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REVIEW ARTICLE

An Updated Review on Nanostructured Lipid Carriers (NLC)

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ABSTRACT

In the last few decades, various drug-delivery technologies have emerged and a fascinating part of this has been the development of nanoscale drug delivery devices. Nanoparticles and other colloidal drug-delivery systems modify the kinetics, drug distribution in the body and release profile of an associated drug. Nanostructured lipid carriers (NLCs) have provoked the incessant impulsion for the development of safe and valuable drug delivery systems due to their exceptional physicochemical and then biocompatible characteristics. Throughout the earlier period, a lot of studies recounting NLCs based formulations have been noticeably increased. They are binary system which contains both solid and liquid lipids aiming to produce less ordered lipid core. Their constituents particularly influence the physicochemical properties and effectiveness of the final product. NLCs can be fabricated by different techniques which are classified according to consumed energy. More utilization NLCs is essential due to overcome barriers surrounded by the technological procedure of lipid-based nanocarriers' formulation and increased information of the core mechanisms of their transport through various routes of administration. They can be used in different applications and by different routes such as oral, cutaneous, ocular and pulmonary. This review article highlights the structure, composition, various formulation methodologies, and characterization of NLCs which are prerequisites in formulating a stable drug delivery system. NLCs hold an eminent potential in pharmaceuticals and cosmetics market because of extensive beneficial effects such as skin hydration, occlusion, enhanced bioavailability, and skin targeting.

Keywords: Application of NLCs, Drug delivery system, Lipid nanocarriers, Nanostructured lipid carriers

INTRODUCTION

At present, the field of nanotechnology is intensely exploited for drug delivery technology for passive and active targeting via various routes of administration. Nanoparticles (NPs) are defined as colloidal particulate systems having dimensions between 10 and 1000 nm.[1] Solid lipid nanoparticles (SLNs) were conceptualized in early 1990s by Professor R.H. Müller (Germany) and Professor M. Gasco (Italy) as a novel formulation possessing several advantages e.g. the use of biocompatible lipids, least use of organic solvents during formulation, high *in vivo* stability and broad application spectrum.[2] SLNs are colloidal particles prepared from solid lipids (solid at room temperature and body temperature), surfactants, active ingredient and water. Still, SLNs experience certain limitations such as poor drug loading capacity, unpredictable gelation tendency, polymorphic transitions, and drug leakage during storage.[1-3] Nanostructured lipid carriers (NLCs) spring up as second generation

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of lipid NPs to overcome the shortcomings of first generation, that is, SLNs. Biodegradable and compatible lipids (solid and liquid) and emulsifiers are used for the preparation of NLCs. Liquid lipids (oil) incorporation causes structural imperfections of solid lipids leading to a less ordered crystalline arrangement which avert drug leakage and furnish a high drug load.[2,4] In last few years, NLCs have gained attention of researchers as an alternative of SLNs, polymeric NPs, emulsions, microparticles, liposomes, etc.[5] These nanocarriers possess the utility in delivery of hydrophilic as well as lipophilic drugs. NLCs have emerged as a promising carrier system for the delivery of pharmaceuticals through oral, parenteral, ocular, pulmonary, topical, and transdermal route. Recently, NLCs are also being exploited in brain targeting, chemotherapy, gene therapy, food industry, and delivery of cosmeceuticals and nutraceuticals.[5]

Pros of nanostructured lipid carrier

- More physical stability.
- More space to accommodate drug.
- Appropriate for loading of lipophilic drug and hydrophilic drugs.
- Easy for large scale production.
- Provide controlled release of encapsulated drugs.
- Avoidance of first pass metabolism.
- Reduction in chances of burst release of drug.
- Better control over release of drug.
- By passing p-glycoprotein efflux pumps.
- Protecting drug from intra-enterocyte metabolism.[6]

Cons of nanostructured lipid carrier

- May cause discomfort by some surfactant.
- May cause cell damage.
- Deficiency of adequate preclinical and clinical study.
- Polymorphic transition of some lipid.
- Particle size growth during storage time.[6]

TYPES OF NLC

Depending on the location of incorporated drug moieties in NLC, following three types of morphological models [Figure 1] has been proposed:

NLC type I (imperfect crystal model)

Imperfect crystal type NLC consists of a highly disordered matrix with many voids and spaces which can accommodate more drug molecules in amorphous clusters. These imperfections in the crystal order are acquired by mixing solid lipids with adequate amount of liquid lipids (oils). Due to varying chain length of fatty acids and the mixture of mono-, di-, and triacylglycerols, the matrix of NLC is not able to form a highly ordered structure. Mixing spatially different lipids increases drug payload capacity however this model offers minimum entrapment efficiency.[7,8]

NLC type II (multiple type)

Multiple type NLC is oil/lipid/water type. Lipophilic drugs are more soluble in liquid lipids than solid lipids. This idea leads to the development of multiple type NLC using high liquid lipid content. Oil moieties, at low concentrations, are effectively dispersed in the lipid matrix. The addition of oil beyond its solubility induces phase separation forming small nano compartments of oil encircled in the solid matrix. Type II model offer advantages such as high drug entrapment efficiency, controlled drug release, and minimized drug leakage.[8,9]

NLC type III (amorphous model)

Amorphous type NLC is formulated by carefully mixing lipids in such a way as to minimize the drug leakage due to process of crystallization. Specific lipids such as hydroxyl octacosanyl, hydroxyl stearate, isopropylmyristate or dibutyladipate form solid yet non-crystalline particles. The lipid matrix exists in a homogenous amorphous state.[7-9]

EXCIPIENTS USED IN FORMULATING NLC

In general, NLCs are composed of lipid (s) (solid as well as liquid), surfactant(s), organic solvent, and other agents such as counter-ions and surface modifiers.

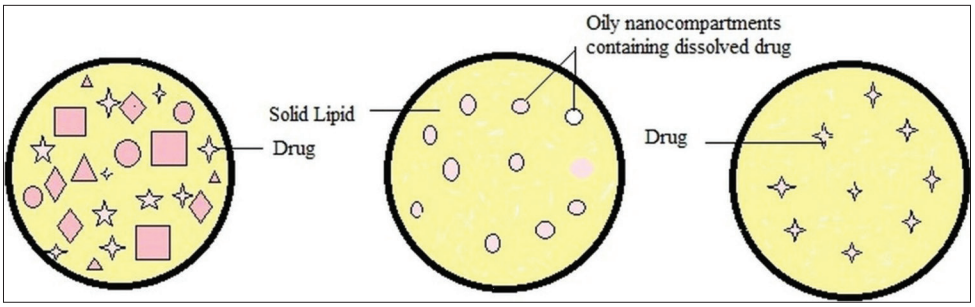

Figure 1: Types of nanostructure lipid carrier[19]

Lipids

The primary component of nanostructure lipid carriers that govern drug loading capacity, prolong action and stability of the formulations is lipid. Solid lipids such as fatty acids, triglyceride, diglyceride, monoglyceride, steroids, and waxes have been used for formulating NLC.[10] Physiologically acceptable, biodegradable, non-toxic, and generally-recognized-as-safe status lipids are preferred for preparation of lipid NPs. Choice of suitable lipids is essential preceding their utilization in preparation of nanoparticulate carriers. The type and structure of lipid affects various characteristics of nanocarriers. Practically, solubility or evident partition coefficient of bioactives in the lipid has been suggested as the best fitting criteria for choosing a suitable lipid. The solubility of the drug molecules in lipid is interpretative as it affects drug loading and encapsulation efficiency.[9] Degree of crystallization of various lipids employed also affect drug entrapment and loading, size and charge, and efficacy.[10] On account of higher viscosity of dispersed phase, because of higher melting lipids, the average particle size of nano dispersion increases. Shape of lipid crystals, lipid hydrophilicity, and variation in composition is additional lipid related parameters that may influence quality of NLC. It has been found that a 5–10% hike in lipid amount leads to larger particle size.[11]

Surfactants

The type and concentrations of surfactant exert influence on quality and efficacy of NLC. It has been found that toxicity, physical stability and crystallinity of NLC are greatly influenced by choice of surfactant.[12] Surfactant systems also have an impact on extent of drug dissolution and drug permeability. Surfactants are chosen based on of route of administration, hydrophilic-lipophilic balance (HLB) value, effect on particle size, and lipid modification. Surface active agents (emulsifiers) are adsorbed on the interface where they reduce the tension between lipid and aqueous phases because of their amphipathic nature. During the formulation of NLC, crystallization of colloid particles goes along with solidification, but the surface area of particle increase remarkably during crystallization so that the whole system become unstable. Hence, surfactant is a requisite to improve interface quality of NPs to attain stability.[13] Modifying the surfactant system compositions may govern the miscibility of chemical components in NLCs, and hence the stability.[12] Required HLB (rHLB) plays an important role while selecting suitable type and amount of surfactant for NLC formulation.[14] rHLB of lipids and lipid matrix is determined to calculate the amount of emulsifiers to be added in formulation. The rHLB value for lipid is the HLB value of emulsifier which is necessary for appropriate emulsification, that is, reduction of interfacial tension between oil and water phase. This also assists in achieving a stable nano system and small particle size of NLCs.[15,16] By determining rHLB a right combination of emulsifiers with least concentration can be employed for formulation. rHLB for lipids (solid and liquid) and lipid matrix is calculated experimentally by dispersing in blends of surfactant with different HLB values. The mixture is put through high pressure homogenization (HPH) and analyzed for least particle size.[14,16,17]

Other ingredients

Organic salts and ionic polymers may be employed as counter-ions in formulation of nano structure

carriers to overcome the challenge of encapsulating water soluble drug molecules. Surface-modifiers are another category of excipients used in formulation of NLC to minimize their phagocytic uptake by the macrophages in reticuloendothelial system. Lipid particles are coated with hydrophilic polymers such as PEG, poloxamines, or poloxamers to increase the residence time of drug molecules in systemic circulation. Surface modification may offer other advantages such as enhanced physical stability and biocompatibility, drug targeting, increased transport across epithelium.[9,18]

METHODS OF PREPARATION NLCS

There are methods used for production of NLCs. Based on the energy required, methods can be categorized into three types [Figure 2].

HIGH ENERGY REQUIRED METHODS

There are many techniques used for production of NLCs and required high energy input such as HPH, high shear homogenization/sonication, supercritical fluids, and microwave based. In this review, I will focus on HPH and high shear homogenization/sonication as they are the most widely used techniques.

HPH

This method is considered as one of the most preferred methods because no solvents are added during the preparation. It has been considered as a consistent and powerful technique for the large scale production of NLCs[20] as it produces highly stable particles and requires no organic solvent addition.[21]

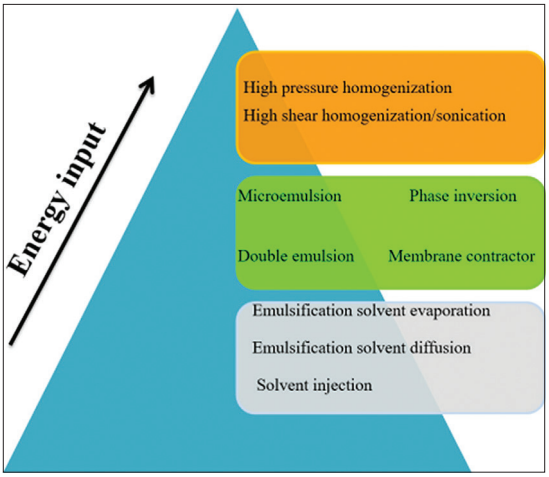

Figure 2: Methods of fabrication NLCs according to energy input

Hot homogenization

During this method, the drug is added to the molten lipid mixture which dispersed in heated aqueous solution of surfactant using high speed stirring. Finally, the preemulsion is further homogenized by high pressure homogenizer. NLCs are formed once the obtained nanoemulsions recrystallized at room temperature. The drawbacks of this method include heat degradation of thermolabile actives, reduction of emulsification power of some surfactants at higher temperatures and low drug encapsulation efficiencies as it may be partitioned in both lipid and aqueous surfactant solution at high temperature which promotes drug escaping into aqueous phase.[22]

Cold homogenization

In this method, the molten lipid mixture with the drug is solidified by rapid cooling under the effect of liquid nitrogen or dry ice. Subsequently, it is micronized and dispersed in a cold aqueous surfactant solution. The obtained dispersion is finally processed by high pressure homogenizer is applied. This technique can relatively overcome the drawbacks of hot methods such as avoiding heating of drugs and surfactants. In addition, desired crystal structure can be obtained by controlling the crystallization process. On the other hand, the produced particles may exhibit higher particle sizes and heterogeneity when compared with hot homogenization method.[23]

High shear homogenization/sonication

In this method, lipophilic drug is dissolved or dispersed in molten solid lipid/liquid lipid mixture. The temperature used should be 10°C above the melting point of solid lipid to make difficult to recrystallize. The aqueous surfactant solution

of the same temperature is poured to lipid phase and pre-microemulsion is formed under the effect of high speed stirrer. The pre-emulsion is further homogenized using high shear homogenizers followed by probe sonicator treatment.

LOW ENERGY REQUIRED METHODS

Microemulsion

Microemulsion is prepared by similar procedure of high shear homogenization/sonication technique. Then the hot microemulsion is added to cold water to form nanoemulsion, which then recrystallizes to form NLC.

Double emulsion

In this method the prepared microemulsion is added to cold water (2–10°C) which facilitate precipitation of uniformly distributed NLCs particles.

Phase inversion

In this method the whole components' mixture are exposed to three heating and cooling cycles. After that, the hot mixture is shocked by dilution with cold water and NLCs are formed by phase inversion.

Membrane contractor

Small lipid droplets are obtained by pressing the molten lipid against porous membrane. Concurrently, they are circulated inside the membrane module and sweeps away from the pore. NLCs are formed after cooling at room temperature.

VERY LOW OR NO ENERGY REQUIRED METHODS

Emulsification solvent evaporation

In this technique, active substance as well as lipids is dissolved in water immiscible solvent. The resultant solution is then emulsified with aqueous surfactant solution. Subsequently, the solvent is evaporated under continuous stirring resulting in NLCs formation. As there is no heat involved, this method is suitable for heat sensitive actives. The main disadvantages of this technique are solvent residue associated toxicity and diluted particles of NLCs due to inadequate solubility of the lipids in the solvents used.[24]

Emulsification solvent diffusion

In this method, active substance and lipids are dissolved inorganic solvent which is saturated with water for thermodynamic equilibrium. The resultant momentary o/w emulsion is distributed into water under stirring until solidification of the dispersed phase.

Solvent injection

In this method, active substance and lipids are dissolved in organic solvent and injected in with aqueous surfactant solution.

DRUG ENCAPSULATION IN NLCS

There are three ways of incorporating or encapsulating drug within the lipid NPs or NLCs. They are homogenous matrix of solid solution, drug-enriched shell, and drug-enriched core.[20]

- Homogenous matrix of solid solution: In this method of encapsulation, the drug is homogenously dispersed into the lipid matrix of the particles and the drug release occurs by diffusion process.
- Drug-enriched shell: In this method, the drug is concentrated on to the outer most layer or shell of the lipid NPs. This type of NPs exhibits burst release of the drug due to precipitation and solubilization mechanism.
- Drug-enriched core: In this method, prolonged release is observed due to the saturation solubility of drug in the lipid.

MECHANISM OF SKIN PENETRATION AND DRUG RELEASE IN NLCS

In general, the rate of drug release depends on various factors such as the solubility of drug,

desorption of the surface bound/adsorbed drug, drug diffusion through the NPs matrix, NPs matrix erosion/degradation, and combination of erosion/diffusion process. Thus solubility, diffusion and biodegradation of the matrix materials govern the release process. This type of release can also be triggered by an impulse when the particles are administered [Figure 3]. The reason behind higher drug loading is the highly unordered lipid structures found in NLCs. NLCs of certain structures can be triggered this way.[25]

The development of a less ordered solid lipid matrix is desired for a sufficiently high drug-load. In general, the drug can be located in between the fatty acids or in between the lipid layers and also in imperfections (e.g., amorphous). In case of spatially very similar lipid compared to the more or less highly ordered matrix molecules, especially when mono acid highly purified glycerides such as tristearin is used, drug load is very limited and drug expulsion occurs within hours or a few days.[26]

STABILITY OF NANOSTRUCTURED LIPID DISPERSIONS

NLCs may contain additional colloidal structures, such as micelles, mixed micelles, liposomes and nanoemulsions which contribute to their stability. There are also some major stability issues during storage, such as particle size enhancement, gelation of the dispersion, and drug expulsion from the lipid matrix. Gelation takes place due to formation of the network and lipid bridges between the particles. The physical stability of these dispersions is generally investigated by measurement of particle size (Photon correlation spectroscopy and Laser diffraction), zeta potential and thermal analysis (Differential scanning calorimetry). Several studies indicated physical stability of SLNs dispersion more than 1 year.[27] The long-term storage of lipid dispersions leads to aggregation and shell formation as reported in case of SLNs.[28] In case of highly concentrated NLC dispersions the particles form a "pearl-like network," thus undergoing collision and perikinetic flocculation. After the administration of NLCs and their dilution with gastrointestinal (GI) fluid, the network is destroyed releasing single, non-aggregated particles. Lipid particle dispersions were produced at identical surfactant concentration, but with low lipid content (below 30%, outside patent coverage) and with 35% lipid. The low particle dispersion aggregated during storage time, the gel-like NLC dispersion remained stable during storage and, after dilution, and single particles were obtained showing no size increase.[29] Freely diffusible NPs in low concentration dispersion can collide and aggregate (upper), while in highly concentrated dispersions the particles are fixed in a network, where further dilution with water releases non-aggregated definite NPs [Figure 4].

STRATEGIES EMPLOYED FOR OVERCOMING THE ISSUES RELATED TO STABILITY OF NLCS

Polyethylene glycol (PEG)

In general, surface modification of colloidal particles by coating with a hydrophilic substance like PEG reported to bring following benefits:

- Providing good physical stability and dispersibility of colloids,

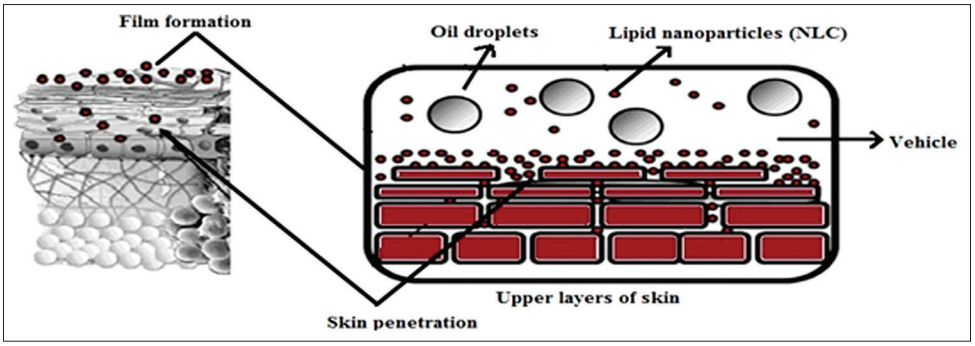

Figure 3: Mechanism of penetration into skin

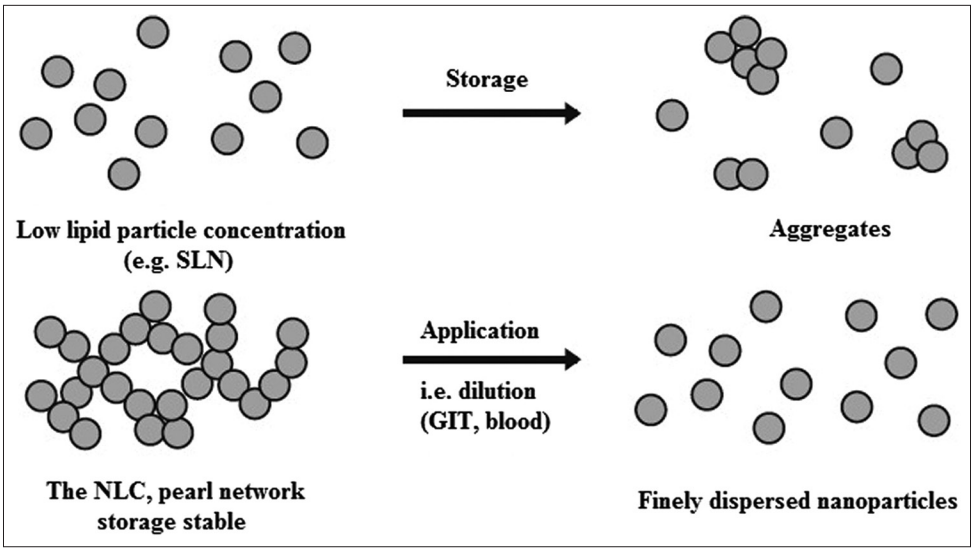

Figure 4: Stabilization effect in highly concentrated lipid particle dispersions; adopted and modified from[31]

- Improving presence of colloids in blood circulation for systemic use,
- Increasing stability of colloids in body fluids such as GI fluids,
- Acceleration of colloid transport across the epithelium,
- Modulation of interaction of colloids with mucosa for specific delivery requirements and drug targeting,
- Increasing biocompatibility and decreasing thrombogenicity of drug carriers, and
- Providing reservoir function to colloid particles carrying hydrophobic drugs due to hydrophilic coating around the particles.

Spray drying

In addition to the optimized storage conditions, SLNs/NLCs dispersions can also be spray dried to increase their stability.
However, melting point of the lipid matrix should be more than 70°C for spray drying.

Lyophilization

Another efficient way to increase stability is lyophilization. However, when SLN are lyophilized without cryoprotectant, the final product commonly results in the aggregation of particles. Some of the most widely used cryoprotectants are trehalose, sorbitol, glucose, sucrose, mannose and maltose. Schwarz and Mehnert reported trehalose as the most effective cryoprotectant in preventing particle growth.[30]

APPLICATIONS

NLCs as nanolipid 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:

Topical delivery

Topical route has been greatly exploited for the drug delivery to dermal areas employing lipid based NPs. In recent years, many studies and experiments have been performed on topical application of NLCs for their unique properties.[32-34] 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.[35] Therefore, NLCs are used for topical application of various categories of drugs for improvement of penetration along with sustained release. Another benefit of NLCs for topical delivery of active

compounds is the short time required to market these products.

Experimental studies have confirmed the significant improvement in therapeutic response and reduction in local side effects of acitretin NLCs loaded gel indicating its effectiveness in the topical treatment of psoriasis. The prepared NLCs were spherical in shape and the release study showed biphasic drug release pattern with an initial sustained release phase for up to 10 h followed by a steady drug release phase. Idebenone loaded NLCs (I-NLCs) were prepared for topical delivery of antioxidant idebenone and evaluation of its sun protection efficacy. Sun protection factor (SPF) value for I-NLCs was found to be 23 which represent that lipid nanocarriers (LNC) have standards of blocking of 94–96% of Ultraviolet-B rays.[36] The potential of NLCs loaded with lipophilic calcipotriol and hydrophilic methotrexate for topical therapy of psoriasis was investigated. The study confirmed that NLC systems are a promising carrier for the topical delivery of anti-psoriatic drugs as revealed by enhanced skin permeation, negligible skin irritation, and the compatibility of the two drugs.[37] In another study, antifungal drug ketoconazole loaded NLCs were physically more stable as compared to SLN as the SLN matrix was not able to protect the chemically labile ketoconazole against degradation under light exposure.[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 dispersivity of NLCs due to which they exhibit a high specific surface area for enzymatic attack by intestinal lipases. 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. The mechanisms involved in the absorption of the NLC from the intestine include direct uptake through 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. 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 activity of CYP3A could be inhibited by oleic acid.[39,40] For example, Lovastatin, a cholesterol-lowering agent used for the treatment of patients with moderate hypercholesterolemia has been incorporated in NLCs that showed increased stability and clinical efficacy.[41]

Parenteral delivery

The nano-drug delivery systems such as nanomicelles, nanoemulsions, and NPs have displayed a great potential in improved parenteral delivery of the hydrophobic 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. Another reported example is NLCs of artemether (Nanoject) that offers significant improvement in the anti-malarial activity and duration of action as compared to the conventional injectable formulation. Nanoject can be considered as a viable alternative to the current injectable intramuscular (IM) formulation.[42,43] Bufadienolides a class C-24 steroid also proved to be effective in terms of enhanced hemolytic activity and cytotoxicity with reduced side effects when incorporated in NLCs.[44]

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. 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 transcorneal passage of drugs by incorporating permeation enhancers into formulations like Gelucire 44/14 a type of solid lipid and Transcutol IP that could enhance drug corneal permeability to some extent while stearylamine 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. Mucoadhesive nanostructured lipid carrier modified by thiolated agent has also been evaluated as a promising carrier for ocular drug delivery *in vitro* and *in vivo.* The *in vivo* distribution investigation indicated that thiolated NLC could prolong pre-corneal residence time, and deliver high cyclosporine level into eye tissues in ocular surface and anterior chamber.[45,46]

Drug delivery to brain

Brain targeting not only increases the cerebro spinal fluid concentration of the drug but also reduces the frequency of dosing and side effects. The major advantages of this administration route 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, bioacceptability 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.[47,48]

Pulmonary drug delivery

Drug delivery through inhalation is also a potential route for the treatment of several pulmonary disorders having advantages over conventional (parenteral and oral) dosage forms such as (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 concentrations. In pulmonary drug delivery systems, surfactants and co-solvents are also often used to prepare stable formulations of highly lipophilic active ingredients. Few attempts have been made to deliver anti-cancer agents using NPs and liposomes through 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 NPs using mixture of solid and liquid lipids where most of the nebulized NPs were able to deposit in the alveolar region of the mice lungs and also enhanced the celecoxib lung residence time.[49]

OTHER APPLICATIONS

Cosmetics

Recently NLCs have been developed based on the controlled nanostructuring of particle matrix which provides immense advantages with respect to loading capacity and long term stability. The various forms in which NLC dispersions can be given are gel, cream, lotion, ointment. 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.[50]

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 (Dr. Rimpler,

Wedemark, Germany), was superior with regard to skin hydration in comparison to a conventional o/w cream having the same composition. NanoLipid Restore CLR (ChemischesLaboratorium, Dr. Kurt Richter, Berlin, Germany) is another semi-finished cosmetic product based on lipid NPs. 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 Surmer (Dr. Rimpler, GmbH, Wedemark, Germany) 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 and insect repellents by incorporating them in NLCs.[51]

Chemotherapy

Recent studies have shown that NLCs not only enhanced the efficacy and stability but also reduced side effects of many cytotoxic drugs. Different nanosystems have been developed with anti-cancer drugs, for example, the albumin-paclitaxel NPs were approved in early 2005 in the chemotherapy 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. As these NLCs avoid some potential problems associated with SLN, such as drug leakage during storage and decreased loading capacity. They act by prolonging the exposure of tumor cells to anti-tumor drug and enhancing permeability and retention effect to further increase the therapeutic effect.[52] It has also been reported that hyaluronic acid coated NLC could prolong the circulation time of paclitaxel (PTX) in blood and increase the accumulation of PTX in the tumor. The results of this experiment indicated that hyaluronic acid-coated, paclitaxel-loaded, and NLCs (HA-NLCs) showed higher anti-tumor efficacy and fewer side effects than Taxolin B16-bearing Kunming mice. The overall targeting efficiency of HA-NLC in the tumor was 14.46%, approximately 1.4 times that of Taxol.[53]

Nutraceuticals

Nutraceuticals are bioactive compounds, which provide medicinal or health benefits, including the 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 flavanones 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.[54]

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. However, there are certain difficulties related to the raw material supply, availability, and environmental factors 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 physicochemical stability and bioavailability.[55]

Gene delivery and gene therapy

Transfer of genes to mammalian cells is the most challenging task to achieve efficient and safe gene 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 such as cationic liposomes, SLNs, nanoemulsions, micelles, and some of the polymer based vectors such as poly-L-lysine, polyethylenimine (PEI), polyamidoaminedendrimer and chitosan, exhibit significant advantages as potential candidates for efficient non-viral gene delivery. 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 endocytosis of the complex.[56] 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.[57] Recently, Zhang *et al*. (2008) demonstrated the contribution of NLCs toward 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 folatenanoliposomes for targeted delivery of siRNA in metastatic neuroblastoma.[58] Similarly, NLCs are used as multifunctional carrier for targeted delivery of siRNA and anti-cancer drugs. Recently, Taratula *et al*. (2013) 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 suppression of tumor growth and prevention of adverse side effects on healthy organs.[59]

SAFETY/TOXICITY ASPECTS

Safety/toxicity of NLCs is considered as one of the main concerns. However, few researchers in the literature paid attention to safety profile. Hence, we have tried to gather a brief report on the safety/toxicity of the NLCs stated in the literature. Regarding oral application, NLCs are considered as relatively safe nano carriers due to the content of biodegradable and physiological lipids which are well tolerated in both *in vitro* cytotocity and *in vivo* studies. On the other hand, NLCs contain less quantity of surfactants and cosurfactants when compared to emulsions which improve their safety profile. Rahman *et al*. studied the toxicity of zerumbone-loaded NLCs on BALB/c mice model after oral administration.[60] Based on histopathological alterations, they reported that NLCs did not exhibit any signs of toxicity on lungs, liver and kidney and higher lethal dose (LD50) dose of NLCs was reported. *In vitro* Caco-2 cells cytotoxicity studies showed that NLCs system did not show significant cytotoxicity and cell viability was >90%.[61] In another study, it was demonstrated that the cytotoxicity (on lymphocytes) of NLCs was dependent on the number particles of NLCs in milliliter; 2.1×10^{11} particles/mL caused a decreased in the viability of lymphocytes (about 55%).[62] Regarding cutaneous application, Bruge *et al*. studied cytotoxic effect of five commonly used solid lipids on human dermal fibroblast. They reported that Compritol TM 888 ATO was the safest lipid owing to its neutral cytotoxiceffect.[63] Fang *et al*. reported that enhancer could generally irritate the skin but not correlated to penetration power of the enhancer. They also reported that fatty acids usually presented the most irritating properties, followed by Azone, Dlimonene, and L-alpha-lecithin. A complete portrait of the efficacy and safety of commonly used enhancers was therefore established in this study.[64] Regarding ocular delivery, NLCs are considered relatively safer for ocular delivery due to the biocompatible lipids, non-ionic, and biocompatible surfactants and organic solvent-free formulations.[65] However, the degree of clearance and the toxicity are mainly basedon the site of administration (topical, intravitreal, intravenous, transscleral, suprochoroidal, or subretinal). Liu *et al*. formulated mangiferin loaded NLCs for the treatment of cataract. They were investigated for its irritancy potential and ocular tolerability. They showed good safety profile.[66] Gonzalez-Mira

optimized flurbiprofen-loaded NLC by central composite factorial design based on concentration of drug, ratio of liquid and solid lipid and concentration of surfactant. The optimized formulation was safe and non-irritant to the eye.[67] Regarding pulmonary delivery, various studies showed minimal *in vitro* cytotoxicity of NLCs. In addition, no inflammation or change in the integrity of alveoli,[68] pulmonary edema or pathological changes in lung and liver[69] were observed after inhalation of NLCs.

APPROACHES TO CLINICAL TRIALS

Although NLCs possess great potential as drug delivery carriers, preclinical and clinical studies are still insufficient. Therefore, there is a need to expand the spectrum of their applications to include clinical trial under appropriate ethical regulations. This might be attributed to the lack of critical analysis on the safety profile of NLCs as drug carriers. However, cutaneous and oral applications were the majors in that regard. For example, lovastatin, antihypercholesterolemic agent used for the treatment of patients with moderate hypercholesterolemia has been formulated in NLCs that showed increased stability and clinical efficacy.[41] Acitretin, has been formulated in NLCs for topical treatment of psoriasis.[70] As a final point, the promising characteristics of NLCs can be further trailed with more studies on their absorption, distribution, metabolism, and excretion. In addition, methods to upscale their production, and on their application in clinical trials in the near future should be also clinically investigated. The results are expected to offer an unconventional way for a safer and more competent delivery system.

PERSPECTIVES AND CONCLUSION

The development of drug delivery system is an unending demanding scheme that combines multidisciplinary study attempts in different areas. NLCs are binary lipid-based nanocarriers containing blend of both solid lipid and liquid lipid which allow the entrapment of lipophilic actives, protecting them from degradation and improving their stability. They are composed of FDA approved surfactants and biocompatible lipids which makes them safe for use. Components of NLCs should be carefully selected as they will directly influence product stability and effectiveness. The easiness of successful fabrication shifted them into convenient large scale production especially by high pressure homogenization. However, they are extensively used in the last decade in the pharmaceutical and biomedical fields as they gather key points of smart formulation such as high drug payload, capability to target specific sites by surface modification and increased knowledge of the fundamental mechanisms of transport via various routes of administration. Consequently, they can be used for treatment and control of various conditions in different applications. Due to reduced particle degradation and extended GIT residence times after oral administration, NLCs represent supreme contenders for enhancing drug bioavailability, treatment of inflammatory bowel diseases and alleviation of drug induced toxicity. With regard to the cutaneous applications, NLCs provide a convenient carrier for dermal and transdermal drug delivery as they can hydrate skin and mix with skin lipid eventually. For pulmonary application, NLCs present favorable aerosolization characteristics and convenient stability. In addition, they can overcome the resident barriers and accumulate in the lung. When applied to the eye, they offer extended residence time increasing ocular bioavailability of the actives with no/little toxic effects. Although the tenacious barrier shielding the brain, NLCs can attain the brain by surface decoration which in turn can pass BBB by receptor mediated transcytosis. Taking into account the growing number of patent in the last few years, NLCs should have a fair chance for clear clinical translation and pharmaceutical marketing in all applications.

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