asbutanol are less commonly used due to their regulatory aspects. The microemulsion is preparedin a large, temperature-controlled tank and then pumped from this tank into a cold-water tank for the precipitation step (24).

Solvent emulsification-evaporation technique

In solvent emulsification-evaporation method, the lipophilic material and hydrophobic drug aredissolved in a water immiscible organic solvent and emulsified in an aqueous phase using highspeed homogenizer. The efficiency of fine emulsification is improved by immediately passing the coarse emulsion through micro fluidizer Further the organic solvent is evaporated by mechanical stirring at room temperature and reduced pressure (e.g. rotary evaporator) leaving lipid precipitates nanoparticles (8).

This technique can be applied both for the aqueous and oily phase where solvent used must bepartially miscible with water. Initially, both the solvent and water are mutually saturated in order to ensure the initial thermodynamic equilibrium of both liquids. During the heating process in order to solubilize the lipid, saturation step is performed at the same temperature.

Then the lipid and drug were dissolved in water saturated solvent and this organic phase (14).

Solvent Dispersion:-

In the solvent dispersion method, solid lipid, liquid lipid, and the drug are dissolved in a water miscible organic solvent (ethanol, acetone, or isopropanol). Then, the organic solution is slowly added to the water containing the emulsifier, and the NLC is obtained by centrifugation.The drug loading of NLCs prepared by this method generally increases with the mass of the liquid. To further increase the drug loading of NLC, the dispersed phase is usually employed to enclose a saturated drug solution. The advantages of this method are speed, simplicity, and the low requirements of the instrument. The disadvantages of this method are that it is not entirely suitable for industrial production, and there is residual organic solvent (35).

NLCs are also prepared by the solvent dispersion method. This method was used. They were prepared with cholesterol as the solid lipid and OA as the liquid lipid, and poloxamer188 and polysorbate 80 as surfactants, achieving an entrapment efficiency of paclitaxel72±11.6%.3.1.4(2).

Film-Ultrasonic Method

In the film-ultrasonic method, the solid lipids, liquid lipids, and drugs are dissolved in an appropriate organic solvent, which is later removed by vacuum evaporation. To form a layer ofmixed lipid films, a surfactant aqueous solution is added. Small and uniform NLCs are then produced using an ultrasound probe for ultrasonic dispersion. This method is most often used due to its simplicity and practicality, and its yield of small, uniform particles. However, toxicological problems may result from solvent residues from the product obtained by this method (2).

Applications

NLCs as nano lipid carriers find potential application in various fields. The applications are divided in two broader aspects covering the therapeutic applications which include the various routes of administrations in drug delivery and the second part describes the applications in other fields including cosmetics, nutraceuticals, food, chemotherapy and gene delivery. These are discussed below:

Therapeutic applicationsTopical delivery

Topical route has been greatly exploited for the drug delivery to dermal areas employing lipid-based nanoparticles. In recent years, many studies and experiments have been performed on topical application of NLCs for their unique properties(36). NLCs can enhance the apparent solubility of entrapped drugs, which can form high concentration gradient on skin to facilitate drug permeation. The nano-sized particles tightly adhere to the skin surface and release the drugs in a more controlled manner (37). Therefore, NLCs are used for topical application of various categories of drugs for improvement of penetration along with sustained release.

Another benefit t of NLCs for topical delivery of active compounds is the short time required to market these products (22). Neither SLN nor NLC lipid nanoparticles surpassed normal nanoemulsions in terms of rate of incorporation.(38).

Oral delivery

NLCs have been proved as one of the beneficial systems for peroral administration of poorly water-soluble drugs having low bioavailability. Another important feature is the high dispersityof NLCs due to which they exhibit a high specific surface area for enzymatic attack by intestinal lipases (39). Other advantages of giving NLC in oral forms include increased drug loading; improved drug inclusion; patient compliance; high particle concentration and cream like consistency of the carrier (33).

The mechanisms involved in the absorption of the NLC from the intestine include direct uptakethrough the GI tract, increase in permeability by surfactants and decreased degradation and clearance. Besides this, the NLCs can also adhere on to the gut wall prolonging the residence time, and consequently the absorption (41). Poloxamer is involved in deforming the cell membrane and opening of the tight junction of intestinal epithelial cell, thus facilitating paracellular transport of NLCs. Poloxamer 407 also restrains p-glycoprotein efflux pump and increases NLC transport across the intestinal mucosa. Recently, it has been reported that enzyme activty of CYP3A could be inhibited by oleic acid. For example, Lovastatin, a cholesterol-lowering agent used for the patients with moderate hypercholesterolemia has been incorporated in NLCs that showed increased stability and clinical efficacy (40).

Parenteral delivery

The nano-drug delivery systems such as nano micelles, nano emulsions and nanoparticles has displayed a great potential in improved parenteral delivery of the hydro-phobic agents since last two decades. NLC has been considered as an alternative to liposomes and emulsions due to improved properties such as ease in manufacturing, high drug loading, increased flexibility in modulating drug release profile, and along with these, their aqueous nature and biocompatibility of the excipients has enabled intravenous delivery of the drug with passive targeting ability and easy abolishment (42). Another reported example is NLCs of artemether (Nano-jet) that offers significant improvement in the anti-malarial activity and duration of action as compared to the conventional injectable formulation. Nano jet can be considered asa viable alternative to the current injectable intramuscular (IM) formulation. Bufadienolides aclass C-24 steroid also proved to be effective in terms of enhanced hemolytic activity and cytotoxicity with reduced side effects when incorporated in NLCs (22,40).

Ocular drug delivery

Ophthalmic drug delivery with long pre-corneal retention time and high penetration into aqueous humor and intraocular tissues is the key-limiting factor for the treatment of ocular diseases and disorders. Recent reports indicated that NLC could increase the ocular bioavailability of lipophilic drug, ibuprofen (43). Our previous research showed that NLC could improve the penetration of bioactive compounds into ocular tissues with a good ocular tolerance. Another approach is to increase the trans corneal passage of drugs by incorporating permeation enhancers into formulations like Gelucire 44/14 a type of solid lipid and transductal IP that could enhance drug corneal permeability to some extent while stearyl Amine could prolong the pre-corneal retention of drug; all the three materials could optimize the formulation of a NLC ocular drug delivery and the preparation showed higher bioavailability comparing with eye drops (22).

Mucoadhesive nanostructured lipid carrier modified by thiolated agent has also been evaluated as a promising carrier for ocular drug delivery invitro and in vivo. The in vivo distributio investigation indicated that thiolated NLC could prolong pre-corneal residence time, and deliver high cyclosporine (CVA) level into eye tissues in ocular surface and anterior chamber (22).

Drug delivery to brain

Brain targeting not only increases the cerebrospinal fluid concentration of the drug but also reduces the frequency of dosing and side effects. The major advantages of this administrationroute are avoidance of first pass metabolism and rapid onset of action as compared to oral administration. LNC (e.g., NLC) of this generation are considered to be one of the major strategies for drug delivery without any modification to the drug molecule because of their rapid uptake by the brain, bio acceptability and biodegradability. Further, the feasibility in scale-up and absence of burst effect make them more promising carriers for drug delivery. In addition, NLC further enhanced the intranasal drug delivery of duloxetine in the brain for the treatment of major depressive disorder. Bromocriptine (BC) a dopamine receptor agonist has been also incorporated in NLCs for controlled delivery of drug to provide long-lasting therapeutic effects possibly extending BC half-life in vivo for the treatment of Parkinson's disease (22,33).

Pulmonary drug delivery

Drug delivery via inhalation is also a potential route for the treatment of several pulmonary disorders having advantages over conventional (parenteral and oral) dosage forms like a) non-invasive b) circumventing first pass metabolism and systemic toxicity c) reduced frequent dosing and d) site specificity by directly reaching to the lung epithelium thereby enhancing local drug(44) concentrations. In pulmonary drug delivery systems, surfactants and co-solventsare also often used to prepare stable formulations of highly lipophilic active ingredients(44).

Few attempts have been made to deliver anti-cancer agents using nanoparticles and liposomes via an inhalation route, but the major limitations being instability during nebulization, biodegradability, drug leakage and adverse side effects of drug. The lipophilic COX-2 inhibitor, celecoxib, was successfully encapsulated in the NLC nanoparticles using mixture of solid and liquid lipids where most of the nebulized nanoparticles were able to deposit in the alveolar region of the mice lungs and also enhanced the celecoxib lung residence time. To avoid deposition in the upper airways and exhalation during drug deposition in the deep lung, ultrafine particles must be tiny enough to settle effectively in thealveolar region.(44).

Other applicationsCosmetics

Recently NLCs have been developed based on the controlled nano structuring of particle matrix which provides immense advantages with respect to loading capacity and long-term stability (45). The various forms in which NLC dispersions can be given are gel, cream, lotion,ointment (46). The beneficial aspects associated with these NLCs in cosmeceuticals are very broad which lies in, enhancing skin bioavailability of active ingredients, film formation and controlled occlusion, UV protection, penetration enhancement and epidermal targeting, enhancement of physical and chemical stability and in vivo skin hydration (22).

NLCs greatly increased the in vitro SPF and erythemal UVA protection factor of oxybenzone more than six- and eight-fold, respectively, with fewer side effects. Investigations proved that NLC containing Cutanova Cream NanoRepair Q10, was superior with regard to skin hydration in comparison to a conventional o/w cream having the same composition. Nano Lipid Restore CLR (Chemisches Laboratorium, Dr. Kurt Richter, Berlin, Germany) is another semi-finished cosmetic product based on lipid nanoparticles. The easily oxidized black currant seed oil is incorporated in NLCs which are able to protect it against oxidation and enhance the stability of the final product. Another product Sturme. increases the occlusion of a day cream without changing its light character, that is, achieving higher occlusive properties without having the glossy skin appearance. A prolonged release profile can also be obtained for the perfumes andinsect repellents by incorporating them in NLCs (22).

Chemotherapy

Recent studies have shown that NLCs not only enhanced the efficacy and stability but also reduced side effects of many cytotoxic drugs.(47) Different nano systems have been developed with anti-cancer drugs, for example, the albumin – paclitaxel nanoparticles were approved in early 2005 in the chemo-therapy for metastatic breast cancer; etoposide NLCs were found to be cytotoxic against human epithelial-like lung carcinoma cells; stabilization and prolonged release of topotecan NLCs in treatment of refractory ovarian and small-cell lung cancer. Advantages of incorporating anti-cancer drugs in NLCs include high drug loading efficiency; prolonged release profile; increased chemical stabilization; increased cytotoxicity (22). Nutraceuticals

Nutraceuticals are bioactive compounds, which provide medicinal or health benefits, includingthe prevention, and treatment of diseases. Among them, the carotenoids are one of the most important groups of natural pigments, because of their wide distribution in plant tissues, structural diversity and numerous functions. Carotene-LNC with highly antioxidant and significant anti-bacterial activities were successfully produced by using natural oils and a versatile high-shear homogenization technique. Hesperetin (5,7,3 ′ -trihydroxy-4 ′ -methoxy flavanone) belonging to flavonones which is useful in chemically induced mammary tumorigenesis, colon carcinogenesis, heart attack and blood pressure was also successfully encapsulated in NLCs that showed good acceptance, homogeneity, improved taste and enhanced therapeutic effects (22).

In food industry

Because of its good stability and high loading capacity, the NLCs are widely applied in the pharmaceutical field. It was seldom reported that the NLC was applied as a nutritional supplement carrier in food industry for the capsule and beverage preparations (48). However, there are certain difficulties related to the raw material supply, availability and environ-mentalfactors due to which there is still a great risk for food industry to invest in this area. Coenzyme Q10-loaded NLCs for food application were developed to enhance the Physico-chemical stability and

bioavailability (22).

Gene delivery and gene therapy

Transfer of genes to mammalian cells is the most challenging task to achieve efficient and safegene therapy. Gene delivery systems are basically divided into two types, viz., viral and non-viral vectors. Viral vectors have been extensively investigated because of their high transfection efficiencies while non-viral vectors have the benefits of low immunogenicity and ease of preparation. However, their efficiency is not quite satisfactory. Colloidal particulate delivery systems like cationic liposomes, SLNs, nano emulsions, micelles, and some of the polymer based vectors like poly-L-lysine, poly-ethylenimine (PEI), polyamidoamine dendrimer and chitosan, exhibit significant advantages as potential candidates for efficient non-viral genedelivery. Among them, cationic liposomes and PEI are the most extensively investigated where cationic liposomes form a complex with anionic DNA molecules and deliver DNA through endosomes after endo-cytosis of the complex. Lipopolyplexes are used as nanomedicines for successful and efficient gene delivery. These are prepared by combination of gene (RNA/DNA), polycations, and lipids. They are mainly preferred for gene delivery in treatment of various cancers (Garc í a et al. 2012). Recently, demonstrated the contribution of NLCs towards gene delivery, by evaluating the in vitro gene transfer properties of polycationic nanostructured lipid carrier (PNLC) loaded with triolein in human lung adenocarcinoma. Enhanced transfection efficiency of PNLC was observed, which proved that PNLC is an effective non-viral gene transfer vector. Zhu et al. (2013) explained the utility of folate nanoliposomes for targeted delivery of RNA in meta-static neuroblastoma. Similarly, NLCs are used as multifunctional carrier for targeted delivery of siRNA and anti-cancer drugs(49). Recently, demonstrated the higher efficiency of NLCs for tumor targeted local delivery by inhalation of anti-cancer drugs and mixture of siRNAs for treatment of lung cancer with efficient sup-pression of tumor growth and prevention of adverse side effects on healthy organs (22).

Conclusions

NLCs has revolutionized the field of lipid-based NPs formulation and presented a wide spectrum of advantages over numerous, commonly-used lipophilic preparations. Using NLCs as drug carrier provided a high loading capacity platform for drug delivery by different routes including parenteral, oral, topical, ophthalmic, and pulmonary routes, while enhancingthe physical and chemical stability of the drugs, providing flexible control over their release, protecting them against degradation, and improving their poor pharmacokinetic parameters. Having such valuable properties has made NLCs highly favorable for use as carriers for toxic chemotherapeutic agents, taking advantage of their minute particle size and ability to passively or actively target tumor sites to enhance their delivery and relieve the patient from their unwanted side effects. In fact, it is evident from multiple invitro and in-vivo studies that NLCs have managed to optimize the delivery of chemotherapeutic agents, resulting in better safety profiles, higher efficacy, and improved pharmacokinetic properties. In comparison to traditional nanoemulsions, neither SLN nor NLC lipid nanoparticles showed any benefit interms of incorporation rate.

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