Review Article

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Nanostructured lipid carrier system: A compendium of their formulation development approaches, optimization strategies by quality by design, and recent applications in drug delivery

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Abstract: The lipid-based colloidal carriers, such as nanostructured lipid carriers (NLCs), solid lipid nanoparticles (SLNs), nanocapsules, liposomes, and microemulsion, are the latest and significant entrants in the development of drug delivery systems owing to their myriad advantages. The NLCs are second-generation SLNs having unstructured matrix, have high drug loading, and provide long-term drug stability in comparison to SLNs and other colloidal systems, which show lower drug loading and experience burst release/drug expulsion during storage. This review is aimed to summarize the formulation development and optimization strategies for NLCs as reported in the literature collected from authentic databases. Various types of NLCs, formulation components, methods of preparation, characterization parameters, optimization (statistical designs) strategies, toxicity, regulatory aspects, and their applications in oral, parenteral, ocular, pulmonary, nose-to-brain, tumor targeting, and transdermal drug delivery have been dealt in detail. Patents granted on the NLCs have also been enlisted.

Keywords: lipid nanocarriers, nanostructured lipid carriers, optimization, novel drug delivery, statistical designs, quality by design

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1 Introduction

The versatility in the field of nanomedicine is evolving with passing time and extensive investigations are being performed by scientists worldwide. The nano-drug delivery systems are classified as polymeric systems, such as nanocapsules, nanospheres, nanofibres, and nanodiscs, and lipid-based systems, such as liposomes, transferosomes, ethosomes, niosomes, virosomes, phytosomes, micelles, solid lipid nanoparticles (SLNs), and nanostructured lipid carrier (NLC) systems.

Lipid nanoparticles (LNPs) gained attention in the early 1990s when the first-generation SLNs were created [1]. The SLNs created a lot of interest worldwide and quite several research groups started working on SLNs in the first years of their discovery, and to date, SLNs remain an alternative approach to current conventional carriers, such as emulsions, liposomes, and polymeric nanoparticles [2,3]. The SLNs are within the range of submicron (50–1,000 nm) and the appropriate selection of lipids and surfactants will affect particle size (PS), long-term storage stability, drug loading (DL), and release behaviors [4,5]. Their major benefit is the prospect of their large-scale industrial development [6,7]. Possible SLN-related problems, such as restricted DL capacity, change in drug release profile, and expulsion of drugs during storage, are either avoided or minimized using the new-generation NLCs [8].

NLCs are the second generation of SLNs, composed of a binary mixture of solid lipid and liquid lipid, the average size in the range of 10–500 nm [9]. Here, the solid lipid is mixed with the liquid lipids preferably in a ratio of

70:30 up to 99.9:0.1. They contain a special nanostructure, which results in the improvement in DL as well as firm incorporation of the drug inside increasing the storage time. The NLCs can be administered to the patients using oral, topical, intravenous (IV), as well as ocular routes [10]. The advantages associated with NLCs include easy scale-up, drug targeting, controlled drug release profile, longer stability profile, high DL, biocompatible, biodegradable, and nontoxicity [11]. The selection of drugs, lipids (solid and liquid), surfactants, and cryoprotectants is crucial in their development and optimization [12,13].

The quality-by-design (QbD) approach has attracted much attention recently for the optimization of manufacturing variables to achieve safe and effective pharmaceutical formulations. It has been applied successfully in the optimization of SLNs and NLCs for the screening of excipients used and in the study of drug–lipid compatibility. Using the QbD, the quality target product profile (QTPP) and critical quality attributes (CQAs) of the product are identified followed by the evaluation of critical material attributes (CMAs) and critical process parameters (CPPs) to ensure the quality of the finished product [14]. Several mathematical models and statistical tests are available to apply to the parameters that influence the physical characteristics of the nanoformulations. These parameters include the variation in the composition of lipids, emulsifiers, and the manufacturing parameters including time and rate of emulsification, time and amplitude of sonication, pressure, and number of cycles of homogenization, etc. The effect of these critical parameters on the PS, polydispersity index (PDI), zeta potential (ZP), drug encapsulation efficiency (EE), and *in vitro* drug release was studied [14].

This review is aimed to summarize and highlight the preparation and characterization techniques of NLCs along with their applications in the biomedical field. The toxicity profile, recent patents, and the regulatory considerations associated with NLCs are also covered.

2 Types of NLCs

The NLCs can be divided into three types: *imperfect*, *amorphous*, and *multiple oil-in-solid fat-in-water* (O/F/W) based on differences in their lipid and oil composition.

2.1 Imperfect NLCs

The preparation of imperfect NLCs requires the mixing of structurally dissimilar lipids, such as glycerides and fatty acids, creating imperfections in the crystal order.

2.2 Amorphous NLCs

In the amorphous form of NLCs, lipids are combined in such a way that they cannot crystallize and a structureless amorphous matrix is obtained. Special lipids, such as hydroxyoctacosanyl hydroxystearte or isopropyl myristate, are mixed with the solid lipid. Consequently, NLCs exist in an amorphous state instead of the ordered one preventing drug exclusion due to β modifications during its storage.

2.3 Multiple O/F/W NLCs

Generally, the solid matrix of many O/F/W-type NLCs contains distributed nanosized liquid oil compartments, which increases the DL as the drug solubility is increased due to these nanosized compartments [15,16]. Preferentially, these lipid particles are suited for the incorporation of lipophilic drugs and the hydrophilic drugs can only be incorporated in low concentrations. In certain cases, a water-insoluble lipid conjugate is formed from the water-soluble drugs by conjugating it with lipids using covalent linkage or salt formation. These lipid conjugates can be melted and processed to afford the lipid–drug conjugate nanoparticles and these lipidic conjugates can have 30–50% DL for water-soluble drugs [17].

3 Components of NLCs

The major components of NLCs include solid lipids, liquid lipids, surfactants, and surface modifiers [18]. Solid lipids form the solid lipid core of the NLCs and act as the matrix-forming lipids. Various solid lipids along with their melting point and compositions used in the NLC formulations are given in Table 1. On the other hand, liquid lipids (oils) are the lipophilic excipients that are used to integrate the solid lipid core and to reduce its crystallinity [19]. In the preparation of NLCs, two types of oils are used: natural oil or synthetic oil, and most of the drugs are dissolved in synthetic oils [20]. Various types of synthetic and natural oils used in the formulation of NLCs along with their examples are summarized in Table 2.

The entrapment efficiency, stability, drug-loading capacity, and the controlled release behavior of NLC formulations are influenced by their lipid component [4]. These lipids are well-tolerated physiologically and are approved as generally regarded-as-safe (GRAS) compounds. Although there are no specific guidelines as such for their selection, however, the

Table 1: List of solid lipids, their melting points, and compositions used in the NLC formulations

Solid lipids	Melting point (±5°C)	Compositions used
Stearic acid/octadecanoic acid/ cetylacetic acid	69.6	Stearic acid + palmitic acid + small concentration of oleic acid (HLB value = 15)
Glyceryl monostearate (GMS)	57–65	Monoglycerides + diglycerides of fatty acids (HLB value = 3.8)
Glyceryl monostearate polyoxylethylene stearates (Gelot™ 64)	55–62	Mixture of glycerol monostearate and PEG-75 (MW 3,500) stearate (C18) (HLB value = 10)
Carnauba wax	82	Hydroxy acid aliphatic ester + *p*-methoxy cinnamic acid aliphatic ester + *p*-hydroxy cinnamic acid aliphatic diester + oxy-polyhydric alcohol (HLB value = 12)
Cetyl palmitate	54	Ester derived from hexadecanoic acid and hexadecanol (HLB value = 10)
Glyceryl palmitostearate (Precirol® ATO 5)/ glyceryl distearate	50–60	Mono- + di- + triglycerides of C16 and C18 fatty acids (HLB value = 2)
Glyceryl behenate (Compritol® 888 ATO)/ glycerol dibehenate	65–77	Mono- + di- + triesters of behenic acid (HLB value = 2)
Compritol® HD5 ATO/behenoyl polyoxyl-8 glycerides	60–67	Mono- + di- + triglycerides of PEG8 and mono- + diesters of behenic acid (HLB value = 5)
Geleol™ mono- and diglycerides NF/mono- and diglycerides	54–64	Mono- + di- + triesters of palmitic acid (C16) and stearic acid (C18) (HLB value = 3)
Grades of Softisan – it is a mixture of triglycerides graded based on even-numbered, saturated, and non-branched natural fatty acids of chain length C8–C18 and are of vegetable origin		
Softisan 100	33–35	Hydrogenated co-glycerides + mixture of C10–C18 fatty acid triglycerides
Softisan 154	53–58	Hydrogenated palm oil + mixture of C10–C18 fatty acid triglycerides
Softisan 142	42–44	Mixture of C10–C18 fatty acid triglycerides of
Softisan 138	NA	Mixture of C10–C18 fatty acid triglycerides and diglycerides
Softisan 645	NA	Mixture of diglycerol with caprylic, isostearic, capric, stearic, and adipic acid
Softisan 378	38	Mixture of caprylic/capric/myristic/stearic triglycerides
Softisan 601	40–45	Hydrogenated coconut oil + ceteareth-25 + triglycerides (glyceryl cocoate)
Softisan 649	35	Mixture of diglycerol, caprylic, isostearic, capric, stearic acid, hydroxystearic, and adipic acid
Grades of Witepsol® – synthetic hard wax, available in different series		
Witepsol®H (5,12,15,19,32,35,37)	31–38	Mixture of triglycerides + diglycerides (15%) + monoglycerides (1%) with low hydroxy value
Witepsol® W (32,25,35,45)	32–35.5	Mixture of triglycerides (65–80%) + diglycerides (10–35%) + monoglycerides (1–5%) with high hydroxy value
Witepsol® S (51,55,58)	30–35.5	Non-ionic ethoxylated emulsifier
Witepsol® E (75,76,85)	37–44	Series E are hard fats having melting point more than the body temperature
Imwitor grades		
Imwitor 372P	62	Glyceryl stearate citrate, HLB = 12
Imwitor 491	66–77	Mixture of GMS and monoglycerides with HLB value 4
Imwitor 900K	61	Glycerol monostearate (40–55%, type I), HLB = 3
Imwitor 928	34	Mixture of glyceryl cocoate and medium-chain partial glycerides
Dynasan grades		
Dynasan® 116	63–68	Tripalmitin
Dynasan® 118	72	Glyceryl tristearate/triglycerides
Dynasan® 114	55–58	Solid triglycerides (triglycerides/trimyristin)
Others		
Lauric acid (dodecanoic acid)	43.2	Mixture of saturated fatty acid with a 12-carbon atom chain
Apifil® CG/PEG-8 Beeswax	59–70	It is a polar beeswax derivative by esterification of beeswax with polyethylene glycol (PEG). PEG group imparts hydrophilic properties to beeswax.

(*Continued*)

Table 1: *Continued*

Solid lipids	Melting point (±5°C)	Compositions used
Tefose® 63	49	Mixture of ethylene glycol palmitostearate + PEG-6 palmitostearate + PEG-32 palmitostearate (HLB value = 9.5)
Tristearin/glyceryl tristearate	54–72	It is derived from three units of stearic acid

drug solubility in lipids is one of the criteria to select an appropriate lipid. The third component surfactant is often a polymeric material that is used to provide stability to the lipid crystal suspension, maintain uniform PS distribution, and prevent particle growth. Various properties of NLCs, such as the viscosity and water-solubilizing capacity, are determined by the surfactants and they play a crucial role in the formulation development. They also prevent the agglomeration and dispersibility of the particles and provide stability to the formulation.

Surfactants are amphiphiles that reduce the surface tension and therefore facilitate the partition of particles between the two phases. The hydrophilic head of the surfactants remains oriented toward the aqueous phase and the lipophilic groups get oriented toward the lipid [21]. Surfactants are selected based on the chosen lipid component as they should be physico-chemically compatible with each other. Other factors that determine the selection of surfactants include the route of administration and the hydrophilic–lipophilic balance (HLB) value of the surfactant. Another compound, known as the co-surfactant, is used to further decrease the interfacial tension and to enhance the drug solubility in SLNs/NLCs to a value lesser than that of the surfactants alone. A few examples of surfactants and co-surfactants used in the preparation of NLCs are listed in Table 3 [22].

Table 2: Synthetic and natural oils used for the formulation of NLCs and their examples

Types of oil used	Examples
Synthetic oils	
Medium-chain mono- and diglycerides of caprylic/capric acid	Capmul MCM Imwitor
Propylene glycol (PG) fatty acid esters including PG monolaurate	Lauroglycol FCC Capmul PG-12 Lauroglycol 90
PG diester of caprylic/capric acid	Labrafac PG
PG dicaprylate	Miglyol 840
Medium-chain triglycerides and their esters including capric/caprylic triglycerides	Akomed R, Akomed E, Miglyol 810, Captex 355, Crodamol GTCC, Neobee M5
Fractionated coconut oil	Miglyol 812, Triacetin, Labrafac CC, Captex 300
Long-chain monoglycerides, such as glyceryl monolinoleate	Maisine 35
Glyceryl monooleate	Peceol, Capmul GMO
Capric/caprylic/diglyceryl succinate	Miglyol 829
Fatty acids, such as oleic acid and caprylic acid	Crossential O94
Fatty acid esters	Ethyl butyrate, ethyl oleate (Cardamol EO), isopropyl myristate, ethyl butyrate, isopropyl palmitate
Mineral oil	Liquid paraffin
Vitamins	Vitamin E/α-tocopherol
Diethylene glycol monoethyl ether	Transcutol HP
Natural oils	
Fixed oils	Sunflower oil, shark liver oil, palm oil, sesame oil, olive oil, rice bran oil, margosa oil, mustard oil, jojoba oil, cod liver oil, cottonseed oil, arachis/peanut oil, castor oil, soyabean oil, chaulmoogra oil
Essential oils	Pumpkin seed oil, lemon grass oil, cinnamon oil, peppermint oil, citronella oil, lavender oil, clove oil, garlic oil, geranium oil

Table 3: Various surfactants, co-surfactants, and surface modifiers used in the formulation of NLCs

Agents	Examples
Surfactants/co-surfactants	Soy lecithin (Lipoid S 75, Lipoid S 100), phosphatidyl choline 95% (Epikuron 200), Poloxamer-188 (Pluronic F-68), egg lecithin (Lipoid E 80), Cremophor EL, Poloxamer 407, lecithin, Poloxamine 908, Solutol HS 15, Tyloxapol, Polysorbate 20 (Tween® 20), Polysorbate 60 (Tween® 60), Polysorbate 80 (Tween® 80), sodium cholate, taurodeoxycholic acid sodium, sodium glycocholate, butyric acid and butanol, sodium dodecyl sulfate, cetylpyridinium chloride, polyvinyl alcohol, and sodium oleate
Surface modifiers	Folate, PEG 2000, biotin, ferritin, transferrin, β-D-galactosides, mannose, L-arginine, oligo-chitosan, hyaluronic acid, wheat germ agglutinin

Another important component of NLCs includes the surface modifiers, which are the ingredients that add special properties to the formulation and influence the *in vivo* effects of NLCs. Several surface modifiers are used to modify the NLCs and a few of them are listed in Table 3. These surface modifiers may be attached with some linkage, such as 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-PEG, succinic acid-PEG 2000, folate-PEG-Chol, and mannan-PEG-phosphatidylethanolamine. The surface modifications of SLNs are generally inspired by the methods used for the functionalization of liposomes. Special functionalized lipids are included in the SLNs/NLCs' formulation using the post-insertion method. Another feasible approach to modify the surface of SLNs/NLCs is by linking the coating agent used in the formulation to an excipient, which is an integral part of the formulation. This is done by using *N*-hydroxy succinimide/1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide chemistry forming covalent bonds and is a well-validated approach in designing polymeric particles systems [23,24].

4 Methods of preparation of NLCs

Techniques used in the preparation of SLNs can also be used for the preparation of NLCs [25]. These preparation techniques are divided into three major categories: high-energy approaches, low-energy approaches, and approaches with organic solvents. Various critical aspects in the formulation development of NLCs are discussed henceforth [26] and are depicted in Figure 1. Various advantages and disadvantages are associated with different techniques and the selection of appropriate methods for the preparation of NLCs depends upon the characteristics of the drug and the PS required. For instance, the high-pressure homogenization (HPH) technique utilizes both high pressure and high temperature and is the most commonly employed method for NLCs' preparation. It has the advantage of utilizing high temperature, which decreases the viscosity of mixed liquids and thereby reduces the PS; however, the disadvantage associated with this method is the probability of degradation of drug and carrier due to higher temperature [27]. Therefore, this method is more suitable for highly lipophilic and insoluble drugs and not for hydrophilic ones. Other advantages of this technique are the avoidance of organic solvents and the scale-up suitability. On the other hand, the emulsion evaporation technique includes evaporation of the oil phase by heating under reduced pressure; therefore, the advantage of this technique is the avoidance of heat; however, the major disadvantage is the use of solvents, which may lead to toxicity due to solvent residues. Similarly, the advantages of the solvent dispersion technique include speed, simplicity, and avoidance of major instruments; however, this method is not suitable for the preparation of NLCs at an industrial scale and also is associated with chances of solvent residue in the final product. The microemulsion technique has the advantage of the usage of a small amount of drug and simplicity; however, it requires high concentrations of emulsifiers.

4.1 High-energy approaches

4.1.1 HPH technique

The HPH technique remains one of the most preferred methods owing to the shorter production time in comparison to other techniques, easier scale-up, and lack of solvents [27]. HPH methods can be divided into hot HPH and cold HPH protocols [28]. The hot HPH process includes melting solid lipids first followed by mixing them with liquid lipids and drugs. This dispersion is then mixed with hot surfactant solution in water resulting in a pre-emulsion, which upon homogenization at up to three cycles at 500 bar at high temperature gives SLNs/NLCs as the end desired product. Generally, the high temperature reduces the PS by decreasing the viscosity of lipids; however, the chances of drug or carrier system degradation increase. Whereas, in cold HPH, the lipid blend is melted and mixed to form lipid microparticle dispersion, which is then mixed with a cold surfactant

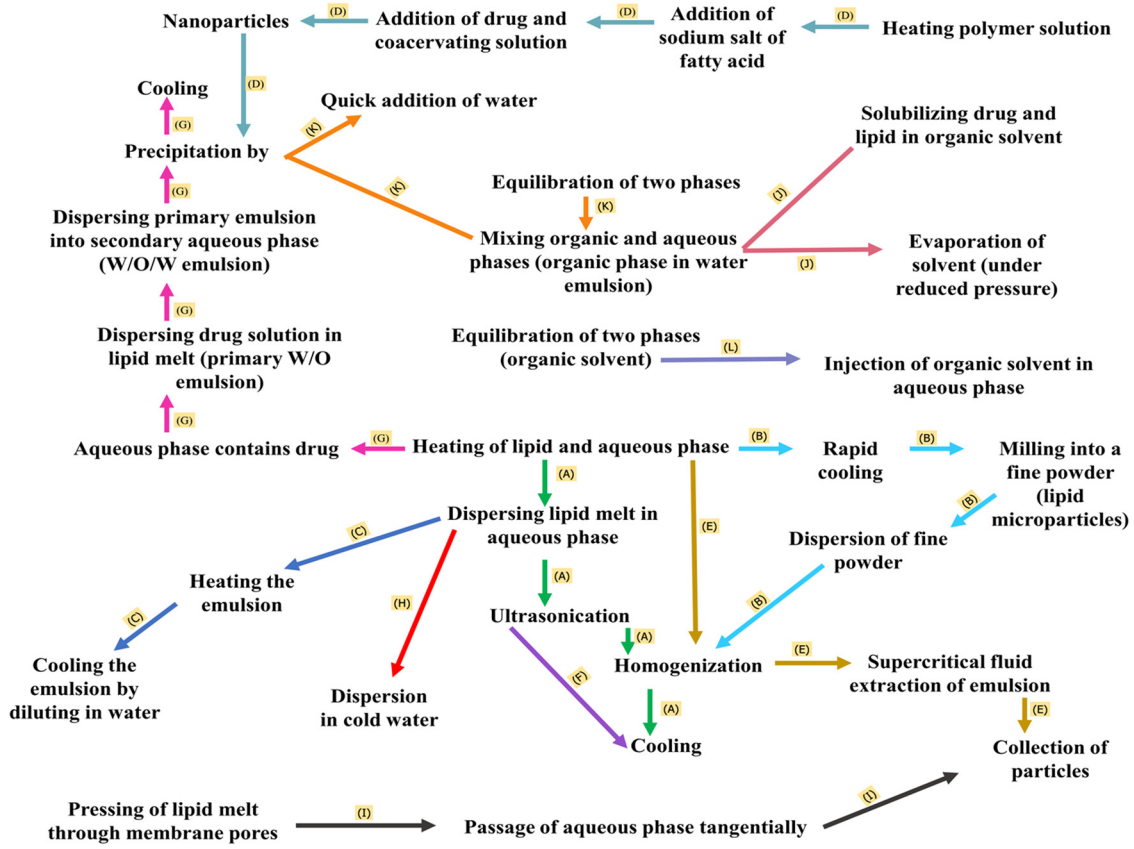

Figure 1: Methods of preparation of NLCs. (a) Hot homogenization technique, (b) cold homogenization technique, (c) phase inversion temperature (PIT) technique, (d) coacervation technique, (e) supercritical fluid (SCF) technique, (f) high shear homogenization/ultrasonication technique, (g) double emulsion technique, (h) microemulsion technique, (i) membrane contactor technique, (j) emulsification–solvent evaporation technique, (k) emulsification solvent diffusion technique, and (l) solvent injection technique.

solution to form a pre-suspension, which upon homogenization at up to 5–10 cycles at 1,500 bar at room temperature gives SLNs/NLCs as the end product.

4.1.2 Melt emulsification homogenization technique

In this technique, the solid lipid, the liquid lipid, and the drug are mixed and dispersed into the aqueous surfactant solution using the probe sonication method. The mixture is then cooled to a low temperature to afford solid NLCs. The important advantage associated with this technique is the avoidance of heat.

4.2 Low-energy approaches

4.2.1 Microemulsion technique

In this technique, the lipid carrier is melted just above its melting point followed by the addition of a drug, auxiliary emulsifier, and deionized water (preheated to the same temperature) to yield a transparent mixture with thermodynamic stability similar to oil-in-water type microemulsion. The obtained mixture is dispersed immediately in ice-cold water (0–4°C) with gentle mechanical stirring forming dispersion of NLCs. The temperature difference between the microemulsion and cold water is the key factor in determining the size of nanoparticles as the rapid cooling prevents the aggregation of these particles resulting in smaller size NLCs. While method simplicity is its advantage, the abundant use of emulsifiers and auxiliary emulsifiers is its disadvantages.

4.2.2 Membrane contractor technique

Lipid is transported through the pores of a membrane at a pressure that keeps the system above the melting temperature of the lipid, resulting in the creation of tiny droplets. The aqueous phase, on the other hand, is cycled within the membrane, and droplets generated at the

holes are swept along with it. When the preparation is cooled to room temperature, LNPs are formed. The size and lipid influx of LNPs are influenced by aqueous phase flow velocity, lipid and aqueous phase temperature, membrane pore size, and lipid phase pressure.

4.2.3 Phase-inversion temperature technique

The phase inversion from O/W to W/O emulsion is used in this process, which is a unique, cost-effective, and solvent-free method for the synthesis of LNPs. Step 1 comprises optimizing the quantities of lipid, surfactant, and water, followed by a 4°C increase in temperature from room temperature to 85°C. To achieve the phase inversion zone, the system is subjected to three temperature cycles. The addition of cold water (0°C) in step 2 produces an irreversible shock to the system, causing nanocapsule production.

4.2.4 Coacervation technique

This is a revolutionary solvent-free approach that can even include thermosensitive pharmaceuticals without the use of expensive equipment or hazardous solvents. In the presence of an appropriate amphiphilic polymeric stabilizing agent, a Micellar solution of a fatty acid sodium salt interacts slowly with an acid solution (coacervating solution).

4.2.5 Double emulsification technique

This method, which is based on the solvent emulsification–evaporation process, is mostly used to make LNPs that are loaded with hydrophilic medicines. The medication and the stabilizer are enclosed in the W/O/W double emulsion's inner aqueous phase. Due to their higher PS than SLNs, these formulations are referred to as lipospheres.

4.3 Approaches with organic solvents

4.3.1 Solvent emulsification evaporation technique

The lipid is dissolved in a water-immiscible organic solvent, such as cyclohexane or chloroform, and then emulsified with continuous stirring in an aqueous phase containing surfactants, resulting in an O/W emulsion. Solvent evaporation and lipid precipitation result from evaporation at decreasing pressure. Although the approach eliminates heat stress, it does have one drawback: the use of an organic solvent.

4.3.2 Emulsification solvent diffusion technique

This approach is similar to the "solvent emulsification–evaporation" method, except that the lipid is dissolved in a somewhat water-miscible organic solvent, such as benzyl alcohol or ethyl formate. The transitory oil-in-water emulsion is poured into the water with constant stirring, causing the dispersed phase to solidify and form LNPs due to organic solvent diffusion. The lipid solidifies as the solvent diffuses to the liquid phase.

4.3.3 Evaporation solvent injection technique

A water-miscible solvent, such as acetone, ethanol, methanol or isopropyl alcohol, or a water-soluble solvent mixture, is used to solubilize lipid, which is quickly injected into an aqueous surfactant solution while being stirred constantly. Excess fat is removed from the resulting dispersion by filtering. This approach has the advantages of being simple to use, efficient, versatile, requiring no specialized equipment (such as a high-pressure homogenizer), and using authorized organic solvents.

4.3.4 Supercritical fluid technique

This technique involves solubilization of the lipid material with the drug in an organic solvent, such as chloroform by adding a suitable surfactant resulting in an organic solution. The organic solution is disseminated in the aqueous phase (that may or may not contain a co-surfactant) and the combination is then homogenized at high pressure to generate an O/W emulsion. The O/W emulsion is injected at a constant flow rate from one end of the extraction column (typically the top), and the supercritical fluid (kept at constant temperature and pressure) is introduced at a constant flow rate counter currently. Continuous extraction of solvent from the O/W emulsions is used for the formulation of LNP dispersions.

5 Lyophilization of NLCs

Lyophilization (freeze-drying) method has been the technique to stabilize the NLCs. One of the purposes of

lyophilization is to prolong the product storage duration and preserve it from chemical and physical degradation. Therefore, a solid-state material is easy to obtain using the lyophilization technique and it would readily re-disperse when required. Lyophilization, in general, is based on the sublimation principle (dehydration process) where the water goes straight from a solid state (ice) to a gaseous state without going through a liquid state [29]. During the lyophilization process, cryoprotectants or lyoprotectants are used as stabilizers, which protect the formulation during freeze-drying (cryoprotectant) against the freezing and drying stresses (lyoprotectant). To protect the product from the high stress created during the lyophilization process, cryoprotectants, such as mannitol, trehalose, fructose, sorbitol, lactose, glucose, sucrose, and aerosil, are added in 5–15% w/w concentration [30].

It is the most dependable drying procedure since it does not alter the product's molecular structure. Cryoprotectants are also known to vitrify at a certain temperature, Tg. Nanoparticles can be immobilized inside a glassy cryoprotectant matrix to avoid the aggregation and protect them from the mechanical stress of ice crystals. Cryoprotectants not only improve the long-term stability of NLCs during storage, but they also serve as bulking agents (which add bulk to the formulation, especially when the product concentration to freeze dry is low) and tonicity adjusters [31]. Surfactant-stabilized NLCs created by an appropriate approach might retain good stability. However, in aqueous solutions, it can have poor chemical and physical stability.

During storage, however, aggregation or other unforeseeable adverse effects may develop. As a result, boosting the stability of NLCs and maintaining their physical qualities is increasingly important. Cryoprotectants were used to lyophilize the NLC solution and protect it against agglomeration during storage, according to many investigations. Tilmicosin NLC suspension was lyophilized using mannitol as a cryoprotectant after being produced by heat homogenization with Compritol 888 ATO, sesame oil, Poloxamer 407, and Tween® 80. The lyophilized products were shown to be more stable over a longer period [32]. NLCs containing lopinavir (LPV) and verapamil were produced utilizing Compritol 888 ATO® and oleic acid as solid and liquid lipids using a hot high-shear homogenization technique. PS and PDI were used to screen many cryoprotectants, including mannitol, sorbitol, sucrose, and trehalose. Trehalose was shown to be superior at preventing LPV-NLC aggregation and improving stability throughout the freeze-drying process. Trehalose has several benefits in comparison to cryoprotectants, such as lesser chemical interactions and a higher glass-transition temperature, both of which may aid nanoparticle stability [33,34]. Rifabutin (RFB)-NLCs for the treatment of tuberculosis were prepared with the help of miglyol-812 (liquid lipid) and Precirol® ATO 5 (solid lipid) *via* high-shear homogenization and ultrasonication techniques. Aerosil was used as a cryoprotectant and made a stable formulation at 2% w/w of its concentration [35]. Olmesartan NLCs prepared by Precirol ATO 5 and Capmul MCM were lyophilized by mannitol as it resulted in the formation of voluminous, easy to redisperse cake with redispersibility index close to one, and ultimately their long-term stability [36]. Mannitol as a cryoprotectant for the preparation of exemestane NLCs using ultrasonication technique is also reported. Flaxseed oil was used as liquid lipid and Precirol ATO 5 was used as solid lipid for the preparation of the NLCs. NLCs' lyophilized formulation showed better stability as compared to their suspension throughout the storage period [37].

6 Characterization of NLCs

Owing to the tiny size of the particles and the intricacy of the system, the characterization of NLCs can be challenging. PS and size distribution, ZP, surface charge, particle morphology, polymorphism, thermal behavior of lipids, degree of crystallinity, and lipid modification are relevant parameters to be considered [38]. The PS and size distribution of NLCs are the most essential characteristics that determine their stability, solubility, release rate, and *in vivo* performance; hence, their accurate measurement is critical [15]. Photon correlation spectroscopy (PCS) and laser diffraction are the most promising and extensively used methods for the PS assessment of LNPs [39,40]. NLCs are generally polydispersed in nature; therefore, the measurement of PDI is vital to understand the size distribution of the nanoparticles. The nanoparticle dispersion is considered to be more monodispersed when the PDI value is low. The majority of studies consider a PDI value of less than 0.3 to be optimal [41,42]. PCS using a Zetasizer NanoZS at 25°C may be used to detect PDI and ZP [43]. The second most essential characteristic parameter is DL and EE, which may be assessed by calculating the amount of drug encapsulated within the nanoparticles. In a nutshell, NLC dispersion is placed in a centrifugal tube and centrifuged for 30 min at 5,000 rpm, after which the quantity of drug in the aqueous phase is evaluated using HPLC [44–46]. The following formulae can be used to compute drug EE and DL:

$$\text{EE}\ (\%) = \frac{\text{Actual amount of drug in the filtered formulation} - \text{Soluble unencapsulated drug}}{\text{Amount of drug added during formulation}} \times 100,$$

$$\text{L}\ (\%) = \frac{\text{Actual amount of encapsulated drug}}{\text{Amount of lipid used to prepare the formulation}} \times 100.$$

Because these factors are significantly connected with drug incorporation and release rates, determining the degree of lipid crystallinity and the alteration of the lipid are also key components of characterizing NLCs. Lipid crystallization and modification modifications may be greatly slowed due to the tiny size of the particles and the presence of emulsifiers. To assess the state of lipids, differential scanning calorimetry (DSC) and X-ray scattering are commonly utilized [47–49]. Varied lipid changes have different melting temperatures and melting enthalpies, which DSC makes use of. The length of the long and short spacing of the lipid lattice may be measured using X-ray scattering. Infrared and Raman spectroscopy are important methods for studying lipid structural characteristics, but their use in characterizing NLC dispersions has yet to be determined [50–54].

7 Optimization of NLCs

Experimental design *via* the QbD system helps develop a design space statistically, which offers approaches to address the formulation-related problems and it is helpful in improving and validating formulation for better efficacy and patient care [55]. In QbD, we combine experimental data and mechanistic knowledge to predict performance, as it allows us to achieve optimal product design by balancing high-risk and medium-risk to low-risk variables. ICH-Q8 (pharmaceutical development), ICH-Q9 (quality-risk management), ICH-Q10 (pharmaceutical quality system), and ICH-Q11 (development and production of pharmacological substances) are some of the ICH recommendations that focus on the conceptual views of QbD and give some support to manufacturers [56–59]. The adoption of the QbD approach in the formulation optimization process has led to statistically designed experiments (DOEs) for determining the impact of multiple parameters and their interactions on the product profile. In the process of optimizing NLCs *via* the QbD approach, much importance is given to develop a relationship between process variables and the method and it involves multiple steps, such as defining the purpose and objectives, QTPP, identifying CMAs, screening of CPPs, identifying working design space, and analyzing CQAs.

At this stage, the QTPP must be well defined through research literature and field experience systematically assessed under the quality risk management strategy, which helps to reduce time and waste of resources. For the NLCs' preparation, QTTP is set for the improvement of its biopharmaceutical performance to provide therapeutic benefits to the patients. CMAs and CPPs are referred to as independent variables defined by simulation and specific statistical techniques [60]. NLC formulations optimized *via* basic knowledge of these process parameters with the aid of appropriate experimental design are a must otherwise undesirable outcomes will be achieved (Figure 2). For the nanolipid carrier system, CMAs include API composition, solid lipid concentration, liquid lipid concentration, excipient ratio, surfactant concentration, drug–lipid ratio, types of solvent ratio, polymer concentration, the ratio of a binary mixture, *etc*. CPPs may include stirring time, homogenization time, preparation technique, temperature, sonication time, lipid type, *etc*. CQAs are PS distribution, phase separation, EE, polydispersibility index, ZP, pH, *in vitro* release, dissolution efficiency, DL, and enthalpy. Table 4 shows all the NLC formulations optimized by various statistical designs along with detailed information for the researchers.

8 Applications of NLCs

The NLCs can be used in a wide variety of drug delivery systems by different routes including oral, transdermal, ocular, pulmonary, and IV delivery systems. Some of the pharmaceutical applications of NLCs are summarized in Figure 3.

8.1 Oral drug delivery

Drug delivery through the oral route is the well-received and cost-effective way of drug administration, with the greatest patient adherence. However, high hepatic first-pass effect, restricted drug solubility and/or efflux pumps of P-glycoprotein (P-gp), and enzymatic and chemical

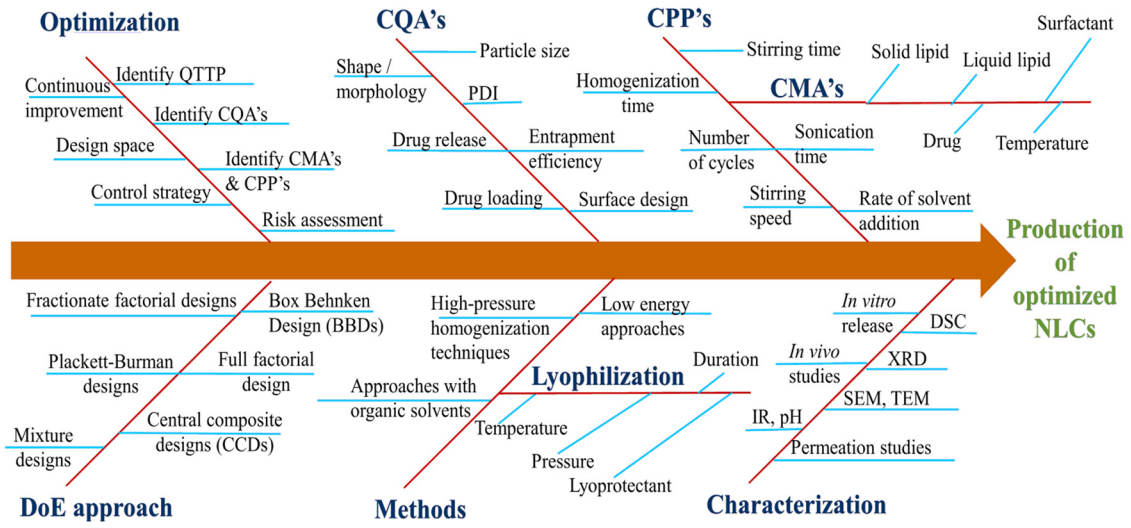

Figure 2: Ishikawa design for production of optimized NLCs.

deterioration all contribute to low oral bioavailability, which must be overcome [93,94]. LNPs, including NLCs and SLNs, have the benefit of being able to maintain steady plasma levels due to their potential for sustained drug release. Because of their larger specific surface area and saturation solubility, they dissolve quickly, hastening the start of pharmacological action. P-gp efflux pumps can be inhibited by certain lipids and surfactants employed in the synthesis of these LNPs. Drugs are less likely to be degraded chemically or enzymatically because they are incorporated in the lipid matrix [95]. According to the diagrammatic representation in Figure 4, lymphatic transport can be increased by LNPs and can bypass the liver, avoiding the hepatic first-pass effect. As a result, adding bioactives into NLCs may aid in improving therapeutic effectiveness and extending release time from these nanocarriers [96].

Piazzini *et al.* reported the synthesis of silymarin (SLM) NLCs for optimal oral absorption and *in vivo* effectiveness in a type 2 diabetes and metabolic syndrome model. Through energy-dependent processes, the formulations were successful in boosting transport through the Caco-2 cell layer. SLM-NLC formulation showed a considerable reduction in blood glucose and lipid levels when compared to free SLM [98]. To improve the oral bioavailability and extend the action and effectiveness of the anti-hyperlipidemic medication simvastatin (SIM), NLCs were synthesized using an emulsification–solvent evaporation method followed by ultrasonication. In contrast to SIM suspension, a single dosage of SIM-NLC resulted in a 4-fold increase in bioavailability, making NLCs a viable drug delivery method in the control of hyperlipidemia [99]. According to other researchers, atorvastatin (AT)-loaded NLCs released more drugs than the drug solution. In comparison to AT suspension and Lipitor, the oral bioavailability of NLC formulation (NLC-1) rose by 3.6 and 2.1 times, respectively. The nanosized formulation, which may have enhanced lymphatic absorption, was implicated in the high plasma levels of AT from NLCs [100].

NLCs of the poorly soluble drug raloxifene hydrochloride (RLX) were prepared in a study using a hot homogenization procedure followed by an ultrasonication technique. In female Wistar rats, high entrapment efficiency of over 90%, sustained drug release, and a 3.19-fold increase in bioavailability of RLX-NLC were indicated when compared to a plain drug suspension, indicating its possibilities as a potential carrier for RLX oral delivery in osteoporosis treatment [101]. Likewise, for the oral administration of docetaxel (DTX), cysteine-modified NLCs (cNLCs) were synthesized and evaluated against unmodified NLCs. In total intestinal segments, the intestinal absorption of cNLCs was significantly enhanced as compared to unchanged NLCs and DTX solution, which can be related to changed cNLCs' increased mucoadhesion capabilities [102]. Finally, the emulsion–evaporation and low temperature–solidification approaches were used to effectively manufacture baicalin-loaded NLCs (BA-NLCs). When compared to BA suspension, BA-NLCs could significantly enhance baicalin bioavailability following oral administration (1.9-fold) [103].

8.2 IV drug delivery

The IV method is typically used for medications that are unable to absorb through the digestive tract or injected

Table 4: Statistical designs applied to NLCs' formulation development and optimization

Drug-loaded NLCs with technique	Lipids used (solid lipid:liquid lipid)	Independent variables applied	Dependent variables applied	No. runs	Characterization parameters	Delivery route	Important conclusions of the study
Central composite designs are among the most commonly used optimization designs as they have five levels of each independent variable with a limited number of required experiments							
Gypenosides–sodium glycocholate-NLCs/hot melting, HPH method [61]	GMS: Labrafil M1944 CS and Maisine 35-1	Total lipid concentration (%), ratio of liquid lipid to total lipid (%), surfactant concentration (%)	PS, encapsulation efficiency (EE), DL	20	DSC, TEM, *in vitro* dissolution, XRD, accelerated stability studies. *in vivo* imaging, pharmacokinetics, and *in situ* intestinal perfusion study	Oral	Improved oral absorption of drugs
Atazanavir NLCs/emulsification–HPH process [62]	—	CMAs and CPPs	PS, PDI, ZP	—	DSC, powder X-ray diffraction, FT-IR analysis	Lymph targeting	Improved drug bioavailability and avoiding first-pass metabolism
Curcumin-NLCs/ultrasonication method [63]	Compritol ATO 888: Olive oil	Liquid lipid concentration, vitamin E tocopheryl polyethylene glycol succinate (TPGS), Poloxamer 188	Speed, sonication time	15	Photon correlation microscopy, TEM, *in vitro* transcorneal release, corneal hydration, corneal histology, stability studies	Ocular	Enhanced the curcumin permeation across corneas
Exemestane-NLCs/ultrasonication technique [64]	Precirol® ATO 5: flaxseed oil	Sonication time (min), weight of solid lipid (mg), weight of surfactant (mg)	PS, EE, PDI	20	SEM, TEM, DSC, XRD, *in vitro* release, DL, *ex vivo* gut permeation study, confocal laser scanning microscopy (CLSM)	Oral	Promising approach for the management of breast cancer
Dihydroartemisinin-NLCs/solvent diffusion method [65]	GMS:Miglyol 812N	Drug concentration (%), lipid concentration (%), ratio of liquid lipid to total lipid	PS, DL, EE	20	ZP, PDI, stability study, TEM, *in vitro* release	Parenteral	Prolonged plasma level of drug
Beta-carotene-NLCs/solvent diffusion method [66]	Palmitic acid:corn oil	Liquid lipid-to-total lipid ratio (%), lipid phase concentration, surfactant concentration (%), aqueous phase temperature	PS and b-carotene degradation	30	TEM	Oral	Enhanced bioavailability
Nepafenac-NLCs/melt-emulsification and ultra-sonication techniques [67]	GMS:Miglyol 812N	Drug concentration (%), Miglyol 812N concentration (%), ratio of Cremophor EL/soy lecithin	PS, PDI, EE	20	Morphological studies, DSC, *in vitro* release cellular uptake, cell cytotoxicity assay on human corneal epithelial cells (HCECs)	Occular	Promising approach for the management of inflammation

(*Continued*)

Table 4: *Continued*

Drug-loaded NLCs with technique	Lipids used (solid lipid:liquid lipid)	Independent variables applied	Dependent variables applied	No. runs	Characterization parameters	Delivery route	Important conclusions of the study
Econazole nitrate-NLCs/solvent injection method [68]	Precirol ATO 5:oleic acid	Solid lipid concentration (5), liquid lipid concentration (%), Poloxamer 407 (% w/v)	PS, PDI, EE, and ZP	15	*In vivo* permeation study by CLSM, recrystallization index, TEM, stability study	Topical	Effective delivery for deep-seated fungal infection
Linalool-NLCs/HPH [69]	GMS:decanoyl/octanoyl-glycerides	Solid lipid (%), liquid lipid concentration, Span 80, Tween® 80	PS	30	ZP, TEM, XRD, DSC, and *in vitro* release study	Oral	Sustained release and enhanced the bioavailability of the drug
Ifosfamide-NLCs/solvent diffusion technique [70]	GMS:oleic acid	Drug/lipid ratio, organic/aqueous phase ratio, surfactant concentration	PS, DL, EE	20	TEM, DL, EE, ZP, DSC, FT-IR, XRD, *in vitro* release, stability studies	Oral	Successfully developed NLCs with high entrapment efficiency and stability
Box Behnken design requires three stages of each variable and all combinations of treatments include at most one midpoint of space design edges and the preparation of NLC-loaded drugs showed that it is a suitable instrument establishing the relationship among factors and expected attributes							
Nobiletin-NLCs/melt-emulsification technique and HPH [71]	Geleol mono- and bi-glycerides NF:Labrafac WL 1349	Emulsifier-to-lipid ratio, lecithin-to-Poloxamer 188 ratio, and liquid lipid-to-solid lipid ratio	PS, EE	17	FT-IR, DSC, XRD, PDI, ZP	Oral	Enhanced bioavailability
Glibenclamide-NLCs/emulsion-solvent diffusion and evaporation method [72]	GMS:Capryol 90	Surfactant (Tween® 80), total lipid, liquid lipid/ total lipid	PS, EE, DL	17	Zeta potential, DSC, TEM, *in vitro* skin permeation study, CLSM	Topical	Enhanced bioavailability and better permeation of drug through rat skin
UcuubaNLCs/emulsification, homogenization and solidification technique [73]	Ucuuba fat: Capryol 90	Surfactant concentration (% w/v), liquid lipid concentration (% w/v), solid lipid concentration (% w/v)	PS, PDI, EE	17	ZP, TEM, *in vitro* release, DSC, XRD, FT-IR	Topical	Successfully developed NLCs and showed antifungal efficacy against onychomycosis
Lurasidone hydrochloride-NLCs/solvent evaporation method [74]	Gelot64:Capryol 90	Total lipid (%), surfactant (%), sonication time (min)	PS, EE, drug release	15	PDI, TEM, SEM, DSC, *in vitro* release, *in vivo* pharmacokinetic and stability study	Intranasal	Promising approach for the management of schizophrenia through intranasal route
Ticagrelor-NLCs/hot melt emulsification ultrasonication method [75]	GMS:Capmul MCM	Total lipid amount, liquid lipid/total lipid, surfactant (%)	PS, PDI, EE	17	Cytotoxicity, cellular uptake, pharmacokinetic study, pharmacodynamic study	Oral	Promising approach for antiplatelet activity and improved oral bioavailability

(*Continued*)

Table 4: *Continued*

Drug-loaded NLCs with technique	Lipids used (solid lipid:liquid lipid)	Independent variables applied	Dependent variables applied	No. runs	Characterization parameters	Delivery route	Important conclusions of the study
Triamcinolone Acetonide-NLCs/hot homogenization method [76]	Spermaceti:soybean oil	Solid lipid (g), liquid lipid (g), Tween® 80 (g)	PS, EE, ZP	17	Field emission scanning electron microscope, energy-dispersive X-ray spectroscopy, *in vitro* release, *in vitro* permeation studies	Buccal	Promising efficiency approach for the buccal drug delivery system
Salicylic acid-NLCs/emulsification method using high-speed homogenization [77]	Stearic acid:Lexol GT-865 (mixture capric/caprylic triglyceride)	Total lipid concentration (%), solid lipid-to-liquid lipid ratio, surfactant concentration (%)	PS	17	SEM, DSC, EE, PDI, ZP	Oral	Reduced side effect and improved efficiency of salicylic acid
Luliconazole-NLCs/sonication technique [78]	NLC (lipids) hydrogel excipients	Lipid content, surfactant concentration, and sonication time	PS, % EE	17	Occlusivity, spreadability, and extrudability	Topical	Increased antifungal activity of the drug, increased patient compliance by reducing the frequency of application
20(S)-Protopanaxadiol-NLCs/melt emulsification method [79]	Cetylpalmitate:Miglyol 812N	Drug concentration, volume of the liquid lipid, and the amount of the surfactant	PS, PDI, EE	15	TEM, XRD, *in vitro* deposition of the drug into human cadaver skin, *in vivo* human skin irritation study	Topical	NLC formulation did not cause any skin irritation, it could be used to enhance the topical skin deposition of 20(*S*)-Protopanaxadiol
Fenofibrate NLCs/hot homogenization – ultrasonication [80]	Solid: liquid lipid	Drug concentration, ratio of solid lipid/liquid lipid, and percentage of emulsifier	PS, % EE	17	DSC, *in vitro* release, *in vivo* studies	Oral	Enhanced dissolution and bioavailability of fenofibrate
Rivastigmine-NLCs/ HPH technique [81]	GMS:castor oil	Solid to liquid lipid, ratio of Tween® 80 and span 80, number of HPH cycles	PS, PDI, Z, EE	17	ATR, TEM, DSC, *in vitro* release, skin irritation study, pharmacokinetic study	Topical	Promising approach for the dementia management
Butenafine HCl-NLCs/HPH method [82]	Compritol 888 ATO 5:Labrasol	Liquid content, surfactant concentration (Tween® 80), and homogenization cycle	PS, EE	17	*In vitro* drug release studies, *ex vivo* drug permeation study	Topical	Enhanced bioavailability of the drug in the treatment of fungal infections

(*Continued*)

Table 4: *Continued*

Drug-loaded NLCs with technique	Lipids used (solid lipid:liquid lipid)	Independent variables applied	Dependent variables applied	No. runs	Characterization parameters	Delivery route	Important conclusions of the study
Olmesartan minoxidil-NLCs/ homogenization method [83]	Precirol® ATO 5: Capmul MCM	Amount of liquid lipids (mg), amount of total lipids (mg), drug concentration (mg), surfactant concentration (%)	PS, PDI, EE	30	DSC, XRD, SEM, TEM, *in vitro* drug release, cellular uptake, pharmacokinetics study	Oral	Enhanced oral bioavailability of the drug
Full factorial three-level statistical design involves two or three input factors with more experiments are needed							
Carvedilol-NLCs/HPH method [84]	GMS:oleic acid	Total lipid concentration, liquid lipid concentration, surfactant concentration	PS, EE	8	DL, FT-IR, DSC, SEM, *in vitro* release, XRD, accelerated stability study	Oral	Enhanced oral bioavailability of the drug
Repaglinide-NLCs/emulsification and ultrasonification technique [85]	GMS:oleic acid	Liquid lipid ratio, surfactant concentration	PS, ZP, EE	9	*In vitro* release, TEM, DSC	Oral	Promising carrier for the controlled release of Repaglinide
Febuxostat NLCs/high shear homogenization [86]	Stearic acid:oleic acid	Liquid-to-solid lipid ratio, surfactant concentration	PS, % EE	12	PDI, ZP	Oral	This system seemed to be suitable for oral delivery of febuxostat for gout treatment
Raloxifene-NLCs/solvent diffusion method [87]	GMS:Capmul MCM C8	Solid-to-liquid lipid ratio (%), concentration of stabilizer (%)	EE	9	DL, *in vitro* drug release studies, FT-IR, DSC, PS, ZP, TEM, XRD, stability study, pharmacokinetic study	Oral	Enhanced oral bioavailability of the drug
5-Flurouracil-NLCs/solvent diffusion method [88]	Cholesteryl stearate:oleic acid	Octanol, oleic acid, cholesteryl stearate (%)	DL, EE, PS, drug release	12	Atomic force microscopy	Oral	A useful optimization method for the production of NLCs
Plackett–Burman designs (unique types of two-level fractionate designs) that analyze $N - 1$ input variables with N number of experiments; this N should be multiplied by 4. This design is seldom used, but this DOE was proved to be more efficient and logical in the optimization of formulations.							
Olmesartan Medoxomil-NLCs/hot-microemulsification homogenization followed by ultrasonication [89]	Stearic acid:oleic acid	Solid lipid concentration, liquid lipid concentration, surfactant concentration	PS, EE	13	ZP, *in vitro* release with model kinetics, TEM, stability study	Oral	Successful development of NLCs using multivariate statistical approaches for improved product and process understanding

(*Continued*)

Table 4: *Continued*

Drug-loaded NLCs with technique	Lipids used (solid lipid:liquid lipid)	Independent variables applied	Dependent variables applied	No. runs	Characterization parameters	Delivery route	Important conclusions of the study
Isradipine-NLCs/solvent evaporation with probe sonication [90]	Emulcire61:Capryol 90	Total lipids (% w/v), solid lipid: liquid lipid, surfactant (% w/v); surfactant: co-surfactant, stirring speed (rpm), sonication time (min), temperature (°C), drug (mg)	PS, EE, drug release	12	DL, TEM, DSC, ZP, FT-IR, stability study, CLSM, *in vitro* permeation study	Oral	This study improved the lymphatic uptake and biodistribution of drugs, thereby promising the approach as *in vivo* prospect and clinical efficacy
Colombian propolis-NLCs/ emulsification–diffusion technique [91]	Labrafac:LipophileWL 1349	Polyvinyl alcohol (%), caprylic/capric triglycerides (%), Poloxamer concentration of sesame oil (%), emulsification time (min), emulsification stirring rate (rpm)	PS, PDI	12	DSC, XRD, FT-IR, NMR, SEM, cytotoxicity, neutral red uptake assay	Cosmetics	This approach can be used pharmaceutical or cosmetic industries for developing innovative products
Mixture designs are used while the total compositions of independent variables are fixed, and variable ratios are often not independent of each other. The proportion of the other components will decrease if the number of one component increases.							
Zingiber zerumbet-NLCs/emulsification method [92]	GMS:coconut oil (virgin)	Zingiber zerumbet oil, solid lipid, liquid lipid	PS, PDI, ZP, EE	11	Penetration study	Topical	NLCs prepared with DOE showed a stable formulation and provide a promising future in various applications, such as drug delivery, food, textile, and cosmetics

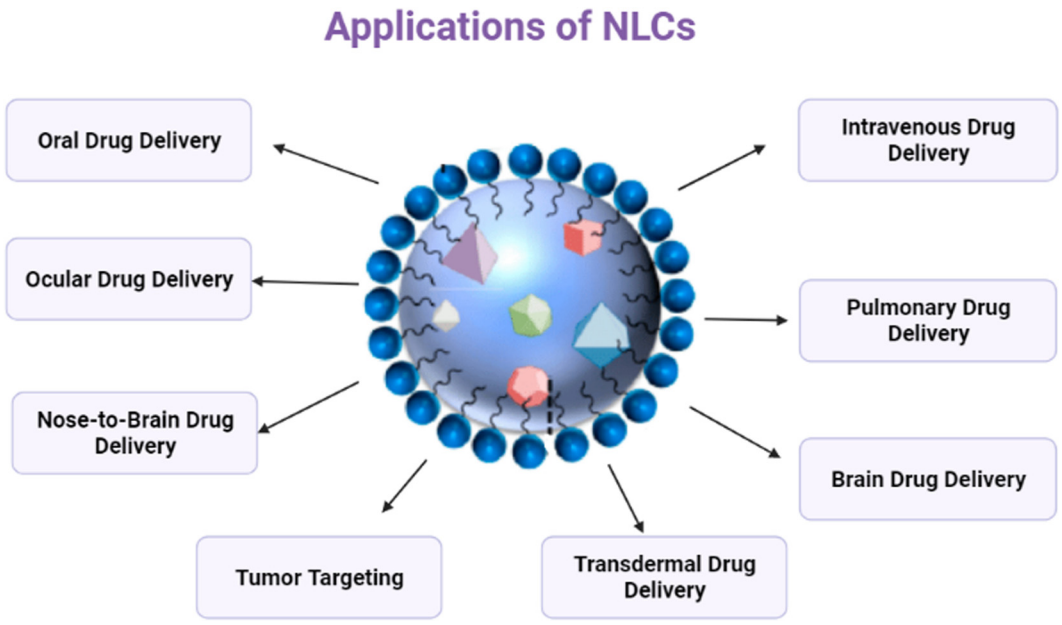

Figure 3: Various pharmaceutical applications of NLCs.

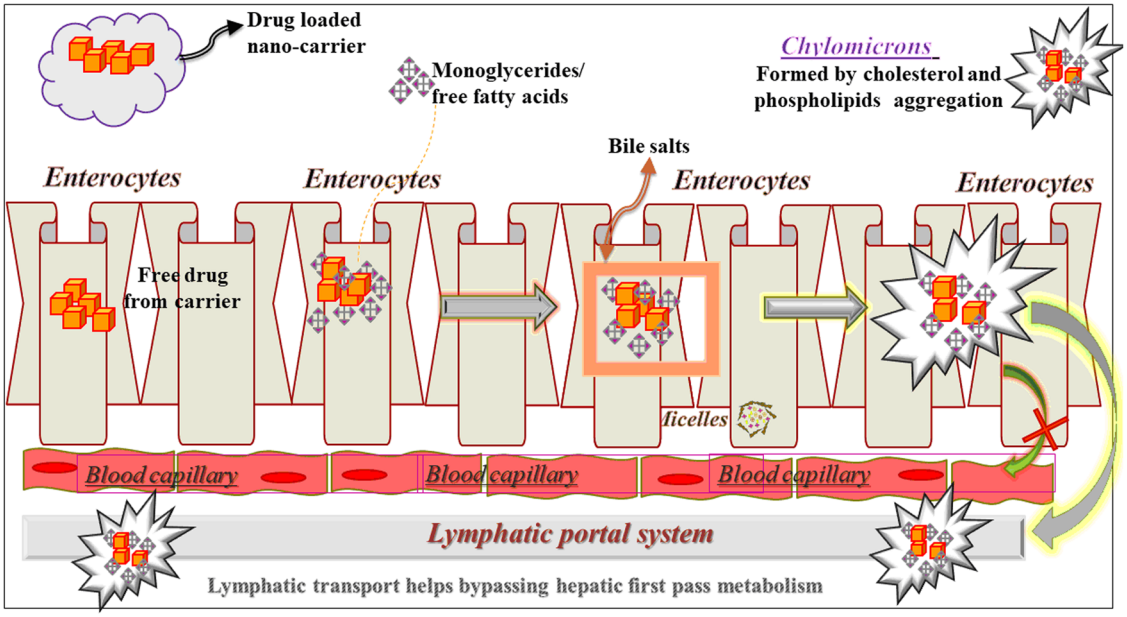

Figure 4: Schematic representation of the absorption of drugs from NLCs in the lymphatic portal system. Drugs incorporated in the NLCs bypass the first-pass metabolism in the liver resulting in increased effectiveness [97].

into muscles or other tissues [104]. From the IV route, drug directly goes into the blood system for systemic action bypassing the first-pass metabolism and absorption phase, making it a reliable quicker route [105,106]. Drugs with short half-lives or duration of action are more suitable to be given *via* this route as they could be delivered at a more uniform rate, and bioavailability of 100% could be achieved. Moreover, IV is a better option for patients who are uncooperative, unconscious, or likely to vomit upon oral administration of a drug [107]. Few significant studies performed on NLCs for their delivery by IV route are enlisted here. Mupirocin-loaded NLCs (M-NLCs) for IV administration were prepared recently, which enhanced the antibacterial activity of the drug. By the storage of almost 3 months at 25°C, all formulations of NLC demonstrated sustained drug release and acceptable

physical properties. M-NLC-1 was shown to be safe in rats at a dosage of 250 mg/kg and to have a considerable rise in plasma concentration in rabbits after IV injection, indicating a better pharmacokinetic profile than free drug [108]. In another study, genistein-loaded NLCs for IV administration were prepared and optimized by the QbD approach for higher entrapment (92.8%) and the ZP of −21.25 mV with excellent stability of the formulation [109]. Recently, commercial artesunate was used to prepare and test intravenously delivered artemether NLCs (ARM-NLCs) for *in vivo* pharmacodynamic effectiveness. In a mouse model, both formulations were shown to be efficient in lowering parasitemia, with ARM-NLC outperforming the others [110]. Furthermore, DTX-loaded NLCs were manufactured using a simplified film ultrasonication–dispersion process to minimize harmful effects and increase therapeutic effectiveness, making them a viable cancer drug delivery system [111].

8.3 Ocular drug delivery

The eyes are very sophisticated and complex organ of the body. Ocular delivery is difficult due to distinct physiological and anatomical aspects of the eyes, as well as many restrictions that must be addressed to attain specific ocular tissue. To eliminate these hurdles and increase ocular tissue bioavailability, LNPs were used as a new innovative drug delivery method [112]. The most frequent route of medication delivery to the anterior portions of the eyes is by topical application to the eyes. The blood ocular barrier, corneal epithelium, conjunctival blood flow, and tear drainage are main hurdles in this system. LNPs can cross the blood–ocular barrier as they are a unique technology and achieve sustained and regulated drug release, prevent medicines from lacrimal enzymes, and extend drug deposition and resident duration in the eyes [113]. Ocular problems, particularly in the posterior region of the eyes, are challenging to cure. When it comes to targeting intraocular tissues, the topical method is not always considered the best option.

Transscleral administration (subconjunctival and retrobulbar injection), intravitreal route, and subretinal injection, among others, are being investigated [114]. Because the majority of these methods are painful, LNPs are an excellent alternative drug delivery strategy for the treatment of ocular disorders. Gene therapy for retinal disease targeting employing non-viral vector gene delivery, such as SLNs and NLCs, has also been suggested as a viable therapeutic strategy for retinal illnesses [115]. For ocular biodistribution, amphotericin B (AmB)-loaded PEGylated NLCs have just recently been developed and improved. In both wild-type and AmB-resistant *Candida* bacteria, AmB-PEG2K-NLCs had considerably superior antifungal efficacy ($p < 0.05$) and were equivalent to, or better than, commercially available parenteral AmB formulations [116]. Melt-emulsification along with ultra-sonification was used to synthesize novel hybrid Genipin-crosslinked dual sensitivity hydrogel/NLCs of baicalin (BN) (BN-NLCs). In comparison to BN eye drops and BN-NLCs, the *in vitro* release investigation revealed that BN-NLC gel enhanced BN release. As a result, this new hydrogel with a lengthy precorneal residence duration has the potential to be used in ocular medication administration [117]. Last to mention here, Timolol maleate™ and Brinzolamide-loaded NLCs were designed to enhance the bioavailability, permeation, and precorneal residence time of these drugs that would result in efficacious treatment of glaucoma. There was a remarkable enhancement in the release pattern and permeation of both the drugs from NLCs as compared to that from their suspension [118].

8.4 Pulmonary drug delivery

A new method of drug delivery used by the researchers is pulmonary drug delivery associated with many advantages, such as non-invasiveness for both local and systemic administration, high drug accumulation in the target site, accelerated onset of action by direct inhalation, the large surface area of pulmonary system, high drug permeability through thin alveolar epithelium, reduction in drug dosage, and consequently reduced drug adverse effects [119,120]. LNPs have been considered for pulmonary delivery as these have shown good results in comparison to the conventional formulations, such as sustained drug release, biodegradability, low toxicity, higher stability, and biocompatibility [121,122]. Here, we have summarized a few marvel research studies performed under this category for the understanding of pulmonary delivery of drug-loaded nanoparticles.

For intramuscular and pulmonary delivery, sodium colistimethate-loaded NCLs (SCM-NLCs) were prepared [123]. Nanoparticles inhibited eight drug-resistant *P. aeruginosa* strains with a minimal inhibitory concentration of 1–2 mg/L *in vivo*. SCM-NLC had much fewer CFU/g lung than saline and was comparable to free SCM, despite the fact that the dosage in the SCM-NLC group was lower than free SCM. The treatment procedures did not cause any tissue injury. After pulmonary or intramuscular

injections, SCM-NLC was effective against *P. aeruginosa in vivo*, was nontoxic, and transported effectively to the liver and lungs [123]. Lipid nanocarrier systems were prepared and evaluated to selectively deliver the RFB to alveolar macrophages to alveolar macrophages. These nanoparticles were engineered to be absorbed by alveolar macrophages, transported to acidified phagosomes and phagolysosomes, and released bactericidal quantities of the antituberculosis drug intracellularly using both mechanisms of passive and active targeting. By using the pulmonary route of delivery, the proposed nanocarrier can be studied as a viable transporter for the safer and more effective treatment of TB [124]. Recently, Montelukast-loaded NLCs (M-NLCs) for pulmonary delivery were developed and tested *in vitro* for aerodynamic evaluation and *in vivo* evaluation done in Wistar rats for pulmokinetics. When compared to the Montelukast-aqueous solution, the pulmonary pharmacokinetic analysis revealed prolonged drug residence in the lungs with better bioavailability and a targeting value of 11.76 for M-NLCs. The research showed that Montelukast lipidic nanoparticulate preparation had the potential to increase efficacy while reducing toxicity, resulting in improved medication performance as M-NLC-DPI for inhalation administration [125]. The literature also suggested that the delivery through the pulmonary route was possible for NLC formulation for beclomethasone dipropionate (BDP). NLC was effectively nebulized, resulting in aerosols with a PS adequate for BDP deep lung administration. The findings showed that LNPs are potential nebulized carriers for BDP, creating opportunities for nebulized lipophilic drug-targeting techniques [126].

8.5 Nose-to-brain drug delivery

The brain is one of the most essential organs and its homeostasis is very critical. The flow of endogenous and exogenous chemicals between the peripheral blood and the cerebrospinal fluid (CSF) is controlled by barriers, which is necessary for appropriate brain function [127]. Due to its anatomy and physiological obstacles, such as the blood–brain barrier (BBB), which is the primary barrier to active molecules entering the central nervous system (CNS) [128], drug delivery to the brain is the most difficult task. Due to the difference in the physicochemical properties from those required for molecular entrance into the CNS, most active CNS medicines (98%) are unable to get through this barrier [129]. Drugs that pass through the BBB are lipophilic drugs with a log *P*-value of 1.5–2.7 and a molecular weight of less than 600 Da [130]. A variety of transporters are present in the BBB including P-gp efflux transporter, which restricts drug entrance into the CNS. One of the delivery approaches for overcoming BBB is nose-to-brain drug delivery. Its benefit is patient compliance, to avoid hepatic first-pass metabolism, and non-invasiveness [131]. There are three ways through which NLCs are transported from the nose to the brain. They include the olfactory pathway, the trigeminal nerve pathway, and the systemic pathway. Blood circulation is also involved in drug absorption into the brain from the nasal cavity [132]. The distribution of active agents happens throughout the systemic circulation, entering nasal blood arteries before being swiftly transported to the carotid artery blood supply to the brain and spinal cord [133].

8.6 Brain drug delivery

In contrast to oral drug delivery, brain targeting enhances the drug's concentration in the CSF, reduces dose frequency, has fewer adverse effects, avoids first-pass metabolism, and has a faster onset of action [134]. The two primary difficulties are decreased drug penetration through the BBB and efflux of delivered medicines from the brain to the blood circulation [135]. The main benefits of LNPs are their high drug encapsulation and small size. That is why they are an ideal choice for targeting specific tissues of the brain. Colloidal drug delivery systems, including SLNs and NLCs, have the benefit of enhancing the retention time of drugs in the blood of brain capillaries and triggering a drug gradient from blood to brain tissues, widening tight junctions to promote entry from BBB and transcytosis of drug-loaded LNPs *via* the endothelium layer, and increasing the retention time of drug in blood of brain capillaries. Furthermore, they can accommodate both hydrophilic and lipophilic drugs [136].

Because of their bio-acceptability, quick absorption by the brain, and biodegradability, NLCs may be regarded as one of the key techniques for drug administration without any alteration to the drug molecule. Furthermore, the ease with which they may be scaled up and the lack of a burst effect make them ideal drug delivery vehicles. Over the last few years, a growing number of research papers have been published in this area, showing NLCs as a potential method in brain delivery. An increasing number of studies have been reported in this regard over the past decade establishing the NLCs as a promising tool in brain delivery. The recent development is of almotriptan maleate (ALM) mucoadhesive chitosan-coated NLCs for the treatment of

migraine since ALM has poor solubility, poor penetration, and low concentration in brain regions, demanding repeated oral administration. Experiments conducted on albino rabbits for *in vivo* pharmacokinetics revealed that the optimized ALM-NLCs (1.54 mg/mL) had a considerably greater C_{max} in plasma than ALM solution (0.25 mg/mL) and ALM oral marketed tablet (0.58 mg/mL). Because NLCs are flexible and lipophilic, they may be a viable technique for drug transport to the brain [137]. The *in vivo* anticonvulsant effect of carbamezipine-loaded NLCs (CBZ-NLCs) in a PTZ-induced seizure model demonstrated a considerable rise in the starting date (134.0 s) and a decrease in the length (17.2 s) of tonic-clonic seizure compared to CBZ dispersion (75.4 and 37.2 s), respectively [138]. Apart from these, NLCs of lurasidone hydrochloride for the treatment of schizophrenia [139], artemisinin NLCs for the treatment of brain tumors and malaria [140], and baclofen NLCs for targeted delivery to the brain [141] are also reported in the literature for their improved brain delivery.

8.7 Transdermal drug delivery

The transdermal drug delivery system (TDDS) has been used for centuries to transfer medications and therapeutics through various layers of the skin, including the surface, epidermis, dermis, and hypodermis, for therapeutics effects [142]. Moreover, it is essential to handle various issues with traditional skin preparations, including skin barrier impermeability, limited effectiveness, and excessive application frequency [143]. One of the many advantages of NLCs is the presence of biologically active and biodegradable lipids, which show less toxicity and provide adhesiveness, skin hydration, lubrication, smoothness, emollience, skin penetration enhancement, and modified drug release characteristics. For increasing progress in the cosmetic and pharmaceutical sector, researchers are currently focusing on manipulating topical and dermal applications of NLCs [144]. The small size of NLCs ensures enhanced skin penetration of active compounds through contact with the stratum corneum and few mechanisms are available in the literature related to it [145].

Understanding the transport pathways that are vital since drug transit through skin necessitates greater lipophilicity. The process of transport of a hydrophilic drug through the skin from NLCs differs from that of a hydrophobic drug. It is predicted that unless water from the NLCs readily penetrates transdermally, a hydrophilic medication would not be accessible for percutaneous transport from NLCs. As a result, significant water mobility within the NLC vehicle and for percutaneous transmission through the skin barrier is proposed [146]. NLCs are also effective in delivering hydrophobic drugs transdermally to the systemic circulation [147]. The major concerns across the world are skin disorders, both infectious and non-infectious. Skin disorders are difficult to treat because of therapeutic limitations, such as limited medication effectiveness due to inadequate skin penetration of drugs from standard formulations. Drug permeation is limited by the stratum corneum of the epidermis, which must be overcome by switching the penetration channel from transcellular to paracellular or follicles [148]. The rapid emergence of LNPs (SLNs and NLCs) had remained successful to increase the skin penetration of drugs as these formulations are prepared by mixing LNPs with the conventional formulations [149]. Biodegradability, adhesiveness, close contact with skin, biocompatibility, regulated drug release profile, hydration of skin, and film formation to enhance skin penetration effects are benefits of LNPs for topical dermal administration [150].

For the treatment of vitiligo, topical SIM-loaded NLCs were produced and proved to be safe, with the drug entrapment of 99.27% and adequate long-term stability [151]. Febuxostat-loaded NLC gel was formulated for the topical treatment of gout. The NLC gel formulation showed 87% release within 6 h in a controlled manner [152]. According to a recent discovery, for skin regeneration in tissue engineering, a new gelatine/hyaluronic acid/polycaprolactone nanocomposite scaffold containing 54.1 wt% AT-loaded NLCs was tested as a suitable target [153], optimized NLC-loaded Apremilast showed PS 157.91 ± 1.267 nm, % entrapment efficiency 69.14 ± 0.278%, and ZP −16.75 ± 1.40 mV, respectively. It was suggested to be explored for topical delivery of drugs for the treatment of psoriasis [154].

The NLC gel of repaglinide (RG), an effective anti-diabetic drug, was formulated to improve the bioavailability by the transdermal route. The optimized gel system (RG-NLC gel) showed sustained release up to 24 h and two times improvement in the bioavailability in comparison to the marketed oral tablet in the rat model [155]. Likewise, NLC-based gel was produced for improved skin administration of donepezil-free base. In Alzheimer's disease, because of many disadvantages of the oral administration of this drug, patients are less likely to stick to their treatment regimen. Drug skin penetration from the new gel was boosted in the *in vitro* skin permeation studies, and lipid nanocarriers provided an additional boosting impact to enhance drug permeability over the skin [156]. An NLC gel loaded with tripterine (TRI) for transdermal delivery was developed in another research.

In another study, NLC gel loaded with TRI for transdermal administration was formulated. Sustained-release

properties of the drug are demonstrated by *in vitro* drug release from the TRI-NLC-gel. The penetration of NLC into the deep skin layers was discovered through dermatokinetic studies and histopathological studies showed that treated skin remained intact indicating better compatibility of the novel gel [157]. It is also important to note that topical administration in the treatment of rheumatoid arthritis, triptolide-loaded NLCs reduced knee edema transdermally by lowering inflammation and modulating TNF-, IL-1, and IL-6 levels, making the drug-NLC system a viable delivery strategy [158].

In contrast to the Exelon® patch, the NLC transdermal system had a remarkable increase in bioavailability and sustained release of rivastigmine. The designed NLC-based transdermal patch was shown to be nonirritating in skin irritation tests. The higher Cmax and AUC0–72 in plasma treated with NLC-loaded transdermal patches compared to standard patches suggested that NLC-based transdermal patches might be used as a possible carrier for improving rivastigmine bioavailability for improved dementia therapy [159].

8.8 Tumor targeting by NLCs

In recent times, tumor targeting has become one of the most important parts of drug delivery. The development of a new carrier system for the transport of numerous anti-cancer medications is still a subject of discussion, and it is a vital step toward the improvement of drug entrapment and tumor targeting [160]. NLCs as nanocarriers can be the drug delivery choice for certain anticancer drugs upon proper research by improving their drug release, chemical stability, and cytotoxicity. In a research study, it was revealed that encapsulating camptothecin and topotecan in the NLC system was found to have higher cytotoxicity and cell uptake against melanoma and leukemia cells. To provide a long circulation impact and excellent tumor targeting, they can be coupled with an amphiphilic copolymer, folate poly PEG-cyanoacrylate-*co*-cholesteryl cyanoacrylate [161].

To combat multidrug resistance and improve cancer treatments, NLCs co-loaded with arginyl-glycyl-aspartic acid were used to co-deliver doxorubicin and sildenafil citrate. In contrast to individual treatment and administration, co-delivery of drugs enhanced uptake and accumulation *via* integrin-mediated endocytosis and potential ABC transporter blockage, making co-delivery an efficient strategy for inducing apoptosis [162]. The capability of multifunctional NLCs in the management of non-small-cell lung carcinoma was recently discovered by the researchers, and the findings have had a substantial impact on the field of drug delivery to enhance the effectiveness of lung cancer therapy [163]. To improve cytotoxic effects in MCF-7 breast cancer cells, researchers developed chrysin-loaded NLCs of doxorubicin. A significant rise in the percentage of apoptosis was observed in chrysin-loaded NLCs from 21.11 ± 5.72% to 27 ± 3.13% ($P < 0.05$). In comparison to the control group, the quantities of mRNA expressions of Nrf2, NQO1, HO1, and MRP1 were significantly lower. As a result, it is possible that delivering chrysin with NLCs could improve doxorubicin effectiveness by inhibiting drug efflux pumps [164]. In animal models, novel NLC-based formulations of di-indolylmethane (DIM) derivatives DIM-10 and DIM-14 increased oral bioavailability and antitumor activity significantly. The anticancer properties of both derivatives were shown to be better than those of free drug-treated groups, indicating that they had promising capabilities for clinical purposes [165].

9 Toxicity studies on NLCs

The rapid emergence of nanomaterials has led to an exponential resurgence of drug delivery systems in the field of pharmacy and medical science. Equipped with useful characteristics, such as being biodegradable, biocompatible, and stable profile, makes NLCs a very suitable drug delivery system [166]. LNPs (both SLNs and NLCs), due to their ability to incorporate lipophilic drugs and maintain their controlled drug release, have garnered a lot of attention recently. It is therefore required to sufficiently investigate their toxicity owing to their extensive applications [167]. The toxicity studies performed on various NLC formulations are summarized in Table 5. The acute toxicity research for Zerumbone (ZER)-loaded NLCs (ZER-NLC) was carried out by orally administering a single dosage of water, olive oil, ZER, NLC, or ZER-NLC to BALB/c mice for 14 days [168]. Clinical and behavioral problems, toxicological effects, feed consumption, and gross appearance were identified in the animals. MMP decrease, lysosomal membrane destabilization, and lipid peroxidation were restored by quercetin-loaded NLCs, which also prevented paraquat-treated change in Bax and Bcl2 gene expression [169]. In rabbits and rats, intravaginal administration of 0.5% podophyllotoxin-NLCs (POD-NLCs) induced only minor discomfort with no evidence of acute or chronic damage to the vaginal mucosa [170].

Table 5: Various drug-loaded NLCs tested using animal models (*ex vivo* and *in vivo* studies)

Drugs	Animal/test model	Outcomes	Ref.
Tocopherol	Skin irritation testing/three-dimensional tissue culture model EpiDerm	Histological evaluation revealed that the prepared tocopherol-loaded NLCs were nontoxic and nonirritant with a relative % cell viability of 92.7%	[171]
Calcipotriol + Methotrexate	Skin irritation testing on female nude mice	Prepared NLCs showed non-significant or negligible skin irritation	[172]
Huperzine A	Draize patch test for skin irritation on albino rabbits	The primary skin irritation index or the irritation score was found to be zero even after 24 h	[173]
Tretinoin	Skin compliance test on female Laca mice	No visual or microscopical signs of irritation were observed with the prepared NLCs, however, the marketed formulation showed signs of marked inflammation	[174]
Cyclosporine A	Cytotoxicity studies on human corneal epithelial cell lines	Cell viability increased upon incubation for 12 h	[175]
Flurbiprofen	Ocular tolerance analysis/Eytex® system	Ocular tolerance analysis/Eytex® system	[176]
Levofloxacin	Modified egg chorion of hen/ocular tolerance test	Absence of irritation	[177]
Cyclosporine A	Ocular irritation/tolerance study	No significant signs of ocular irritation	[178]
Flurbiprofen	Ocular irritation/tolerance study	No evidence of inflammation or tissue edema	[179]
Bixin	Bixin 2.5 mg/kg given to Wistar rats p.o.	The treated group showed better protection against the Paracetamol-induced lipo-oxidation as well as reversal of AST and ALT liver enzymes to normal level	[180]
Halofantrine	Halofantrine given to Swiss albino mice p.o. in different doses	Normal glomerulus, normal peri-portal mononuclear infiltration	[181]
Artemether	Swiss albino mice	No change in the morphology of kidney and liver tissues	[182]
DTX	Administered to ICR mice IV	Showed signs of shortness of breath for 1–2 min immediately after drug administration which came back to normal after 5 min	[183]

10 Stability and safety of NLCs

The SLNs have problems with stability; however, other lipid-based nanoformulations, such as NLCs, have been developed to alleviate the problems associated with SLNs. NLCs are sometimes referred to as new unstructured-matrix SLNs. It is a misconception to assert that NLCs will not be employed in the future; every problem has a remedy. The HPH process utilized for the preparation of NLCs can be easily transferred from a small-batch production to a large-batch production in the pharmaceutical industry while avoiding the use of any organic solvent in their preparation [184]. Altogether, the use of GRAS components, the large-scalable production methods for their preparation, and the improved drug safety demonstrated by the use of lipid-based nanocarriers make NLCs an ideal drug delivery system candidate for the pharmaceutical market.

The long-term physical stability of NLCs has some issues owing to the presence of water content in it. However, there are now a plethora of options for preserving them, such as freezing the suspension to turn it into a solid. A freeze-dried nanoparticle should ideally preserve stability while keeping the fundamental nanoparticle characteristics and maintain water in the dispersion and include a preservative. Moreover, NLCs are considered generally safe nanocarriers for oral, dermal, pulmonary, and ocular administration due to the presence of biodegradable and physiological lipids, which have been shown to be well tolerated in both *in vitro* and *in vivo* investigations [185]. NLCs contain lesser quantities of surfactants and cosurfactants when compared to emulsions, which further improve their safety profile.

11 Patents on NLCs

In this section, recent patents concerning NLCs over a period of time are summarized. The nanosized NLC formulations for dermal, nasal, topical, and those directed toward the CNS and peripheral nervous system have already been patented. The enhanced bioavailability, ease of crossing BBB, and stability as compared to the conventional formulations have them interesting enough for their patentability. Table 6 summarizes the recent

Table 6: Patents granted on NLCs in the field of applications in drug delivery

Patent number	Title of patent	Inventors	Ref.
US20080020058-A1	Lipid nanoparticles-based compositions and methods for the delivery of biologically active molecules	Chen *et al.*	[186]
EP2229936-A1	Nanonized testosterone formulations for improved bioavailability	Keck and Muchow	[187]
US20090238878-A1	Solid nanoparticle formulation of water-insoluble pharmaceutical substances with reduced ostwald ripening	Singh	[188]
US20100047297-A1	Nano-crystals for use in topical cosmetic formulations and method of production thereof	Petersen	[189]
US20100247619-A1	Nano-structured lipid carriers containing Riluzole and pharmaceutical formulations containing said particles	Bondi *et al.*	[190]
US20110059157-A1	Anionic lipids and lipid nano-structures and methods of producing and using the same	Awasthi and Lagisetty	[191]
US20110097392-A1	Antibody bound synthetic vesicle containing molecules for delivery to central and peripheral nervous system cells	Wang *et al.*	[192]
WO2011116963-A2	Lipid nanoparticle capsules	Petit *et al.*	[193]

patents of NLCs for various drug delivery applications. Chen *et al.* developed cationic charged LNPs, which delivered major active biomolecules including proteins, peptides, vitamins, antibodies, and nucleic acids [186]. Keck and Muchow prepared testosterone undecanoate NLCs using stearic acid as solid lipid. These prepared NLCs enhanced the oral bioavailability of the drug as compared with the marketed product. Furthermore, these NLCs can be used for dermal or nasal delivery [187]. DTX NLCs with reduced Ostwald ripening were developed by Singh using whey protein as an emulsifier. A specific characteristic of this patent was lactoglobulin/albumin, immunoglobulin, and bovine serum albumin, which was used as whey protein (emulsifier) [188].

Petersen developed rutin nanoparticles with size 300–800 nm and demonstrated that nanodelivery, such as liposomes, NLCs, and SLNs, was significantly long term and electrolyte stable in contrast to existing conventional formulations [189]. Bondi *et al.* prepared riluzole in NLCs using compritol for the amyotrophic lateral sclerosis (neuroprotective action) treatment. The size of prepared NLCs was found to be less than 100 nm, which can easily cross BBB and showed clinical signs of allergic encephalomyelitis in rats as compared with the group treated with the free riluzole [190]. Awasthi and Lagisetty claimed the novel NLCs' preparation using cholesteryl hemisuccinate, 2-carboxyheptadecanoylheptadecylamide, 1,4-dipalmitoyl-tartarate-2,3-diglutaric acid, and 1,4-dipalmitoyl-tartarate-2,3-disuccinic acid as solid lipid and vitamin E as liquid lipid [191]. Wang *et al.* prepared nanoparticles, such as NLCs, of biotinylated protein for targeting CNS or PNS neuronal cells. NLC-loaded protein recognizes a receptor expressed on a neuronal cell's surface [192]. There are few references related to the use of LNPs to deliver peptides/proteins because of the matrix's hydrophobic nature. Proteins/peptides containing LNPs provide proteolytic degradation protection in the GIT. Petit *et al.* improved protein/peptide delivery with the use of polymeric-coated NLCs or SLNs. These polymer-coated nanoparticles provided extra protection by enhancing their stability against chemical degradation and have a greater capacity for skin penetration than conventional LNPs [193]. Lutz *et al.* filed a patent on the process and method for the development of NLCs. They prepared NLCs using murumuru seed butter and bisdiglyceryl polyacyladipate-2 and have been used in cosmeceutical and skincare industries [194].

12 Regulatory considerations and marketed formulations

The main objective of keeping a vigilant eye on any formulation by the regulatory bodies is to ensure, promote, and protect public health. Important commodities, such as pharmaceuticals, foods, medical devices, *in vitro* diagnostics, biological, nutritional products, veterinary medicines, cosmetics, and agrochemicals, are under mandatory and strict control of regulatory bodies to ensure their safety and efficacy. In the case of polymeric nanoparticles, sometimes the excipients used do not belong to the GRAS category as they are not regulatorily accepted by the FDA. They might give good experimental results but cannot be used in products in the market [195]. Regulatory bodies ensure that pharmaceutical companies perform all sorts of toxicity studies on any new drug or new

polymer before creating formulations from them and there should not be any hindrance in the development of a new drug delivery system [196]. All the emerging nanocarrier systems, such as nanoparticles, dendrimers, carbon nanotubes, liquid crystals, and others, have raised the importance of regulations for a new pharmaceutical product [197]. All the ingredients, components, and constituents of NLCs, such as lipids and emulsifiers, are considered to be physiologically safe, nontoxic, non-immunogenic, biodegradable, and biocompatible in nature and easily fit in the category of GRAS by the regulatory authorities. They are already being used in the food industry and sometimes for encapsulating pharmaceutical compounds in a safe and accepted range [198–200]. Minor regulatory obstacles are the significant reason for the wide acceptance and commercial success of the NLCs.

Several products containing NLCs received regulatory approval and are available on market. A few of them are summarized in Table 7 along with other marketed lipid-based formulations, excipients used in the preparation, and surfactants.

Table 7: Marketed NLCs containing formulations, excipients, and surfactants

Marketed products	Country
Products containing NLC	
Cutanova Nanorepair Q10 cream	France
FloraGlo®	Netherlands
NanoLipid Restore CLR®	France
NLC deep effect eye serum	Germany
extra moist softener	Korea
Cutanova Nanovital Q10 cream	France
Lipid-based formulations	
Sandimmune®	United States
Sandimmune Neoral®	United States
Norvir®	United States
Fortovase®	Switzerland
Marketed excipients used for NLC formulation	
Solid lipids	
Xifaxan®	United States
Azelex®	Ireland
Viokace™	United States
Survanta®	United States
Liquid lipid	
Avodart™	United Kingdom
Lipofen®	Canada
Terramycin®	United States
Baycip®	Germany
Surfactants	
Targretin®	California
Rapamune®	United States
Oxidize®	Romania
Dermazene™	United States
Kaletra®	United States

13 Recent advancements of NLCs in delivering diverse therapeutics

13.1 Anticancer agents

In contrast to pure drug, the design and preclinical testing of microneedle-assisted Resveratrol NLCs for localized administration to breast cancer treatment revealed greater anticancer efficacy and improved internalization of MDA-MB-231 breast cancer cells [201]. The hot microemulsion technology was used to deliver sorafenib and ganoderic acid NLCs for hepatocellular cancer, and the dual drug-loaded NLCs beat the plain medicines in chemoprotection, signaling superior activity [202]. Miltefosine given *via* NLCs *in vivo* in tumor-bearing BALB/c mice for the breast cancer treatment showed antitumor potential, improved pharmacokinetics, and higher apoptotic effects [203]. In MCF-7 cells, NLCs loaded with imatinib were produced and their *in vitro* effectiveness was assessed. The cytotoxicity of the optimized drug–NLC combination (IC_{50} = 6 M) was found to be 8.75 times higher than that of the drug alone (IC_{50} = 52.5 M) [204]. In another study, nanoencapsulated resveratrol possessed anticancer and apoptotic effects on cell proliferation and exerted a protective effect against cytotoxicity induced by paraquat [205]. A multi-component cancer-targeting delivery system was recently reported for the treatment of non-small-cell lung cancer by NLCs enclosing luteinizing hormone-release hormone (cancer-targeting moiety), gefitinib (EFG-TK inhibitor), paclitaxel (anticancer drug), siRNA targeted to EGF receptor mRNA as an EGF receptor suppressor, and rhodamine (imaging agent).

In comparison to the individual components used accordingly, the system has dramatically improved anticancer action [206]. Cabazitaxel (CAB) was created using expert design, NLCs of a taxane derivative, and the antimicrotubule compound, and different formulation features including the ratio of liquids and concentration of surfactants, homogenization speed, and time were adjusted. The NLCs were successful in delivering the extremely lipophilic medication CAB as a potential drug carrier in the treatment of breast cancer [207]. Folic acid-conjugated NLCs were described as an effective delivery approach for doxorubicin targeted to breast cancer cells [208]. Optimized dual drug-loaded NLCs of quercetin and piperine were found to be effective against oral squamous

cell carcinoma [209]. Co-delivery of rituximab-conjugated NLCs of curcumin and imatinib in the treatment of non-Hodgkin lymphoma is reported recently [210]. Optimized NLCs of 5-Fluorouracil were prepared using a QbD approach using a 32 factorial design as effective chemotherapy for colon cancer. The oral administration of drug-loaded NLCs had higher bioavailability as compared to drug solution [211].

13.2 Phytochemicals

Active plant constituents have manifested their pharmacological activities against many diseases from time immemorial, but due to their physicochemical properties, they often have solubility, permeability, and poor bioavailability issues. Such phytoconstituents require an advanced and novel lipid carrier as a formulation to improve their biopharmaceutical problems. NLCs have been formulated for active plant constituents and these lipid-based nanocarriers have emerged as novel drug delivery systems [212]. Development of 1′-acetoxychavicol acetate NLCs for prostate cancer [213], naringenin-loaded NLCs against nonalcoholic fatty liver disease [214], cannabidiol NLCs for nasal administration for the treatment of neuropathic pain [215], nanolipoidal α-terpineol in combating keratitis induced by *Pseudomonas aeruginosa* [216], and bergamot oil NLCs was seen as promising means for photothermal treatment of vitiligo [217]. Curcumin-NLCs improved the neuroprotective effect of curcumin as a therapeutic for anxiety and depression [218], magnetic curcumin NLCs reported no mitochondrial toxicity when tested *in vitro* [219], NLCs were able to improve stability and release profile of lycopene from watermelon extract [220], and NLCs of nicergoline, a semi-synthetic ergot alkaloid based on sesame oil for intranasal delivery for brain targeting, showed augmented neuroprotective action for the treatment of dementia [221].

Curcumin-loaded NLC smart gels with the encapsulation efficiency of 72.15% and ZP of −21.67 mV were found to be biocompatible and effective for the treatment of rheumatoid arthritis [222]. Corylin-NLC gel was prepared and was found to be a promising strategy for the treatment of UV-induced skin aging [223]. Heparin-decorated NLCs of artemether-protoporphyrin IX-transferrin combinatorial delivery system were made for the treatment of malaria [224]. To address the failure of single antibiotic therapies, co-delivery of hesperidin and clarithromycin in NLCs was tried against *Helicobacter pylori*. NLCs were reported to interact with the microorganism membrane by adhering to the outer cell membrane and causing leakage of cytoplasmic contents [225]. Very recently, puerarin-loaded NLCs were prepared by a solvent evaporation method. This system showed an enhanced therapeutic effect on alcohol-induced cell injury of BRL-3A cells. The optimized NLC composition consisting of GMS, olive oil, poloxamer, and puerarin has an average PS (159 ± 1.1 nm), %EE (92.16%), DL (5.75%), and ZP (−28.3 mV), respectively [226]. Biotin-conjugated NLCs for oral delivery of chrysin distinctly improved the biopharmaceutical performance of chrysin, a vital flavonoid from nature [227].

13.3 Therapeutics

Miltefosine-loaded NLCs for the treatment of cutaneous leishmaniasis orally are recently prepared for a better safety profile and reduced hemolytic potential [228]. The oral bioavailability of perphenazine-loaded NLCs was enhanced as NLCs showed the potential to surmount the oral delivery drawbacks of this poorly water-soluble drug [229]. The low solubility and poor stability issues of hydrochlorothiazide suitable for pediatric oral therapy were tackled by formulating it into SLNs and NLCs. The NLCs showed better performance than SLNs, with 90% entrapped drug compared to 80% in SLNs. The NLC formulation showed good physical stability during 6-month storage at 4°C (*vs* SLNs) [230]. Eplerenone-loaded NLCs were successful as oral targeting delivery carriers in the treatment of chronic serous chorioretinopathy. The optimized drug-loaded NLCs showed a PS (134 nm), PDI (0.31), %EE (76 ± 6.56% w/w), and ZP (−32.37 mV) [231]. The oral bioavailability of nintedanib esylate in NLC was ameliorated over 26.31-fold compared to drug suspension resulting in increased oral bioavailability of the drug *via* lymphatic uptake [232]. Incorporation of olanzapine in mesoporous NLC showed a significant improvement in the oral bioavailability of olanzapine over the plain drug suspension [233]. Mannosylated NLCs of clofazimine can be used as a promising carrier for the safe delivery of drugs *via* inhalation route for the treatment of tuberculosis disease. A two-fold greater bioavailability was seen compared to drug dispersion [234].

Novel inhalable ciprofloxacin-NLCs formulated into nanocomposite microparticles emerged as a new approach to improve the targetability of ciprofloxacin in noncystic fibrosis bronchiectasis, thereby overcoming poor lung targeting issues of the drug after oral inhalation [235]. Itraconazole PEGylated NLCs for the treatment of pulmonary aspergillosis *via* inhalational delivery are reported [236].

NLC of BDP for pulmonary drug delivery *via* medical nebulizers is reported [237]. Intranasal administration of NLCs of Artesunate was reported to have great potential and a satisfactory alternative to parenteral administration in the treatment of severe and cerebral malaria in remote areas of sub-Saharan Africa [238]. Transferrin-functionalized curcumin NLCs were found to be promising in brain delivery [239].

14 Conclusions

In the present pharmaceutical world of research and development, numerous sophisticated nano-scaled drug delivery systems are being explored both on the laboratory and industry scale extensively. The nanosized drug delivery system offers a good stability profile to both the drug and the carrier system giving them desirable characteristics in the dosage form development. NLCs are the latest and new-generation LNP formulations, poised with flexibility in DL, modulation of their release profiles, and improved pharmaceutical performances. These special characteristic features of NLCs are attributed to their unique composition, which is the blend of solid and liquid lipids. In this review, we have tried to sum up all the information regarding the newest LNPs aka the NLCs. We have managed to throw light on all the aspects, such as components of NLCs, their preparation techniques, characterization parameters, and their optimization process, by giving numerous examples at each step for the complete understanding of the young formulator/researcher. The NLCs offer a wide range of applications in the field of medical science for the treatment of various diseases as they can administer both the hydrophilic and hydrophobic drugs by different routes, such as oral, parenteral, topical, ocular, pulmonary, and drug delivery, to the brain very efficiently. Their nontoxicity upon systemic exposure can open their new role as diagnostic tools in the field of imaging. Their specificity and targeting ability make them a very promising nanosized drug delivery system of the present times. The design and development of multifunctional NLCs loaded with combinations of drugs and biological actives and targeting them specifically to the required site will soon open better research options for the scientists and researchers worldwide in the fields of the cosmetic and pharmaceutical industry, which will be a successful step toward a new technological era in human clinical trials. Some key advantages of these nanosized carrier systems are decreased drug payload, enhanced patient compliance, reduced toxicity of the drug, cheap large-scale production, biocompatibility, biodegradability of their constituents, ease of manufacturing, and improvised chemical stabilization of the active ingredients making them even more promising and near to perfect all-rounded nanocarriers in their generation.

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