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Review Article

Nanostructured Lipid Carriers for Improved Delivery of Therapeutics via the Oral Route

Alok Kumar Mahor,[1] **Prem Prakash Singh**,[1] **Rishikesh Gupta**,[1] **Peeyush Bhardwaj**,[1] **Priyanka Rathore**,[1] **Ankita Kishore**,[1] **Rohit Goyal**,[2] **Neeraj Sharma**,[3] **Jyoti Verma**,[4] **Jessica M. Rosenholm**,[4] **and Kuldeep K. Bansal**[4]

[1]*Institute of Pharmacy, Bundelkhand University, Jhansi, India*
[2]*School of Pharmaceutical Sciences, Shoolini University, Solan, India*
[3]*Department of Pharmacy, Madhyanchal Professional University, Bhopal, India*
[4]*Pharmaceutical Sciences Laboratory, Faculty of Science and Engineering, Åbo Akademi University, 20520 Turku, Finland*

Correspondence should be addressed to Alok Kumar Mahor; alokmahor522@bujhansi.ac.in and Kuldeep K. Bansal; kuldeep.bansal@abo.fi

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Drug delivery via the oral route has always been challenging for poorly soluble drugs. Acid-induced hydrolysis, enzymatic degradation, and poor mucosal absorbency remain the primary hiccups for effective oral delivery of medications. With the advent of nanotechnology, nanostructured lipid carriers (NLCs) have emerged as a promising delivery carrier that can circumvent gastrointestinal tract (GIT) barriers hindering the solubility and bioavailability of such drugs. These NLCs can efficiently transport drug moieties across intestinal membranes shielding medications from intestinal pH and enzymatic degradation. Because they are composed of lipidic materials, they can be easily absorbed or taken up by various pathways such as transcellular absorption, paracellular transport, and M-cell uptake. Such mechanisms not only improve the absorption and solubility of drugs but also augment bioavailability and residence time and may bypass first-pass metabolism. This review explores the diverse applications of nanostructured lipid carriers (NLCs) in oral drug delivery for various medical conditions, shedding light on their current regulatory status, including FDA-approved options and those in pre/clinical stages. The review also features patented NLC formulations. It provides valuable insights into how NLCs can be harnessed for effective oral drug delivery and outlines recent advancements in optimizing their performance to tackle gastrointestinal barriers, thus opening new possibilities for NLCs in future pharmaceutical applications.

1. Introduction

Delivery of medications via the oral route is considered the ideal way to achieve therapeutic and prophylactic effects against many ailments in treating both acute and chronic conditions [1]. In addition, oral delivery possesses several benefits such as noninvasiveness, ease of administration, patient compliance, and economical and ease of large-scale manufacturing. Drugs that are capable of sustaining stability in the stomach acidic environment and do not pose gastrointestinal (GI) irritation and toxicity challenges are preferred for oral delivery [2, 3]. Although most drugs are lipophilic, poor absorption leading to low bioavailability is a major concern regarding the formulation of a successful oral dosage form.

Furthermore, poor permeability across the GI membrane [2, 3], intrinsic dissolution rate (mass of the drug dissolved per time unit and area), where dissolution is the rate-limiting step in the absorption of hydrophobic drugs (especially drugs of BCS class II and IV) [4], acidic environment [5], first-pass metabolism, intraenterocyte metabolism, and enzymatic degradation [6] limit the absorption

of the therapeutic agent. Also, drug eviction from the drug transporter (P-glycoprotein: P-gp) and interaction with the food present in the GIT leads to variable absorption of the drug, finally reaching systemic circulation [6]. In addition, the age, gender, and pathological condition of patients affect the absorption of the drug.

Several approaches have been explored to augment the solubility of BCS class II and IV drugs. One such method is the transformation of the drug into a solubilized state, enabling the absorption profile of the drug to be close to that of the BCS class I drug [7]. Another approach is the use of nanotechnological approaches such as polymeric nanoparticles, microspheres, lipid-polymer hybrid nanoparticles (LPHNPs) [8], lipid-based nanoparticles [9] such as liposomes, niosomes, and solid lipid nanoparticles (SLNs) [10], and nanostructured lipid carriers (NLCs) for improving the solubility of drugs [11, 12].

NLCs are colloidal structures comprising an amalgamation of solid and liquid lipids that constitute an amorphous lipid matrix fenced by a solid lipid coat. A combination of solid and liquid lipids provides structural integrity to NLCs where less organized structures have been created that accord a steadier enclosure of the drug in the lipid matrix lending long-term shelf life to the formulation [13]. In addition, NLCs can incorporate both hydrophilic and hydrophobic therapeutics [14].

NLCs present several advantages such as easy manufacturing, low toxicity, physical stability, custom-tailored release, high drug entrapment, no drug leaching during storage, and improvement of drug's solubility and stability, which are some excellent features that grant them an upper hand over other drug delivery systems. NLCs, by their biocompatible nature, can be administered via the oral, parenteral, topical, rectal, and pulmonary routes [15–17].

Owing to the several advantages of NLCs, this review focuses on the events that occurred late and recently in the successful oral delivery of poorly soluble medications using NLCs. In this article, we present an understanding of the mechanism of drug protection in terms of *in vitro-in vivo* capacity, preparation methods of NLCs, and applicability in conveying a variety of medications, proteins and peptides, bioactive compounds, etc., across biomembranes. In addition, insights on the clinical trials and patents granted on the potential implications of NLCs in the delivery of therapeutics are provided.

Lipid-based nanoparticles have been utilized in the delivery of poorly soluble drugs of late with solid lipid nanoparticles being the earlier drug delivery system that has shown great potential in the delivery of medications across the GIT. The use of biodegradable natural lipids and surfactants and toxic solvent-free methodologies in their preparation enable lipid-based nanoparticles to be the foremost selection for the delivery of poorly soluble drugs. The natural lipids used can withstand the degradation factors both *in vitro* and *in vivo* rendering them the first choice for nanoparticulate drug delivery systems that deliver medications across biological membranes [14]. According to the data available on Google Scholar, more than twenty-four thousand pieces of literature are available on lipid nanoparticles for oral delivery from 2010 to date, indicating their extensive use in the transport of biological molecules.

Lipid nanoparticles are now being considered as a substitutional food supplement post-COVID-19 [18] and are now the fast-growing sector worldwide with a compound annual growth rate (CAGR) of 13.6% between 2022 and 2029, which is expected to rise from $777.4 million in 2022 to $1,895.1 million by 2029, due to increasing health alarms [19]. People are interested in using these lipid nanoparticles because of the presence of omega-3 and omega-6 as main components. Therefore, lipid-based nanoparticles have huge potential for use in pharmaceutical, healthcare, dietary supplements, functional food/beverages, and cosmetics/personal care sectors. SLNs share the market with a market share of 45% [20].

2. Types of Nanoparticles

Based on their compositions, nanoparticles can be categorized into inorganic/polymeric/lipid nanoparticles. Owing to their electrical, physical, optical, or magnetic properties, inorganic nanoparticles have excellent stability and the ability to deliver therapeutics, diagnostics, etc. [21]. Metallic nanoparticles such as gold (AuNps) [22], silver (AgNps) [23], copper (CuNps) [24], iron (FeNps) [25], and silica (SiNps) [26] have found applicability in the vast biomedical sector because of their exceptional biochemical, optical, and electrical properties. However, these inorganic nanoparticles have low aqueous solubility and threaten toxicity concerns, limiting their clinical use [21].

Similarly, polymeric nanoparticles such as polymeric micelles [27], dendrimers, and micro/nanospheres have their share in the delivery of medications, proteins/peptides, and diagnostics. Because of their composition of biodegradable, biocompatible material, surface modification attributes, and ability to deliver both hydrophilic and lipophilic drugs at the application site, they remain the first-choice delivery vehicle [28–31]. However, toxicity concerns, aggregation of particles, and stability issues restrict their use as delivery partners [32].

Lipid-based nanoparticles such as liposomes, niosomes, micelles, and nanoemulsions were developed keeping in mind the necessity of efficiently delivering poorly soluble, low-permeability drugs across the biological membranes, especially through the GIT. The use of natural or synthetic lipids renders them quick formulation and scale-up ability and easy evaluation using various techniques. Versatile delivery via various routes, biocompatibility, augmented drug-loading efficiency and controlled release, and minimal fluctuations in the plasma profile of the drug make them ideal drug delivery systems. Even with such exciting attributes, these lipid-based drug delivery systems suffer from stability issues both *in vitro* and *in vivo*. Shelf-life degradation, degradation in the acidic environment of the stomach, and enzymatic degradation due to bile salts are the major drawbacks that hinder their efficacy.

Considering this, Muller et al. devised a more stable, nanosized lipid-based drug delivery system comprising solid lipids dispersed in water and stabilized with the aid of surfactants and cosurfactants. They utilized various

biodegradable/biocompatible, natural lipids for the fabrication of lipid nanoparticles and termed them solid lipid nanoparticles (SLNs) [33, 34]. SLNs can be easily absorbed through the GIT because of their lipidic nature; thus, they possess an enhanced intestinal permeability, possess bioadhesive attributes to the intestinal wall, and can also kindle intestinal lymphatic transference, ultimately leading to the expansion of the bioavailability of both hydrophilic and hydrophobic drugs. SLNs can hold a high drug payload, tunable drug release (controlled/sustained), augmented drug stability, sterilizable formulations, cost-effective scale-up, and diverse applicability via oral, pulmonary, ocular, transdermal, and intravenous administration [28]. In addition, they can adapt to resistance during first-pass metabolism because their byproducts or metabolites are substantially innocuous and are normally excreted [35].

SLNs have been extensively used in the delivery of nutraceuticals, pharmaceuticals, and therapeutic delivery engaged in transporting drugs [36], biomacromolecules (polysaccharides etc.), genetic components [37], vaccines [38], anticancer drugs [39–41], radionuclide therapy and theranostics [42], and antimicrobial agents [43]. They are also employed in the delivery of drugs across the blood-brain barrier [44, 45].

Even with such exhilarating attributes and implications, SLNs composed of solid lipids face challenges in terms of stability. Solid lipids undergo polymorphic transformation due to their crystalline nature at low temperatures. These lipids crystallize into α and β' form after formulation and into β_i and β form during storage. The drug is sandwiched between the free fatty acid chains or in the amorphous cluster in the crystal imperfection of the solid lipid matrix. During storage, these imperfect crystals transform into perfect crystalline structures, allowing little space for the entrapped drug, which ultimately leads to drug leaching and an irrational drug release mechanism. In addition, physical instability, inadequate loading efficiency, and poor absorption of hydrophobic drugs are other major hindrances incidental to the utility of solid lipid nanoparticles [13, 46].

3. Nanostructured Lipid Carriers (NLCs)

Lipid-based nanoparticles such as SLNs and NLCs have revolutionized the way of hydrophobic drug delivery. With the abovediscussed drawbacks, SLNs have limited scope in enhancing the solubility of poorly aqueous soluble medications, and thus, NLCs have emerged as second-generation lipid nanoparticles [47]. NLCs are colloidal structures comprising a blend of both solid and liquid lipids, prepared with various techniques, resulting in the construction of an unstructured lipid matrix [47]. NLCs have several advantages over other lipid-based delivery systems, such as low toxicity due to the employability of biodegradable/biocompatible lipids, minimal use of surfactants/cosurfactants, ease of manufacture using low-cost material, ease of sterilization, and large-scale production [16]. Furthermore, the advantages of NLCs can be elaborated to high entrapment efficiency and drug payload, customized release, safeguarding the encapsulated contents from acidic and enzymatic depletion in the GIT, and bypassing hepatic metabolism.

NLCs also block P-gp efflux of the therapeutic agent, thereby sustaining drug availability in the systemic circulation. Active transport of the drug at the site of action can also be achieved by ligand-mediated NLCs, thereby lessening the side effects and toxicity of medications as the ligand engages the receptor-mediated pathway and thus facilitates the internalization processes via endocytosis and translocation of attached NLCs or enter by disrupting the intestinal membrane [48]. The presence of liquid lipids alters the core structure of NLCs, causing a commotion in the lattice structure and creating an imperfect crystal lipid structure. These disrupted structures can accommodate a high amount of drugs in the oil. Figure 1 illustrates the numerous benefits associated with oral drug delivery using NLCs.

3.1. Types of NLCs. Based on the fabrication process and the combination of lipidic composition, NLCs can be classified into imperfect, amorphous/structureless, and multiple types.

3.1.1. Imperfect NLCs. They are fabricated by blending a variety of lipids, such as glycerides, which aid in lipid crystallization along with a minimal amount of oils, creating many voids and spaces that can lodge medications in shapeless collections. High drug loading can be attained by augmenting imperfections by using a mix of glycerides of diverse saturation and varying carbon chain length [49, 50].

3.1.2. Amorphous NLCs. They hold a noncrystalline solidified lipid matrix with special lipids, such as medium-chain triglycerides, hydroxy octacosanol, hydroxystearate, isopropyl myristate, or dibutyl adipate which render them a structureless amorphous form, thus averting the development of β-modification. This deters drug eviction from the NLCs throughout long-term storage [49–51].

3.1.3. Multiple NLCs. They encompass oil nanocubicles or compartments distributed in the solid lipid matrix. High liquid lipid composition is used with the idea of solubilization of lipophilic medications, rendering them with high drug content. High lipid content causes phase separation and creates nanocubicles in the solid lipid matrix which prolongs the release of the drug [52]. Multiple NLCs exhibit outstanding drug entrapment efficiency with minimal drug expulsion. High drug content can be ascribed to the structural homogeneity between the two lipids, whereas high entrapment efficiency can be accredited to the imperfections in their structures along with solidification where large amounts of drug could be entrapped [1, 11, 53]. Figure 2 describes the assembly of solid and liquid lipids that create imperfect, amorphous, and multiple NLCs.

3.2. Composition of NLCs. NLCs comprise a lipid phase that is a blend of both solid and liquid lipids in varying ratios (99.9 : 0.1 and vice versa) formed with the aid of emulsifiers (1.5–5% w/v) [1]. A wide variety of both solid and liquid

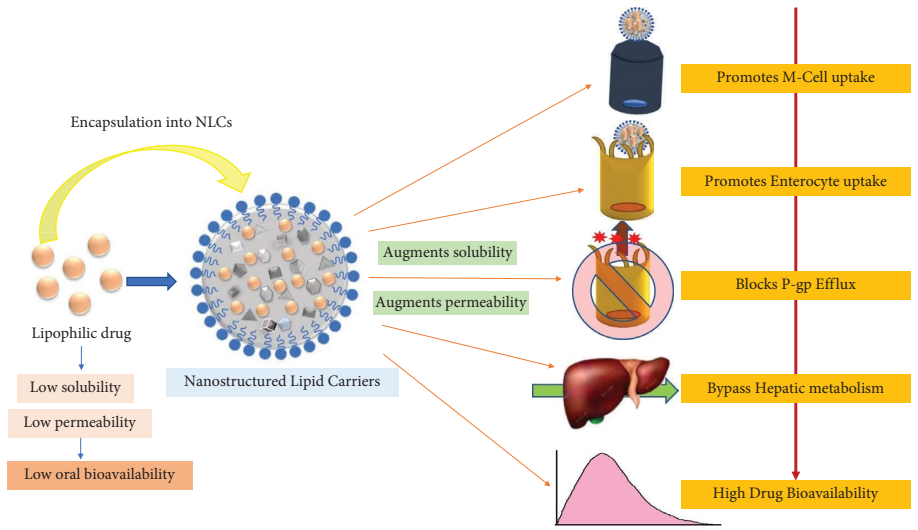

Figure 1: Advantages of NLC-based oral drug delivery.

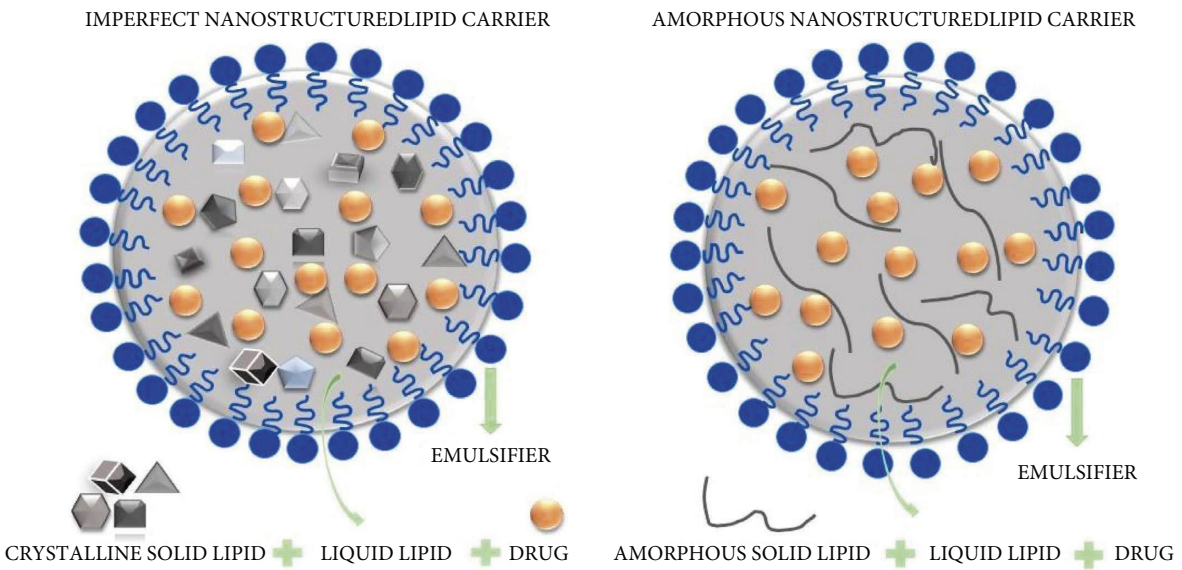

Figure 2: NLC classification based on the lipid matrix structure.

lipids are employed in the fabrication of the lipid phase which creates an imperfect solid lipid matrix. NLCs are identical to SLNs, in which lipids are dispersed in water as a continuous phase and are stabilized by emulsifiers. In NLCs, like SLNs, solid lipids are added to the liquid lipids that remain in a liquid or amorphous state, rendering them stable both *in vitro* and *in vivo* [16]. Liquid lipids partly disrupt the matrix assembly and create crystal-disordered structures in NLCs with voids and spaces to entrap high quantities of therapeutics compared with SLNs [54]. The lipid matrix avoids any polymorphic alteration of solid lipids by inhibiting recrystallization, enabling them to efficiently encapsulate the drug [13, 29].

A wide variety of lipids are employed in the fabrication of NLCs that impact their overall performance in the enhancement of oral bioavailability of poorly soluble drugs using various mechanisms. Selection could be made depending on physiological tolerance, physicochemical properties, drug solubility, and miscibility in solid and liquid lipids [42]. The choice of solid and liquid lipids should be carefully scrutinized by inspecting their compatibility and miscibility by inspecting the macroscopic lipid phase homogeneity/parting below the melting point of fat. The selected lipids should be biodegradable/biocompatible (GRAS: generally recognized as safe), withstand environmental/temperature conditions, and be able to solubilize a wide variety of drugs. If not, lipid should be versatile enough to attach the drug molecule at the surface of the nanoparticle or be able to integrate the drug into micelles in the aqueous phase. Ashkar et al. described various structured edible lipids that could be used in the fabrication of lipid-based nanoparticles, which can upgrade membrane permeability and ameliorate the solubility of therapeutic agents [55].

Elmowafy et al. reported Miglyol incompatibility with Suppocire A, Geleol, Cacao Butter, and Witepsol E75. Miglyol was found to be compatible with Compritol 888 ATO and Gelucire$_{43/01}$ [56]. Garg et al. fabricated aceclofenac-loaded NLCs using three different methods and observed formulation effects on entrapment efficiency. Their lipid phase consists of solid lipids (glyceryl monostearate (GMS), stearic acid (SA), and cetyl alcohol (CA)), liquid lipids (Transcutol, Labrafac, and Labrasol), and surfactants and cosurfactants (ethanol, poloxamer, and tween 80). They employed homogenization, probe sonication, and sonication with homogenization methods to prepare NLCs. High entrapment efficiency (>80%) and uniform size (151.5 ± 11.5) were observed in the latter method of NLC-3, indicating the solubility of aceclofenac in the CA-lipid matrix. Other lipids and preparation methods displayed entrapment efficacy between 60 and 80% and higher particle sizes [57]. Jyoti et al. fabricated paclitaxel-laden Precirol®ATO-5 and Capmul MCM NLCs with high drug entrapment efficiency and extended drug release. They reported that the high entrapment efficiency of PTX-NLCs (>80%) could be attributed to the formation of liquid nanocompartments created by liquid lipids and the action of surface active agents used in formulations [58].

Varying chain lengths of fatty acids and triglycerides affected the particle size of NLCs on the augmented flexibility of internal lipids and fluidity of the emulsifier film. Long-chain fatty acids (lauric acid as solid lipid) generated optimum-sized particle rosuvastatin NLCs (192 ± 2.5 nm) with a %entrapment efficacy of 88.06 ± 5.24% in comparison to medium-chain fatty acids (stearic acid), which produced NLCs of particle size 198 ± 3.4 and a %entrapment efficacy of 92.81 ± 3.74%. Long-chain fatty acid NLCs augmented the oral bioavailability of rosuvastatin by 1.5 fold compared with medium-chain fatty acid-composed NLCs, while both displayed ten times higher C_{max} than pure rosuvastatin aqueous dispersion [59]. Many studies [53, 60–63] have described the use of various solid and liquid lipids along with surfactants in the fabrication of NLCs (refer to Table 1).

The abovelisted components aid in the formulation of NLCs and further add commendable attributes in drug entrapment/loading efficiency, drug release characteristics, stability, and both *in vitro* and *in vivo*. Solid and liquid lipids directly impact the particle size of nanoparticles, control the drug release pattern, and also improve the solubility and bioavailability of poorly soluble drugs.

Raquel Vieira et al. used sucupira oil for the control of diabetes and used it as a liquid lipid in the fabrication of NLCs. They used Imwitor 900 K (glycerol monostearate, type II), Dynasan 116 (tripalmitin), Kolliwax GMS II (glycerol monostearate), Compritol 888 ATO (glyceryl dibehenate), and cetostearyl as the solid lipid components of NLCs. As surfactants, they used d-α-tocopherol polyethylene glycol succinate, vitamin E polyethylene glycol succinate or vitamin E-TPGS, poloxamer 188, Tween® 80, and optimized sucupira oil loaded NLCs by a 2^2 factorial design to establish the correct blend of solid/liquid lipids and developed using a hot high-pressure homogenization technique. Various solid lipids imparted effects on the particle size and polydispersity index with good loading (9.6%)/entrapment efficiency (99.98%). D-α-tocopherol polyethylene glycol succinate ensured a stable formulation, while cell cytotoxicity studies against Caco-2 cell lines exhibited >90% cell viability, rendering sucupira oil-loaded NLCs to be nontoxic [64].

3.3. Fabrication processes of NLCs. Several methods are available to fabricate NLCs, depending on the input of energy such as high/low-energy (Figure 3) emulsifiers and solvents.

3.3.1. High-Energy Method. This method involves hot and cold high-pressure homogenization tactics to develop NLCs. Another method that falls under this category is the high-shear/high-speed homogenization method.

(1) Hot High-Pressure Homogenization Method. Here, solid lipids are melted by heating usually 5–10°C above the melting temperature, and then, liquid lipids are added along with the drug(s), followed by adding this dispersion to a hot surfactant solution in water [65]. The mixture is then homogenized using high pressure (100–2000 bar), leading to the formation of a hot oil in water primary emulsion, which after cooling (liquid nitrogen or dry ice) settles into a form (NLCs) [66]. Elevated temperatures help in reducing the particle size as the viscidness of lipids gradually drops. To

Table 1: List of solid/liquid lipids, surfactants, and surface modifiers commonly used in the fabrication of NLCs.

Solid lipids	Melting point (°C)
Stearic acid	67–69
Behenic acid	80
Palmitic acid	83
Lauric acid	43
Carnauba wax	78–88
Goat wax	40–50
Beeswax	62–64
Theobroma oil	35–37
Tristearin/glyceryl tristearate	70–74
Cetyl palmitate	54
Glyceryl monostearate	54–64
Imwitor 372P, 491, 900k, 928	61–77, 34
Glyceryl palmitostearate	50–60
Glyceryl behenate	65–77
Trilaurin (Dynasan 112)	43–46
Trimyristin (Dynasan 114)	55–58
Tristearin (Dynasan 118)	70–73
Tripalmitin (Dynasan 116)	61–65
Tribehenate (Dynasan 122)	81–85
Hydrogenated palm oil (Dynasan P60)	58–62
Hydrogenated palm oil (Softisan 154)	53–58
Softisan 100, 138, 142, 154, 378, 601, 645, 649	33–58
Witepsol (E, W, S, H)	31–44
Liquid lipids	**Viscosity (mPas at 20–30°C)**
Miglyol (808, 810N, 812N, 818)	23–33
Capric acid	25–32
Caprylic acid	26–32
Oleic acid	40
Fatty acid esters	6.57
Propylene glycol fatty acid esters	6.98
Mineral oil	95–100
Vitamin E	NA
Olive oil	85
Castor oil	390
Palm oil	130
Coconut oil	85
Soybean oil	55
Jojoba oil	33.3
Mustard oil	117
Garlic oil	80
Clove oil	9
Emulsifiers/coemulsifiers	**HLB value**
Polysorbate 20, 80	15–17
Solutol HS	15
Poloxamer 188	29
Poloxamine 908	31
Cremophor El	12–17
Sodium cholate	18
Sodium dodecyl sulfate	40
Polyvinyl alcohol	18
Sodium oleate	18
Soy lecithin	4
Egg lecithin	6.6
Lecithin	3–5
Surface innovators	
Wheat germ agglutinin	
Hyaluronic acid	
Mannose	
β-d-galactosides	
Ferritin	
Transferrin	
Biotin	
L-arginine	
Oligochitosan	
Polyethylene glycol	
Folic acid	

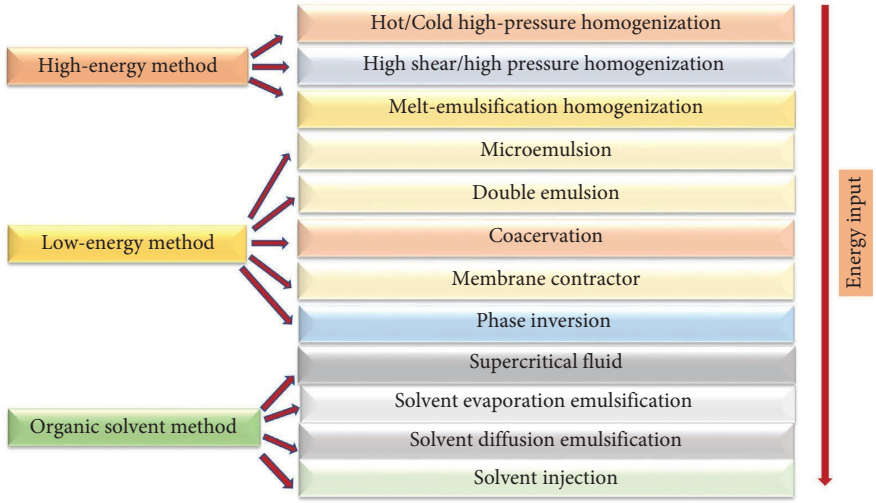

Figure 3: Various methods for the preparation of NLCs.

obtain a narrow particle size distribution, the emulsion is usually subjected to ultrasonication.

(2) Cold High-Pressure Homogenization Method. The cold high-pressure homogenization method involves the melting of solid/liquid lipids/drug(s) and solidification by liquid nitrogen or dry ice [34]. The mixture is then milled and assorted to a cold surfactant solution, resulting in the formation of a presuspension, which is then subjected to high-pressure homogenization (5–10 cycles, 1500 bar pressure), leading to the formation of NLCs [61, 67]. Both these methods offer advantages such as the use of minimal toxic solvents, easy scale-up technique, and quick NLC formulation.

(3) High Shear/High-Pressure Homogenization Method. Solid and liquid lipids are melted 5–10°C higher than their melting points and then mixed with the drug. To this, simultaneously heated to an equivalent temperature, a surfactant solution is added. This mixture is then homogenized at a higher shear pressure to yield low-particle-sized hot oil in water nanoemulsion, which after cooling and ultrasonication settles into a homogeneous NLC formulation [58, 59].

(4) Melt Emulsification Homogenization Method. This method involves dispersing solid and liquid lipids along with the drug into an aqueous surfactant solution, which is then subjected to probe sonication. Later, the blend is cooled to obtain NLCs [61].

3.3.2. Low-Energy Method

(1) Microemulsion Method. This method involves a simpler method to fabricate NLCs in which the molten lipid is blended into the mild hot liquid lipid along with the addition of medication. Then, under constant stirring, the aqueous emulsifier solution and lipid blend are added to the melted lipid mix, leading to the formulation of microemulsion. The microemulsion is disseminated instantly in ice-cold water (0–4°C) around 20–50 times the volume of the microemulsion, which leads to the precipitation of microemulsion globules, creating NLCs [67]. Ice-cold water supports the formation of smaller particles without aggregation and homogeneous preparation. High-volume water may lead to dilution, which may be countered by lyophilization. Although the method is simple, it requires a high volume of emulsifiers and coemulsifiers [68].

(2) Double Emulsion Method. The aqueous phase containing the hydrophilic drug is dispersed into the organic phase (melted solid lipid and liquid lipid) forming primary water in oil emulsion. This primary emulsion is again dispersed into the aqueous phase, forming a w/o/w double emulsion in which the hydrophilic drug is enclosed in the inner watery continuous phase [69]. This method involves a solvent evaporation method but generates large particle-sized nanoparticles.

(3) Membrane Contractor Method. In this method, the melted lipid is pressed through the pores of the membrane at a pressure that leaves the temperature of the system higher than the melting points of the lipids used. The lipid globules coming out of the pores are distributed away by the aqueous surfactant under turbulence, flowing just below the membrane. Upon cooling at room temperature, NLCs are formed. The process is complex and involves the use of sophisticated instruments, and particle size depends on the rate of flow of the watery phase, its temperature, lipid phase pressure, and membrane pore size that may be susceptible to blockage [68].

(4) Phase Inversion Method. The phase inversion method involves two-step processes, where the first step involves formulation of w/o emulsions by integrating lipids, water, and emulsifiers at elevated temperatures (up to 85°C). Then, phase inversion is attained (from w/o to o/w emulsion) by sudden cooling of the emulsion with constant stirring followed by a further drop in temperature by adding cold water

(0°C). This causes the transition of minute lipid droplets to recrystallize into nanolipid carriers. The stability of such a system relies on the regulation of the temperature cycle [70–72]. This method produces particles below 50 nm but involves a high ratio of emulsifiers. In addition, this method is cost effective and is devoid of toxic solvent involvement.

(5) Coacervation Method. This method allows thermosensitive lipids to be developed as NLCs without the use of toxic solvents. Here, an amphiphilic emulsifier is added to the lipidic blend in an acidic environment (coacervation solution) to form NLCs [68].

3.3.3. Organic Solvent Employed Method

(1) Solvent Evaporation Emulsification Method. This method involves the use of water-immiscible organic solvents, such as example dimethyl sulfoxide (DMSO), chloroform, cyclohexane, and dichloromethane (DCM), in which drugs and lipids are dissolved. The blend is then stirred in an emulsifying aqueous phase and further sonicated or homogenized to obtain a homogeneous NLC formulation with uniform particle size and size distribution [73]. The use of an organic solvent is an obvious disadvantage.

(2) Solvent Diffusion Emulsification Method. Water-mixable organic solvents are employed, for example, methanol, ethanol, acetone, benzyl alcohol, and ethyl formate, to dissolve the lipids and drug. The process involves sonication of the mixture at an elevated temperature to create a distinct lipid phase. Subsequently, this lipid phase is blended with an aqueous surfactant solution, which is also maintained at a similar temperature as the lipid phase, with continuous stirring. Dispersion was stirred at room temperature to cool off and evaporate the organic solvent to obtain nanosized lipid carriers [74, 75].

(3) Solvent Injection Method. The method is quite similar to the solvent diffusion method where water-miscible organic solvents are used. The difference is that lipid is injected into the aqueous surfactant solution, and the globules are injected out of the needle [76]. The surfactant solution was kept under turbulence using a stirrer to aid in the quick solubilization of the lipid. The emulsion so formed is filtered to eradicate superfluous fat. The selection of solvent and surfactant concentrations impacts the size and size distribution proportionately. The method itself is unique as it allows the formulator to use simple techniques without the use of sophisticated instruments [77].

(4) Supercritical Fluid Method. The medication and lipids are solubilized in an organic solvent with the emulsifier, leading to the formation of an organic solution. This is then dispersed into the watery phase, followed by high-pressure homogenization, which creates an oil-in-water emulsion. Lipid nanoparticles are formulated by injecting the o/w emulsion from the top of an extraction column while simultaneously introducing a supercritical fluid, typically carbon dioxide, at a constant flow rate to ensure complete solvent removal [78, 79].

3.4. Improvisation of Fabricated NLCs

3.4.1. Surface modification of Lipid-Based Nanoparticles and NLCs. Surface modification is intended for site-specific delivery of the drug at the target and cellular sites. For this, innovators are attached to the NLC surface via some linkage such as succinic acid-PEG, folate PEG-Chol, or by linking the coating agent such as oleoyl-quaternized chitosan to the NLC component [80]. Polymers such as Eudragit RS100 [81], polyacrylamide, poly(ethylene oxide)-modified poly(epsilon-caprolactone), or polyvinylpyrrolidone [82], antibodies such as anticarbonic anhydrase (CA) IX [83], proteins such as low-density lipoproteins [84], and aptamers, for example, HER2 and ATP, have been used as guiding moieties to deliver therapeutics to the target sites [85]. Small molecules such as folate and transferrin are also employed as the targeting agents in NLCs [86]. Priya Sakshi et al. discussed the role of various surface modifiers that can guide various lipid-based nanoparticles such as SLNs and NLCs to the target sites for the effective delivery of medications [87].

Targeted delivery of drugs with poor solubility is another challenge that can be effectively attained by employing NLCs, as these can play a major role in cancer therapy. Alicia Fernandez-Fernandez et al. discussed the potential of nanotherapeutics in the management of cancer [88]. Folic acid-conjugated chitosan-modified NLCs were used to transport umbelliprenin to cancer cells. Modified NLCs reduce the expression of angiogenesis genes and tumor volumes over 19 days [89]. Drugs such as curcumin have been successfully targeted for brain delivery via transferrin conjugation with nanolipid carriers [90].

Orally administered doxorubicin was efficiently targeted to breast cancer cells by folic acid-coupled NLCs [91]. Luiz discussed the use of NLCs treating cancer via hybrid magnetic lipid-based nanoparticles [92]. Supramagnetic iron oxide magnetic NLCs have been employed in the accurate targeting of hepatocytes, providing a ray of hope for future magnetic resonance contrast imaging tools in the detection of liver diseases [93]. Ascorbyl palmitate (AP), a lipophilic derivative of ascorbic acid, is formulated in NLCs that are integrated into magnetic nanoparticles for cancer treatment [94].

3.4.2. Hydrophobic Ion Pairing/Surface Charge Adjustment. The surface charge on nanoparticles plays a critical role in their stability and cellular uptake. Factors such as flocculation, creaming, coalescence, sedimentation, and Ostwald ripening directly affect the stability of nanoemulsions. Surface modification of lipid carriers with charged ionic compounds maintains electrostatic repulsion. In addition, positively charged ions allow extended retention time at the negatively charged biomembrane surface, creating an enhanced cellular uptake environment.

In a study, NLCs were surface decorated to safeguard encapsulated insulin from enzymatic degradation. Polyethylene glycol ester, polyethylene glycol ether, and polyglycerol ester were used as surfactants to fabricate three NLC formulations using the solvent diffusion method. Insulin lipophilicity was upgraded from (−)1.8 to 2.1, which upheld high drug loading in lipid carriers. The NLC surface possessed a negative surface charge with a size distribution of 0.2–0.5 and nanometer-sized particles ranging between 64 and 217 nm with a polydispersity index of 0.2–0.5 and a negative surface charge. From proteolysis analysis, PEG-ether NLCs provided maximum protection to encapsulated peptide, followed by PG/PEG-ester NLCs, indicating that surface modification aids in safeguarding the peptide from GI proteases. The protection to insulin could be attributed to bond formation between PEG-ether NLCs lacking the ester substructures on surfaces that are susceptible to lipid-digesting enzymes of pancreatin, thus exhibiting resistance to pancreatic lipase and shielding peptide. The protective effect could also be due to the medium-chain length of surfactants and the particle size of the so-formed NLCs [95].

3.5. Characterization Techniques of NLCs. Characterization techniques for NLCs are essential for assessing their physical, chemical, and biological properties. These techniques provide valuable information for optimizing NLC formulations and ensuring their quality. Here are some common characterization techniques for NLCs, which are discussed in details in literature [49, 96, 97]:

(1) Particle size analysis:
   (a) Dynamic light scattering (DLS): DLS measures the hydrodynamic diameter of NLC particles in suspension. It provides information about particle size distribution and polydispersity.

(2) Zeta potential measurement:
   (a) Electrophoretic light scattering (ELS): ELS measures the zeta potential of NLCs. Zeta potential reflects the surface charge of particles and can indicate their stability and potential for aggregation.

(3) Morphological analysis:
   (a) Transmission electron microscopy (TEM): TEM allows for high-resolution imaging of NLCs, providing information about particle shape, size, and morphology.
   (b) Scanning electron microscopy (SEM): SEM can also be used to visualize NLCs although it provides surface morphology information.

(4) Drug loading and encapsulation efficiency: HPLC and UV-Vis spectroscopy are frequently used to quantify the drug content in NLC formulations and to calculate encapsulation efficiency.

(5) Physical stability:
   (a) Centrifugation: centrifugation tests can assess the physical stability of NLC dispersions by monitoring particle sedimentation or creaming.
   (b) Freeze-thaw cycling: repeated freeze-thaw cycles can be used to assess the stability of NLC formulations under stress conditions.

(6) *In vitro* drug release studies:
   (a) Dialysis or membrane diffusion: these techniques are used to study the release kinetics of drugs from NLCs over time, simulating drug release behaviour *in vivo*.
   (b) Franz diffusion cell: this allows for the measurement of drug release from NLCs through a synthetic or biological membrane.

(7) Thermal analysis:
   (a) Differential scanning calorimetry (DSC): DSC can determine the thermal behaviour of NLC components, including lipid melting points and drug-lipid interactions.
   (b) Thermogravimetric analysis (TGA): TGA assesses the thermal stability and decomposition patterns of NLC formulations.

(8) X-ray diffraction (XRD): XRD helps in understanding the crystalline structure of lipid components and any changes in drug crystallinity within NLCs.

(9) Nuclear magnetic resonance (NMR): NMR spectroscopy can be used to study drug-lipid interactions and assess the distribution of drug molecules within the lipid matrix.

(10) Fourier-transform infrared spectroscopy (FTIR): FTIR spectroscopy is used to analyze chemical bonds and functional groups in NLC components and assess drug-lipid interactions.

(11) Rheological analysis: rheological tests can provide information about the viscosity and flow behaviour of NLC dispersions, which is important for formulation stability and administration.

(12) Biological studies:
   (a) Cellular uptake studies: these studies assess the cellular internalization of NLCs loaded with drugs using techniques such as confocal microscopy or flow cytometry.
   (b) *In vivo* studies: animal or human studies can evaluate the pharmacokinetics, biodistribution, and therapeutic efficacy of NLC-based drug delivery systems.

(13) Stability and shelf-life testing: accelerated stability studies can assess the long-term stability of NLC formulations under various storage conditions.

The choice of characterization techniques depends on the specific properties and objectives of the NLC formulation, and a combination of these techniques is often used to comprehensively assess NLCs for drug delivery applications.

3.6. Mechanism behind the Improvement in Oral Absorption of Therapeutics via NLCs. NLCs promote the medication's oral bioavailability by augmenting the uptake of drugs by microfold cells (M-cells) in the intestinal membrane and, also, can bypass first-pass hepatic metabolism. Lipid nanocarriers can be transported across the intestinal wall via several pathways such as transcellular absorption, paracellular transport, P-glycoprotein, and cytochrome 450 inhibition. In addition, lipidic compounds instigate the production of chylomicrons, which help in their transfer across the membranes [98, 99].

Many factors impact the effective absorption of drugs that are encapsulated in lipid nanoparticles. The lipids of NLCs are assimilated partly in the stomach, followed by digestion in the small intestine. Lipids in the stomach stimulate the secretion of gastric lipase enzyme, which hydrolyzes the acyl chain of lipids. The pancreatic lipase and colipase in the small intestine help in the digestion of most lipids, where they are converted into digestible diglycerides and free fatty acids. Lipidic nature protracts the exit of the drug in the GIT, thus augmenting its absorption. Owing to nanosize, the increased surface area aids lipids to stay in contact with the biomembrane, supplementing higher absorption.

The various mechanisms by which NLCs augment the bioavailability of poorly soluble drugs are as follows:

(1) Direct uptake: NLC enhances the bioavailability of lipophilic drugs through intestinal lymphatic transport. By using triglycerides, NLCs can stimulate the formation of chylomicrons, facilitating transcellular absorption. This allows lipophilic drugs to follow the route of the intestinal lymphatic system and bypass the first-pass effect. The hydrolysis of triglycerides is initiated in the GIT, aided by lingual lipase and gastric lipase, resulting in the formation of a triglyceride emulsion. This emulsion, in turn, triggers the secretion of bile salts, pancreatic juice, and biliary lipids. Biliary lipids are adsorbed onto the surface of the triglyceride emulsion and stabilized, and the triglyceride droplet undergoes a transformation through the action of pancreatic lipase. It evolves into monoglycerides and fatty acids, which are subsequently absorbed by enterocytes. These components are then processed to constitute the lipid core of chylomicrons and are further stabilized through the addition of phospholipids and apolipoproteins. These lipoproteins are then secreted into the lamina propria and mesenteric lymph nodes, ultimately entering lymphatic circulation [100, 101].

(2) Adherence to the mucosal membrane: NLC adheres to the mucus, leading to extended residence duration, consequently resulting in an elevated drug release from NLC. The tight epithelial cells of GIT are enveloped by a hydrophilic and negatively charged protective mucus layer, which acts as a barrier, limiting the transit of foreign particles. Nevertheless, scientists have harnessed mucus as a valuable strategy to enhance the plasma concentration and therapeutic effectiveness of drugs. This is accomplished by designing engineered nanoparticles that can adhere to mucus. The binding of nanoparticles to mucus extends their presence in the GIT, making it easier for drugs to passively transport and ultimately improving their absorption [102].

Lipid-based nanoparticles and NLCs with a coating of agents such as positively charged polymers such as chitosan and benzalkonium chloride could bind to the negatively charged intestinal mucosa and thus transport drugs passively [103]. A covalent bond formed between the mucus membrane and thiomers decorated over NLCs aids in the site-specific delivery as the covalent binding of the thiol group of the polymer and cysteine-rich components of the mucous membrane slows down the peristalsis of the intestine [104, 105]. Also, thiomers can bind to the transmembrane domain of P-gp and could improve the delivery of P-gp substrate drugs when conjugated with NLCs [106].

(3) Upsurged permeability: NLCs are comprised of surfactants that alter intestinal permeability through various mechanisms. For instance, the surfactant poloxamer induces structural changes in the cell membrane, resulting in the opening of tight junctions in intestinal epithelial cells, thereby facilitating paracellular transport. In addition, it inhibits P-glycoprotein efflux, consequently enhancing the transport of NLC [107, 108].

(4) Formation of mixed micelles: Lipid content in NLC induces bile secretion within the small intestine. As enzymes degrade this lipid, it combines with bile to create mixed micelles. NLCs trigger the secretion of bile, bile salts, phospholipids, and cholesterol from the gall bladder, leading to the formation of micelles, averting lipid precipitation, and thus easing NLC lipid and drug solubilization. They also aid in the carrier transfer across the stationary layer lying amid the intestinal bulk fluid and the brush-border membrane of enterocytes, further enhancing the absorption of the medication [49, 109, 110].

(5) Bypassing first-pass metabolism: Well-designed NLCs can serve as a delivery system, effectively shielding drugs from early degradation while they traverse the GIT, thereby evading the effects of first-pass metabolism. NLCs engage with bile salts within the GIT, resulting in the formation of mixed micelles. The lymphatic system selectively takes up these micelles, effectively bypassing the liver [111]. In addition, these mixed micelles facilitate the solubilization of lipid digestion products within the gut

lumen, establishing a concentration gradient that aids absorption. This versatile capability of NLCs to bypass hepatic metabolism enhances the therapeutic efficacy of drugs that undergo extensive liver metabolism while reducing their dosing frequency and associated dose-related side effects [102].

Figure 4 illustrates the diverse processes through which NLCs are absorbed within the GIT, their ability to circumvent first-pass metabolism, and their capacity to maintain stability against both acidic conditions and enzymatic degradation.

3.6.1. Drug Release Mechanism from NLCs. Drug release from a nanoparticulate formulation can follow the diverse processes that rely primarily on their composition. These mechanisms may be categorized into matrix erosion, diffusion, or swallowing, where lipid nanoparticles exhibit matrix diffusion and erosion release patterns [112].

Particularly in NLCs, the liquid lipid component incidents into a highly unordered lipid-matrix structure that could incorporate a high amount of medications and restrict drug exile. Typically, the drug might be situated in the middle of fatty acids, within lipid layers, or even within structural irregularities such as amorphous regions. When dealing with lipid structures that closely resemble more organized matrix molecules, particularly when utilizing extensively purified monounsaturated glycerides such as tristearin, the drug-loading capacity is notably constrained, leading to drug release within hours to a few days. The mechanism of drug release from NLCs involves various factors and processes, which are as follows:

(1) Diffusion: Drug molecules can diffuse through the lipid matrix depending on their solubility in lipids. Lipid diffusion is generally slower than aqueous diffusion, which contributes to controlled release.

(2) Matrix erosion: Over time, the lipid matrix can erode or degrade due to factors such as water penetration, enzymatic activity, or hydrolysis of lipid components. As the matrix erodes, it releases the encapsulated drug.

(3) Partitioning and solubility: The drug's solubility in the lipid matrix plays a significant role in its release. If the drug has a high affinity to the lipid matrix, it may be released slowly. Conversely, if the drug is more soluble in the surrounding aqueous environment, it can be released quickly.

(4) Lipid mobility: The mobility of lipid molecules within the matrix affects drug release. Higher mobility can lead to faster drug release as the lipid matrix becomes more permeable.

(5) Surfactants and cosurfactants: Surfactants and cosurfactants are often added to NLC formulations to improve drug loading and release. These additives can alter the lipid matrix's structure, affecting drug release kinetics.

(6) Particle size: The size of NLCs plays a role in drug release. Smaller particles generally have a larger surface area-to-volume ratio, leading to faster drug release than larger particles.

(7) pH and ionic strength: Changes in the pH and ionic strength of the surrounding environment can influence drug release from NLCs. These factors can alter the stability of the lipid matrix and affect drug partitioning.

(8) External stimuli: Some NLC formulations are designed to respond to external stimuli, such as temperature, light, or magnetic fields. These stimuli can trigger changes in the lipid matrix's structure and, consequently, drug release.

(9) Targeting ligands: NLCs can be engineered to include targeting ligands on their surface, allowing them to specifically bind to certain cell types or tissues. This can influence the interaction between the carrier and the biological environment, thereby affecting drug release at the targeted site.

In summary, drug release from NLCs is a complex process influenced by the composition of the lipid matrix, drug solubility, particle size, environmental factors, and potential external stimuli. These factors can be tailored to design NLCs with specific drug release profiles for various therapeutic applications.

3.7. Applications of NLCs

3.7.1. In Vivo Enhancement of the Bioavailability/Solubility of Lipophilic Drugs. The mechanism could be explained by Figure 4, where NLCs are depicted to remain stable from enzymatic degradation, proteolysis, and bowel peristalsis following absorption. After coming in contact with the mucus layer in the GIT, lipids are absorbed via various pathways such as transcellular, paracellular, and lymphatic transport. The enterocytes of the villi help in the absorption of lipids via transcytosis and endocytosis by passing through the apical cell membrane into the cell [113, 114]. Many findings demonstrate that lipid-based nanoparticles significantly ameliorate the oral solubility of hydrophobic drugs.

Because of their lipid nature, large surface area, and bioadhesive attributes, NLCs are better absorbed in the lymphatic system. Fathi et al. developed simvastatin-loaded NLCs to improve the oral bioavailability (>5% in neat form) of the loaded antihyperlipidemic drug. They prepared the formulation using an emulsification solvent evaporation technique and stated that preparation abridged total cholesterol and low-density lipoprotein cholesterol levels, whereas drug nanosuspension failed to lower cholesterol levels. In addition, a four-fold augmentation in the oral bioavailability of simvastatin by NLCs was reported [115].

Nintedanib (BIBF), an oral triple tyrosinase inhibitor, is a P-glycoprotein substrate that undergoes hepatic first-pass metabolism that may cause efflux during intestinal absorption, leading to its poor bioavailability. Yunjing Zhu explored the potential of NLCs for improving the intestinal absorption and oral bioavailability of BIBF. They reported

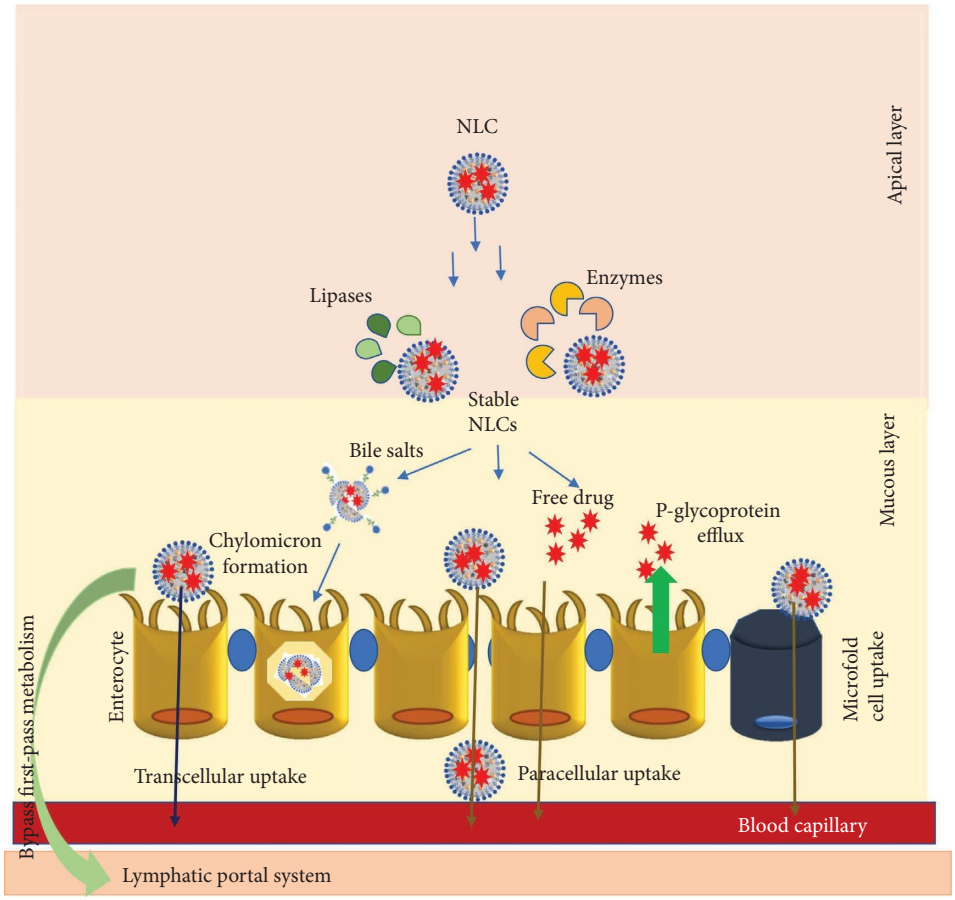

Figure 4: The drug-loaded NLC absorption pathway via the intestinal wall.

that BIBF-NLC-1 and BIBF-NLC-2 augmented the oral bioavailability by 3.13- and 2.39-fold, respectively. The tiny particle size of BIBF-NLC-2, measuring 7.99 ± 0.06 nm, presented a large surface area. This, in turn, caused the nanoparticles to agglomerate within the rat intestine, thereby reducing necessary bending energy during endocytosis. Hence, agglomerated small-sized particles may have been taken up by clathrin-mediated endocytic pathways [116]. BIBF-NLC absorption could also be aided by the degradation of lipid nanoparticles by local enzymes that might have formed mixed micelles along with bile salts, which can be easily absorbed by the intestine [1].

Gefitinib (GEF), with a log *P* value of 3.2, is a hydrophobic drug with low bioavailability. GEF-NLCs [117] were developed for the treatment of metastatic lung cancer as lipidic carriers that upgraded the lymphatic uptake of drug-loaded NLCs by enterocytes through receptor-mediated endocytosis or phagocytosis. This could be due to lymphatic uptake by M cells of small particles below 500 nm [118, 119]. Praziquantel (PZQ) is the preferred drug for treating human schistosomiasis [120]. However, its hydrophobic nature limited dissolution properties with inconsistent and low bioavailability following oral administration [121]. Said et al. investigated the effect of charge on PQZ-NLCs for its oral bioavailability enhancement and effective schistosomicidal activity after oral administration and compared its efficacy with aqueous drug suspension. Upon oral administration, positively charged PQZ-NLCs showed a significant reduction in the number of ova cells (90%) and granuloma size and number (62.1% and 42.55%) in the histopathological examination of the Swiss albino male mice compared to the PQZ suspension, negatively charged PQZ-NLCs, and traditional drug-loaded NLCs. SEM micrographs demonstrated improved ultrastructural changes in histopathological features of the liver of the *S. mansoni*-infected mice when compared with other formulations. These changes can be attributed to electrostatic interactions that permitted higher concentrations of PQZ to interact more with the worm surface by positively charged PQZ-NLCs [122].

Etxebeste-Mitxeltorena et al. in their investigation developed 2m-NLCs for the treatment of visceral leishmaniasis. 2 m is a trypanothione reductase (TR) inhibitor and is categorized as a BCS class IV drug. The group developed 2 m loaded glyceryl palmitostearate and diethylene glycol monoethyl ether-based NLCs (2m-NLCs) for oral bioavailability enhancement. Loading 2 m into NLC significantly improved its intestinal permeability and resulted in plasma levels surpassing its effective concentration (IC_{50}). In BALB/c mice infected with L. infantum, 2m-NLC achieved a reduction of at least 95% in parasite burden in the spleen, liver, and bone marrow after 5 doses, showcasing comparable effectiveness to intravenous administration of the marketed formulation (Fungizone) [123].

Velmurugan and Selvamuthukumar developed NLCs for the oral delivery of ifosfamide (IFS) using response surface methodology. Their *in vitro* study demonstrated a gradual release of ifosfamide from the NLCs over a 72-hour period, suggesting that IFS-NLCs are capable of delivering an initial dose and maintaining prolonged plasma levels *in vivo* [124]. Table 2 illustrates the numerous oral solubility enrichment studies undertaken by these versatile carriers.

3.7.2. NLCs for Site-Specific Delivery of Medications at the GIT. NLCs have been extensively used to enhance low solubility and permeability across the intestinal membrane. Shrestha et al. verified NLC passage capacity across the intestine via Caco-2 intestinal monolayer cells (enterocell-like models). The researchers encapsulated two peptides exenatide and liraglutide in NLCs and conjectured that these can activate endogenous glucagon-like peptide-1 (GLP-1) secretion. Exenatide was completely released because of its hydrophilic nature owing to its higher affinity to bile salt-rich fasted state simulated intestinal fluids. Liraglutide release from Lira-NLC was limited due to the high hydrophobic interactions between the fatty acid chain in the liraglutide structure and the lipidic matrix. Placebo and loaded NLCs induced GLP-1 secretion from enteroendocrinal L-cells (GLUTag) under *in vitro* conditions. NLCs exhibited a 2.9-fold increase in the permeability of exenatide across the Caco-2 intestinal monolayer and can also act as GLP-1 agonists [141].

In a similar study, oral absorption of khellin, a natural pleiotropic molecule, was tested *ex vivo* using Caco-2 cell lines. Khellin-loaded stearic acid, hempseed oil, Brij S20, and Labrafil M 1944 CS-composed NLCs were developed by the emulsification-ultrasonication method. Studies have reported a two-fold increase in the membrane permeability of khellin by nanolipid carriers, suggesting the possibility of oral bioavailability enhancement [142].

In situ intestinal absorption studies conducted by Dudhipala et al. showcased the superiority of NLCs compared with SLNs in transporting nisoldipine across the rat intestine. Nisoldipine-loaded NLCs exhibited a permeation coefficient of $2.95 \pm 0.025 \times 10^{-2}$, whereas SLNs showed $2.36 \pm 0.018 \times 10^{-2}$ in comparison to the nisoldipine nanosuspension permeation coefficient of $1.02 \pm 0.015 \times 10^{-2}$. This indicated that drug-loaded lipid carriers could circumvent the intestinal barriers while transporting the drug across the biomembrane. An enhancement ratio above 1 marked an enhanced perfusion rate of NLCs and SLNs over nisoldipine nanosuspensions. In addition, drug permeation was augmented twofold as the drug accumulated in the intestinal folds of the rats.

A 2.46- and 2.24-fold increment in the bioavailability of nisoldipine was more pragmatic by NLCs and SLNs than drug suspension. The oral bioavailability of the medication for NLCs was superior by 1.09 times that of SLNs as the lipid composition and size of NLCs persuaded longer duration adhesion to the GI tract. They further explained the role of formulation excipients in the increment of permeation, drug transport, and uptake of lipid content by the lymphatic system, thus minimizing drug degradation by the first-pass effect [143].

Nintedanib esylate (NE) is a poorly soluble drug with extremely low oral bioavailability. Being a P-gp substrate, it undergoes hepatic metabolism by esterases. Thus, to evade these glitches, NE-NLCs were fabricated using a high-speed homogenization technique followed by probe sonication [144]. Cellular uptake showed that FITC-labeled NE-NLCs penetrated better than FITC dye into Caco-2 cells. *In vivo* studies revealed that NLCs increased the C_{max} of NE by 4 times compared with its suspension. MRT and $t_{1/2}$ were also extended due to the sluggish release of NE from NLCs as well as an increment in bioavailability was observed to 26.31-fold. The author asserted that NLCs were likely taken up by the lymphatic system, thereby bypassing hepatic metabolism.

In addition, the use of tocopheryl polyethylene glycol succinate (TPGs) and poloxamer inhibited the P-gp efflux of the drug. Sodium deoxycholate as a permeation enhancer may have destabilized the intestinal membrane and aided in the efficient absorption of nintedanib. The plasma concentration-time profile after oral administration of NE-NLCs to cycloheximide- (CHX-) treated and nontreated (control) mice demonstrated that the C_{max} of CHX-treated NE-NLCs declined from 1190.11 ± 191.02 ng/mL to 582.82 ± 20.67 ng/mL, indicating a reduction in the intestinal absorption of NE compared with CHX-treated rats because cycloheximide blocked intestinal lymphatic transport. The chylomicron flow-blocking study exhibited that nintedanib nanolipid carriers were absorbed over the lymphatic alleyway [144]. Table 3 summarizes the recent trends in the delivery of medications using NLCs in the GIT.

3.7.3. NLCs for Oral Delivery of Drugs. NLCs, owing to the excellent virtues discussed above, can reduce the solubility and bioavailability of various drugs *in vivo* and have found applicability in transporting medications and bioactives across intestinal membranes. Shaimaa S. Ibrahim developed NLCs loaded with prednisolone acetate (PA) for enhanced anti-inflammatory activity via the oral route. NLCs were fabricated with Compritol as the solid lipid, oleic acid as the liquid lipid, and Tween 80 or Pluronic F68 as the surfactant using the solvent injection method. They observed excellent suppression of inflammation by PA-loaded NLCs ($83.9 \pm 4.46\%$) in comparison to PA suspension ($40.5 \pm 7.03\%$), whereas drug-free NLCs showed an anti-inflammatory activity of $54.7 \pm 6.12\%$ [156]. Several drugs such as raloxifene [157], ifosfamide [124], baicalin [158, 159], and artemether-lumefantrine [160, 161] have been explored for oral delivery via NLCs.

Trimyristin as a solid lipid was intended to provide stability to resveratrol from enzymatic and pH degradation in glycerol tricaprylate and glyceryl trioleate as liquid lipid nanostructured nanocarriers [162]. Hydrochlorothiazide (HCT) demonstrates low solubility and permeability; hence, an attempt was made to upsurge its bioavailability by incorporating it into NLCs [163]. HCT nanocarriers were found to be stable under simulated gastric fluidic conditions,

Table 2: NLCs and their role in improving the oral bioavailability of pharmaceuticals.

Drug/bioactive molecule	Activity	Model	Enhancement of oral bioavailability	References
Candesartan cilexetil (CC)	Antihypertensive	*In situ* model for determining drug absorption	2-fold increase in oral bioavailability	[125]
Gypenosides (GPS)	Anti-inflammatory	*In situ* intestinal perfusion/*in vivo* pharmacokinetic studies	8.5-fold increase in bioavailability of the GPS-SGC-NLCs	[126]
Nimodipine (NMP)	Calcium channel blocker	*In situ* intestinal perfusion/*in vivo* pharmacokinetic studies	161% increased bioavailability than nimodipine suspension	[127]
Ezetimibe (EMB)	Antihyperlipidemic	*In vivo* pharmacokinetic studies	2.63-and 2.33-fold increase in oral bioavailability in comparison with EMB suspension and marketing product	[128]
Ritonavir (RTV)	Highly active antiretroviral therapy (HAART)	*In vivo* pharmacokinetic study	2.86-fold augmentation in oral bioavailability of RTV in comparison to RTV suspension	[129]
Felodipine (FDP)	Antihypertensive	*In vivo* pharmacokinetic studies	A 2-fold oral bioavailability enhancement of FDP in comparison to marketed formulation	[130]
Apixaban (APX)	Anticoagulant	*In vivo* pharmacokinetic study	2.67-fold increase in oral bioavailability of APX in comparison to pure drug suspension	[131]
Levosulpiride (LSP)	Dyspepsia	*In vivo* pharmacokinetic study	4.38-fold improvement in oral bioavailability of LSP than drug dispersion	[132]
Amisulpride (AMS)	Antipsychotic	*In vivo* pharmacokinetic study	The relative bioavailability of AMS-NLCs capsules was found to be 252.78% as equated with commercial Amipride® tablets	[133]
Triptolide (TP)	Antiinflammatory	*In vivo* pharmacokinetic study	Longer residence time, sustained drug release, 1.54 times increased bioavailability than free drug	[134]
Ergosterol	Antidiabetic nephropathy	*In vivo* pharmacokinetic study	The comparative oral bioavailability of ERG-NLCs was 277.56% higher than that of pure ergosterol	[135]
Raloxifene (RLN)	Anticancer	*In vivo* pharmacokinetic study	A 4.79-fold increase in oral bioavailability of RLN equated to RLN suspension	[136]
Raloxifene (RLX)	Osteoporosis	*In vivo* pharmacokinetic study	3.19-fold enhancement in oral bioavailability of RLX in comparison to free RLX suspension	[137]
Fenofibrate	Antihyperlipidemic	*In vivo* pharmacokinetic study	Four times increase in oral bioavailability	[138]
Transferulic acid (TFA)	Antioxidative, anti-inflammatory, and cardioprotective	*In vivo* pharmacokinetic study	Augmented oral bioavailability than TFA-SLNs	[139]
Ticagrelor (TGL)	Antiplatelet activity	*In vivo* pharmacokinetic/pharmacodynamic study	The oral bioavailability of TGL-NLC was augmented by 254.99% in comparison to raw TGL	[140]

Table 3: Site-specific delivery of medications via NLCs to the GIT.

Drug	Site of action	Model	Outcomes	References
Pumpkin seed oil	Stomach	*In vivo* pharmacokinetic studies	Pumpkin seed oil NLCs significantly reduced the ulcer index as compared to indomethacin and lessened mucosal lesions in comparison to pure pumpkin oil	[145]
Thymoquinone (THQ)	Stomach	*In vivo* efficacy studies against gastric ulcers	THQ suspension, blank coconut oil NLCs, and THQ-NLCs subdued the ulcerative index and hemorrhagic erosions on the gastric mucosa of mice due to ethanol-induced gastric ulcers	[146]
Sirolimus (SRL)	Intestine	*In vitro* lipolysis model/*in vivo* pharmacokinetic study	Nearly 100% drug loading, lipolysis caused fast digestion of NLCs, a 1.82-fold rise in oral bioavailability in comparison to conventional tablets in beagle dogs	[147]
Vincristine sulfate (VCR)	Intestine	Cellular uptake studies/*in vivo* pharmacokinetic study	Boosted two-fold oral bioavailability of VCR by prolonging the interaction between positively charged hyaluronic acid-modified NLCs (HA-NLCs) and negatively charged mucous membranes. The smaller size of NLCs enabled internalization into the gastric mucosa	[148]
Nabumetone (NBM)	Intestine	*In vivo* pharmacokinetic study	*In vivo* studies confirmed that NBM-NLCs can improve intestinal absorption of NEM when administered orally	[149]
Tacrolimus (TL)	Intestine	*In vitro* lipolysis study/*in vivo* pharmacokinetic/organ distribution studies	Higher drug solubility was observed than drug suspension alone. Bioavailability was significantly higher than the market formulation. Lymphatic uptake of NLC-N2 and NLC-C2 was significantly ($^{***}P < 0.001$) enhanced by 19.25- and 14.5- fold, at 1 h; 7.5- and 6.8-fold after 24 h, respectively, in comparison to TL suspension	[150]
Simvastatin and nifedipine	Intestine	*In vivo* pharmacodynamic studies	Simvastatin and nifedipine-loaded NLCs, both in combination significantly lowered the hypercholesteremic levels, and poloxamer 407 might also have improved the intestinal absorption of NLCs	[151]
Isradipine (ISD)	Intestine	*In vitro* gut permeation and *in vivo* solubilization	Cycloheximide (a lymphatic uptake inhibitor) absence increased the oral bioavailability of ISD by 4.2-fold, improved gut permeation, solubility, lymphatic uptake, and biodistribution	[152]
Budesonide (BSD)	Colon	*In vitro* (J774 murine macrophages)/*in vivo* colon localization study	BSD-NLCs and blank NLCs were retained for a longer duration in the mucosa, reduced TNF-α secretion, and coumarin-6-NLCs were localized for a prolonged time at the colon even after dextran sulfate-(DS-) induced colitis in mice	[153]
Celecoxib (CLX)	Colon	*In vivo* therapeutic efficacy study	Eudragit RS100-CLX-NLCs regained the disruption caused by DS-induced colitis in the mucosa, submucosa, and muscular layers of the colon of mice	[154]
Oleuropein (OPN)	Colon	*In vivo* efficacy studies against acute colitis	OPN suspension and OPN-NLCs regained the disruption caused in the structure of the colon by DS-induced colitis in mice	[155]

whereas *in vivo* pharmacodynamic experiments on adult male rats indicated augmented diuresis from 1 h to 6 h in comparison with the control. Table 4 lists some orally delivered therapeutic agents with the aid of NLCs.

3.7.4. NLCs for Reaching the Brain via the Oral Route. The literature has evidenced NLC involvement in the delivery of medication via several routes, including oral with the aid of various approaches to the brain [182, 183]. Nonetheless, the blood-brain barrier (BBB) acts as a shield against the infiltration of foreign substances into the brain. BBB protects the brain by isolating it from regular systemic circulation and restricting the entry of toxins, pathogens, and other harmful substances. It only offers selective entry to some agents, which are regulated by the monolayer of tightly packed endothelial cells. Endothelial cells are again sealed sturdily by a tight junction, rendering them impermeable to pathogenic organisms and unwanted substances and molecules while permitting the supply of oxygen, entry of highly lipophilic smaller substances, and other nutrients needed for brain functioning [184].

NLCs can be transported across the BBB via various mechanisms such as passive diffusion through paracellular and transcellular routes and active diffusion through receptor and carrier-mediated transport [185]. Reports have confirmed that NLCs augment the permeability of the BBB by easing the partial opening of the tight junction. In addition, the surfactant or permeation enhancer present as a component of NLC dissolves the lipids of endothelial cells and facilitates the transcellular diffusion of the therapeutic/biologics [186, 187]. Improvization of the NLC surface could be another approach where target-specific guiding moieties such as lactoferrin, transferrin, CPP28, LDL29, and other peptides can aid in crossing and reaching the target site by guiding the NLCs to a specific receptor at BBB via a receptor-mediated endocytosis mechanism [188].

Khan et al. improved the oral bioavailability of atazanavir (ATZ), which is used in the treatment of neuroAIDs (acquired immunodeficiency disease) with the aid of NLCs. They developed ATZ-NLCs using the quality-by-design approach and further optimized the formulation by the Box–Behnken design. ATZ-NLCs displayed a 2.36-increase in ATZ permeation across the rat intestine. From their pharmacokinetic evaluation, they reported a 2.75-fold upsurge in C_{max} in the brain and a 4-fold enhancement in brain bioavailability compared to ATZ suspension, signifying NLCs capability of transporting drug to the brain circumventing resistance offered by the BBB [189].

Zotepine (ZT), a BCS class II drug, is an antipsychotic drug with poor solubility, oral bioavailability of nearly 10% (Log P 4), and undergoes hepatic metabolism. Tirumalesh et al. attempted an oral bioavailability enhancement of ZT by incorporating it into NLCs. They formulated ZT-NLCs using hot homogenization with the probe sonication method and reported a 1.8-fold increment ($P < 0.05$) in the oral bioavailability of ZT in male Wistar rats compared to ZT coarse suspension [190]. Olanzipine is also a BCS class II drug that has been successfully formulated in NLCs for an oral bioavailability enhancement of a 5½-fold by formulating it with NLCs, indicating the potential of these carriers for the transport of lipophilic drugs for treating ailments such as schizophrenia [191].

Temazepam (TZP) NLCs were formulated for the treatment of insomnia through oral administration. TZP-NLCs were found to accumulate in higher concentrations in the brain after 4 h, as evidenced by gamma scintigraphy images. In addition, the brain biodistribution of TZP by NLCs was 10 times higher than that of plain drug suspension [177]. Lopinavir (LPV) is used in the treatment of HIV-associated neurocognitive disorder (HAND), where its potential was adjudged by comparing oral optimized LPV-NLCs with intravenous LPV-NLCs. Tween 80-coated LPV-NLCs were able to breach the BBB when administered via the oral route with a brain biodistribution of ~2.35-fold compared, which was similar to the LPV concentration when administered via the intravenous route (~2.8 fold). This finding indicated that upon intravenous administration, the concentration of LPV in the brain was higher than that after oral administration, although therapeutic performance of oral LPV-NLCs (16.5-fold increase in AUC) was better than that of plain drug suspension [192].

Raju et al. designed berberine-loaded NLCs (Berb-NLCs) by the melt emulsification and ultrasonication method by using Geleol, Miglyol 812 N, and Solutol HS 15 as a solid lipid, liquid lipid, and surfactant, respectively. Berb, an isoquinoline alkaloid, which is reported for the treatment of Alzheimer's disease, has a low therapeutic window, poor absorption, and low oral bioavailability and limited permeability to the brain. Pharmacodynamic studies included behavioural evaluation by the locomotor activity, passive avoidance test, and elevated plus maze test, and spatial memory assessment by Morris water maze indicates augmentation in behavioural parameters *in vivo* by Berb-NLCs as equated with pure berberine in albino Wistar rats [193].

3.7.5. NLCs for the Delivery of Biologics via the Oral Route. The delivery of proteins and peptides demands a safer vehicle so that their degradation and integrity are safely guarded from the *in vivo* environment. NLCs can deliver these agents by protecting proteins and peptides from the body's enzymes, transporting them efficiently across biological membranes, augmenting their systemic circulation time. Shahzadi et al. used the contemporary ratio of a solid/liquid lipid, i.e., 70 : 30, for the oral delivery of insulin. They assessed the impact of surfactants on the safeguarding of the peptide in three different NLC formulations containing polyethylene glycol ester (PEG-ester), polyethylene glycol ether (PEG-ether), and polyglycerol ester (PG-ester) surfactants prepared by the solvent diffusion method. Researchers have reported that cleavable substructures of surfactants on the NLC surface provide cumulative protection to insulin from the degrading enzymes of the GIT [95].

Calderón-Colón et al. encapsulated insulin B-chain peptide sequence 9–23 (Bpep) into sodium deoxycholate NLCs using the phase inversion temperature method

Table 4: NLCs for the oral delivery of drugs to treat various diseases.

Drug	Disease/activity	NLC components	Model	Outcomes	References
Thymoquinone (TQ)	Antioxidant, anti-inflammatory, and neuroprotective	Hydrogenated palm oil, lecithin, Lipoid GmbH, olive oil	Pharmacokinetic/pharmacodynamic	Enhanced intestinal absorption via the oral route of TQ. Higher bioavailability was observed via the oral route than i.v. administered TQ-NLCs. Due to lower $AUC_{0-\infty}$, oral administration had slower absorption and better bioavailability compared to intravenous administration	[164]
Bromelain (BR)	Antiarthritic activity	Soya lecithin, stearic acid, palmitic acid, myristic acid	Pharmacodynamic studies	BR and Br-NLCs, after oral administration, significantly lessened the complete Freund's adjuvant- (CFA-) induced paw inflammation around the lateral tibial joint and soft tissue thickening as equated to (CFA) control	[165]
Chrysin (CHN)	Antitumor activity	Capmul PG-12, GMS, and S 100	Cellular uptake studies and *in vivo* pharmacokinetics studies	CHN-loaded biotin-modified NLC (Bio-NLC) displayed excellent uptake by Caco-2 cell lines compared to plain NLCs. CHN-Bio-NLCs showed a 7.46-fold increase in AUC0-∞ in comparison to CHN suspension	[166]
Auraptene (APN)	Benign prostate hyperplasia	Compritol, almond oil, phospholipid	Histopathological investigations, oxidative stress, inflammation, and apoptotic assessments	APN-NLCs demonstrated superlative results by safeguarding testosterone-induced BPH in animal models from oxidative stress, inflammation, and apoptosis	[167]
Amphotericin B (AmpB)	Antifungal activity	Chitosan, beeswax, coconut oil, soya lecithin	Mucoadhesion studies	ChiAmpB-NLC enhanced the adsorption of positively charged chitosan to negatively charged porcine gastric mucin protein	[168]
Perphenazine (PPZ)	Schizophrenia	Glyceryl monostearate 900 K, Dynasan 118, oleic acid	*In vivo* pharmacokinetic studies	The oral bioavailability of PPZ-loaded NLC-6 and NLC-12 was enhanced about 3.12- and 2.49-fold, respectively, compared to the plain drug suspension	[169]
Silymarin (SLM)	Hepatoprotective, antidiabetic	Cetyl palmitate (CP) Capryol 90, Lauroglycol 90, Labrafac PG, Labrafac WL 1349, Labrafil M 1944 CS, Precirol ATO 5, oleic acid, hemp oil, borage oil	Transcellular passive permeability by the parallel artificial membrane permeability Assay (PAMPA) and cell uptake studies (Caco-2)	Amplified the passive permeation of SLM by around 10-fold, CP as a solid lipid increased NLC cellular uptake by Caco-2 cells	[170]
Berberine (BB)	Antidiabetic	Oleic acid, glyceryl distearate	Cellular uptake, pharmacokinetic/ pharmacodynamic studies	396.87% and 663.65% augmented oral bioavailability of BB-NLCs and selenium conjugated-BB-NLCs as compared with BB solution. More profound hypoglycemic effect of Se-BB-NLCs than BB-NLCs and BB solution. Fast cellular uptake was seen in Se-BB-NLCs and BB-NLCs than in the BB solution	[171]

Table 4: Continued.

Drug	Disease/activity	NLC components	Model	Outcomes	References
Ezetimibe (EZE)	Hypercholesterolemia	Geleol™, Compritol® 888 ATO, Precirol® ATO 5, Monosteol™, Transcutol® HP, Peceol™, Lauroglycol™ FCC	*In vivo* pharmacokinetic/ pharmacodynamic studies	After oral administration, EZE-NLC showed increased oral bioavailability in comparison to a drug suspension and tablet. A substantial decline in cholesterol levels was observed in a rat model administered with EZE-NLCs	[172]
Rosuvastatin (RST)	Hypercholesterolemia and dyslipidemia	Precirol® ATO 5, oleic acid	*In vivo* pharmacokinetic and pharmacodynamic model	1.65-fold increased absorption of RST-NLC as compared to RST tablet powder indicated oral solubility enhancement	[173]
Atorvastatin (AT)	Hypercholesterolemia	Gelucire® 43/01, glyceryl monostearate (GMS), Compritol® 888 ATO, and Capryol® PGMC	*In vivo* pharmacokinetic/dynamic studies	A 3.6- and 2.1-fold upsurge in bioavailability as equated to AT suspension and market product, AT-NLC-1 significantly reduced rats' serum levels of total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) and raised high-density lipoprotein (HDL) levels	[174]
Naringenin (NGN)	Nonalcoholic fatty liver Disease	Stearic acid, oleic acid	Transepithelial transport, *in vivo* pharmacokinetic studies	High permeability coefficients of NGN-NLC indicated improved transport of NGN via MDCK cells, NGN-NLCs augmented 1.2-fold oral bioavailability	[175]
Adefovir dipivoxil (AD)	Hepatitis B	Cremophor RH 40, Pluronic F68 (poloxamer 188), egg yolk L-α phosphatidylcholine (PC)	*In vivo* hepatoprotective evaluation	Oral administered AD-NLC exhibited higher uptake as indicated by radioiodinated rose Bengal dye to the thioacetamide-induced liver injury	[176]
Temazepam (TZP)	Insomnia	Capryol® 90, Labrasol®, Compritol®888, ATO, oleic acid	*In vivo* gamma scintigraphy imaging and brain biodistribution study	TZP-NLCs accumulated at higher concentrations than the TZP suspension, as evident from gamma scintigraphy. TZP-NLCs augmented relative drug bioavailability by 292.7% after oral administration in comparison to TZP suspension	[177]
Olmesartan (OLM)	Hypertension	Compritol 888, Peceol, Maisine 35-1, Lauroglycol FCC, Lauroglycol 90, stearic acid, GMS, IPM, cetyl palmitate, oleic acid, and ethyl oleate	Cellular uptake and transport studies, *in vivo* pharmacokinetics, *in vivo* biodistribution study	High cellular uptake for OLM-NLCs and OLM-ConA-NLCs compared to a drug suspension. 2.88- and 4.62-fold improvement in oral bioavailability was evident for OLM-NLCs and OLM-ConA-NLCs in comparison to drug suspension, 37% reduction in blood pressure was observed for drug-loaded NLCs in comparison to pure drug suspension	[178]

Table 4: Continued.

Drug	Disease/activity	NLC components	Model	Outcomes	References
Isradipine (ISD)	Hypertension	Glyceryl monostearate, glycerol distearate, glycerol dibehenate, PEG-8 beeswax, PEG-75 stearate, propylene glycol dicaprylocaprate, propylene glycol monocaprylate, and macrogol 15 hydroxy stearate	*In vitro* permeation/*in vivo* pharmacodynamic study	Verapamil (a P-gp efflux inhibitor) significantly enhanced the permeation of ISD from NLCs through the rat intestine by inhibiting the drawing back of the drug inside the intestine. ISD solubility was augmented due to micelle formation owing to the lipid digestion of NLC components	[152]
Quercetin (QCT)	Nutraceutical delivery	Glyceryl monostearate, polyglycerol-6 monostearate	*In vitro* antioxidant activity	Improved solubility and antioxidant activity of QCT-NLCs, which also lessened the lipid oxidation as compared to emulsion	[179]
Quercetin and piperine	Oral squamous cell carcinoma	Glyceryl behenate, squalene	*In vivo* pharmacokinetic study	Enhanced biodistribution of both drugs in oral cavity parts upon oral administration of drug-loaded NLCs	[180]
Eplerenone	Chronic central serous chorioretinopathy	Cremophor® RH40, Miglyol®81N2	*Ex vivo* permeation study	Twofold higher drug permeation through the rabbit intestine compared to aqueous drug suspension after 24 h	[181]

[194, 195] and design of experiment (DOE) methods for antigen-specific immunotherapy for diabetes. They reported that the solubility of peptide was improved when encapsulated into NLCs with high encapsulation efficiency and stability. Gellucire 44/14 was used as a surfactant that aided in dosing flexibility, the achievement of therapeutic concentrations, and minimal systemic toxicity. They demonstrated that, upon oral delivery, NLC formulation accumulated in gut-draining lymphatic tissues such as mesenteric and pancreatic lymph nodes in mice [196]. In another study, the lipophilicity of two peptides, desmopressin (DES) and leuprolide (LEU), was increased by the formation of a hydrophobic ion pair (HIP) with sodium docusate as a surfactant in NLC formulations for oral delivery [197].

NLCs and lipid-based nanoparticles have been utilized in the delivery of small molecules such as RNA, and siRNA [198], replicating viral RNA (rvRNA), and s-glutathione [199], offering them safety from enzymatic degradation and *in vivo* environment. Pfizer/BioNtech and Moderna developed lipid-based nanoparticle-mediated delivery of two approved COVID-19 mRNA vaccines that displayed remarkable disease control efficacy [200, 201]. The ionizable lipids of both vaccines provided a positive charge that enabled RNA complexation and were neutral at physiological pH that lowered toxic effects and triggered payload release. PEGylated lipid composition diminished opsonization by serum proteins and clearance by phagocytes, allowing them to remain for a longer period in systemic circulation.

Vaccine delivery via NLCs has also been shown in the picture where ovalbumin was efficiently taken up by macrophages when delivered by encapsulating an antigen into chitosan-modified NLCs [202]. RNA vaccines against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) must be stowed under cold storage conditions before use. Gerhardt et al. devised lyophilized NLCs complexed with RNA vaccines that exhibited long-term stability at room temperature (for a period of greater than eight months) and refrigerated conditions (for a period of greater than twenty-one month) and reported the efficacy of these vaccines to be viable for protein expression *in vivo* [203].

Emily A. Voigt et al. developed a potent self-amplifying RNA (saRNA) vaccine (stable itself at room temperature) against SARS-CoV-2 complexed with NLCs with improved stability and degradation. In their preclinical studies, they demonstrated that the saRNA/NLC vaccine produced potent humoral immunity by performing pseudovirus neutralization titer to α, β, and Δ variants and inducing bone marrow-secreting cells. When lyophilzed, saRNA/NLCs were stable when stored for at least six months at room temperature and for at least ten months in refrigerated settings and present a horizon for RNA vaccines in the treatment of COVID-19 and other pandemics [204].

Oral vaccine (peptide RNA or DNA-based) delivery via the oral route has presented challenges such as poor bioavailability due to low oral absorption of vaccines, possibly due to enzymatic degradation, poor membrane permeability, first-pass metabolism, and complex GI environment. Thus, lipid-based nanoparticles and possibly NLCs could solicit the abovementioned drawbacks by shielding the antigens from degradation in GIT, transporting antigens to the inductive mucosal surface, promoting antigen uptake (M-cells and Peyer's patches), activating immune cells, and triggering sustained mucosal and systemic immune responses.

3.7.6. NLCs for Delivering Herbal Medications. NLCs have also been found to be applicable in delivering herbal/phytomedicines. Lacatusu et al. adopted diosgenin and *Glycyrrhiza glabra* extracts in NLCs to upgrade the antioxidant and anti-inflammatory properties of the two herbal medicines [175]. Silymarin (SLM), derived from the seeds of *Silybum marianum* L. Gaertn., has been employed as a hepatoprotectant for many years. SLM is a drug with low water solubility (BCS class IV) and restricted oral bioavailability. In their study, Piazzini et al. proposed that employing NLCs could enhance solubility and absorption in the intestine. The permeation study showed that SLM-NLCs effectively improved the permeation of SLM. Furthermore, the transportation of NLCs through the cell monolayer relied on energy and involved pathways associated with clathrin, caveolae, or lipid rafts [170].

NLCs could also be employed to enhance the bioavailability of lipophilic functional biocompounds, vitamins, minerals, plant-derived constituents (polyphenols and carotenoids), prebiotics, probiotics, and postbiotics [205].

Current chemotherapy regimens for breast cancer are primarily focused on eradicating cancer cells or slowing down their division. Unfortunately, these treatments often lead to undesirable side effects and exhibit limited efficacy. For instance, they can result in conditions such as anemia, which causes fatigue and negatively impacts patients' overall quality of life. In addition, issues such as the development of drug resistance, the presence of P-glycoprotein efflux transporters, and substantial first-pass metabolism further complicate the delivery of an effective therapeutic dose to the intended target site. In this challenging landscape, NLCs present a promising solution to address the shortcomings associated with traditional breast cancer chemotherapy. Many herbal drugs have been delivered via the oral route including resveratrol [206], curcumin [207], gambogic acid [208], thymoquinone [209], and exemestane [210]. A few patents have also been documented, which are given in Table 5.

3.8. Patents on NLCs. NLC constituent natural lipids and biodegradable emulsifiers have been considered safe by regulatory bodies, and hence, their use as drug delivery systems has expanded recently. Several patents have been granted on NLCs that have been involved in the delivery of medications/cosmetics via various routes such as oral parenteral, ophthalmic, nasal, topical, CNS, and transdermal. Table 5 summarizes the granted/pending patents on NLCs.

Table 5: NLC and patents granted/pending.

Patent number	Title	Applicant/inventor	Publication date	Lipid components	Application area	Proposed administration route	References
US20220054416A1	Nanostructured lipid carriers and stable emulsions and uses thereof	Infectious Disease Research Institute, Seattle, WA (US)	24.02.2022	Capric/caprylic triglyceride, vitamin E, lauroyl polyoxylglyceride, trimystin, polyethylene glycol	Immunogenic	Intramuscular	[211]
US11141377B2	Nanostructured lipid carriers and stable emulsions and uses thereof	Infectious Disease Research Institute, Seattle, WA (US)	12.10.2021	313-[N–(N′,N′-Dimethylaminoethane)-carbamoyl]Cholesterol (DC cholesterol)	Immunogenic	Intramuscular	[212]
CN108853056B	Folic acid-targeted modification carried doxorubicin hydrochloride and gambogic acid nanostructure lipid carrier preparation and preparation method thereof	Tianjin University of Traditional Chinese Medicine	14.09.2021	Stearic acid, behenic acid, glyceryl behenate, glyceryl monostearate, isopropyl palmitate, isopropyl myristate, soybean oil, and oleic acid	Anticancer	Oral	[213]
AU2021106678A4	A method of preparation of trimcinolone acetonide encapsulated NLCs for psoriasis treatment	Madhulika Pradhan, K. K. Sahu, Deependra Singh, Manju Rawat Singh, Krishna Yadav	16.12.2021	Docosahexaenoic acid (DHA), cetostearyl alcohol (CSA), poloxamer 188	Antipsoriatic activity	Transepidermal	[214]
BR102019026730A2	Antimicrobial nanostructured lipid carriers	Simone Ramos De Castro, Eneida De Paula, Talita Cesarim Mendonça Lígia, Nunes De Morais, Ribeiro Marcelo, Lancellotti Márcia Cristina, Breitkreitz	29.06.2021	Beeswax, tucumã butter and ucuuba butter, sesame oil, andiroba oil, copaiba oil, and rosemary oil	Antimicrobial	Oral	[215]
WO2019068161A1	Method for obtaining nanostructured lipid carriers, NLCs obtained and the use thereof	Nádia Araci Bou Chacra, Paulo Cesar Cotrimlis, Marie Monteiro Nikoletta, Fotaki Raimar Löbenberg	11.04.2019	Hydrogenated palm oil, hydrogenated coconut mono, di, and triglycerides, stearoyl macrogol-32-glycerides	Antileishmaniasis	Oral	[216]
WO2018232257A1	Nanostructured lipid carriers and stable emulsions and uses thereof	Christopher B. Fox, Amit Praful Khandhar, Neal Van Hoeven, Jesse H. Erasmus, Susan S. Lin	20.12.2018	Murumuru seed butter, bis-diglyceryl polyacyladipate-2	Skin care application	Topical	[217]
CN104367549A	Psoralen-doxorubicin-loaded composite nanostructured lipid carrier preparation and preparation method thereof	Liaoning University	12.09.2017	Stearic acid, cholesterol, palmic acid, Labrasol, Miglyol 812N	Antitumor activity	Topical	[218]

Table 5: Continued.

Patent number	Title	Applicant/inventor	Publication date	Lipid components	Application area	Proposed administration route	References
CA2963872A1	Nanostructured formulations for the delivery of silibinin and other active ingredients for treating ocular diseases	Anna Rita, Blanco Maria Luisa, Bondi'gennara, Cavallaro Grazia, Maria Letizia, Consoli Emanuela Fabiola, Craparo Gaetano, Giammona Mariano, Licciardi Giovanna, Pitarresi Giuseppe, Granata Patrizia, Saladino Clara, La Marca Irene, Deidda Salvatore, Papasergi Patrizia, Guarneri Salvatore, Cuzzocrea Emanuela, Esposito Santa Viola	14.04.2016	Tristearin, tripalmitin, caprylic/capric acid triglycerides, Compritol HD-5-ATO	Ocular diseases	Ophthalmic	[219]
US20160015703A1	Nano-micro delivery systems for oromucosal delivery of an active ingredient	Ioannis S. Chronakis, Lars Jorgensen, Maria Ahlm Mattebjerg	21.01.2016	Nicotine	Analgesic	Oral mucosa	[220]
IN276/MUM/2014	Idebenone lipid nanocarrier composition for the treatment of neurodegenerative disorders	Sachin Subhash Salunkhe, Neela Manish Bhatia, Manish Sudesh Bharia	11.09.2015	Glyceryl palmitostearate, propylene glycol dicaprylate/dicaprate	Neurodegenerative disorders	Oral	[221]
CN103263671A	Preparation and application of antitumor activator nanostructure lipid carrier	Cui Guohui, Cui Chunying, Liu Xiaozheng	15.04.2015	17-Allylamino-17-demethoxygeldanamycin, glyceryl monostearate, octanoic acid/capric acid triglyceride, tween 80	Antitumor	Topical	[222]
WO2015039199	Microstructured and nanostructured pharmaceutical and veterinary compositions, containing benznidazole and derivatives thereof, which form microstructures and nanostructures in the gastrointestinal tract and biological uses thereof	Mosqueira, Vanessa Carla Furtado Oliveira, Liliam Teixeira Castanheira, Raquel Gomes	26.03.2015	Labrasol, Cremophor E	Antiprotozoals, chagas disease	Oral	[223]
CN104172184A	Quercetin nanostructured lipid carrier and preparation method thereof	Southeast University	03.12.2014	Polyglycereol monolaurates, tripolyglycerol monostearate, caprylic acid capric acid, camellia seed oil	Functional food field	Oral	[224]

Table 5: Continued.

Patent number	Title	Applicant/inventor	Publication date	Lipid components	Application area	Proposed administration route	References
WO2014123406A1	A composition for treating leukemia	Rasedeemat Abdullahheshu, Sulaiman Rahamn Ahmad, Bustamam Abdulhow, Chee Wunyeap Swee Keong	14.08.2014	Zerumbone, hydrogenated palm oil, phosphatidylcholine (Lipoid S 100), and olive oil	Leukemia	Parenteral/oral	[225]
CN103893167A	Podophyllotoxin preparation resisting condyloma acuminata relapse and HPV latent infection	Nanfang Hospital of Southern Medical University	02.07.2014	Glyceryl monostearate, lecithin	HPV latent infection	Topical	[226]
CN103860389A	Nanostructured lipid carrier loaded with phenyl ethyl resorcinol, preparation method thereof, and cosmetic containing the same	Beinong biochemical (Suzhou Industrial Park) Co., Ltd. Suzhou Nanohealth Biotech Co., Ltd	18.06.2014	Glyceryl monostearate, decanoyl/octanoyl glycerides	Skincare activity	Topical	[227]
US20120195957A1	Novel nanoparticle formulations for skin delivery	Mandip Singh, Sachdeva, Ram Patlolla	06.05.2014	Compritol 888, Miglyol 812	Transdermal delivery, skin diseases	Topical	[228]
CN104688715A	Resveratrol nanostructured lipid carrier and preparation method thereof	Shanghai Traditional Chinese Medicine Hospital	22.01.2014	Glyceryl monolaurate, acetylation monoglyceride, *Fructus persicae* resin	Resveratrol-containing cosmetics	Topical	[229]
MYPI 2012001818	Thymoquinone-loaded nanostructured lipid carriers (TQ-NLC) and uses thereof	Universiti Putra Malaysia	25.10.2013	—	Dermal diseases	Topical	[230]
CN102670510	Tripterine nanostructure lipid carrier modified by lentiviral vect or and appliance for preparing and treating prostatic cancer, lung cancer, and breast cancer drug	Jiangsu Provincial Insititute of Traditional Chinese Medicine	18.09.2013	Tripterine, phospholipid, poloxamer 188, vitamin E, tocopherol and polyethylene glycol succinate	Breast cancer	Transdermal	[231]
IN422/MUM/2011	Nanotechnology-based herbal composition for safe and effective treatment of psoriasis	Kamalinder Singh Kaur, Medha Chetan Patel	12.07.2013	Glycerylbehenate, trimyristin, tristearin, tripalmitin, glyceryl monostearate, cetyl alcohol, stearic acid	Antipsoriatic activity	Topical	[232]
CN102274187B	Nanostructural lipid carrier and preparation method and application thereof	Shanghai University of Traditional Chinese	07.08.2013	Glyceryl behenate-oleic acid, polyethylene glycol glyceride, glyceryl behenate-single glyceryl linoleate	—	Oral	[233]

3.9. Toxicity Concerns of NLCs via the Oral Route. Although, during NLC formulation, biodegradable, biocompatible, physiological lipids are employed, which are generally regarded as safe, the toxicity aspect has to be considered for their *in vivo* use. Many studies have been performed addressing their safe use in both *in vitro*, *ex vivo*, and *in vivo* environments. Alhalmi et al. fabricated raloxifene and naringin- (RLX/NRG NLCs-) loaded NLCs for breast cancer. RLX/NRG NLCs were prepared using Compritol 888 ATO and oleic acid using a hot homogenization-sonication technique optimized by a central composite design. Their results indicated that the oral gavage delivery of dual drug-loaded NLCs produced no acute toxicological effects on vital organs on repetitive delivery of the formulations compared with control-treated Wistar rats [234].

In another study, thymoquinone (TQ) was incorporated into NLCs to lower its toxicity when administered orally. In their subacute toxicity study, it was found that upon oral administration of 100 mg/kg of TQNLC and TQ, it did not cause mortality to either male or female but resulted in minor toxicity to the liver and not to the extent of altering the functions of the organ [235]. Nordin et al. developed citral-loaded NLCs for cancer treatment and reported that NLC-citral showed no toxic effects toward the proliferation of mice splenocytes, and no mortality or toxic signs were reported in the treated groups after 28 days of treatment [236]. Zhou et al. demonstrated that tripterine-NLCs did not exhibit substantial cytotoxicity and that cell viability was >90% in Caco-2 cells [237]. Thus, based on the above few studies, it could be inferred that NLCs, being composed of biodegradable and physiological lipids, remained stable and did not impart toxic effects during *in vitro* and *in vivo* cytotoxicity studies.

3.10. Stability of NLCs. Although NLCs are significantly more stable than SLNs, aggregation has the potential to impede their long-term physical stability. In addition, the presence of water in NLCs could also contribute to stability problems. These problems can be resolved by converting the nanosuspension into a solid powder through methods such as spray drying, freeze-drying, or lyophilization [29, 238].

Spray drying changes liquid into powder form, thus stabilizing particles by restricting mobility inside molecules, facilitating transport, and reducing cost. A careful selection of NLC components should be made as the lipids used may be exposed to high temperatures during spray drying, making them susceptible to polymorphism changes. The use of sodium chloride as an excipient in NLC formulations dried by spray drying rendered excellent flowability to the formulation with optimum nanoparticle particle size [239].

Freeze-dying could lend them a long-term stability asset while maintaining the integrity of nanoparticles. In a previous study, freeze-dried lopinavir-laden NLCs remained stable in a long-term stability chamber with no further increase in particle size and no reported significant change in the polydispersity index, zeta potential, and drug content [240]. However, the redispersibility of freeze-dried powder could be a determining factor, but cryoprotectants such as aerosil (rifabuin-NLCs) [241], carbohydrate trehalose (verapamil-NLCs) [242], mannitol (tilmicosin-lipid nanoparticles) [243], sucrose, and glucose might be helpful to achieve this [244]. A careful selection of lipid components, surfactants and preservatives could also provide them with physical stability for longer use. Figure 5 shows the layout of various stability governing factors of NLCs.

3.11. Regulatory challenges and Marketed NLCs. Regulations governing the constituents used in NLC formulation are essential to ensure their safety for various applications, including pharmaceuticals, biologics delivery, diagnostics, nutritional food, and cosmetics [102, 245, 246]. Most of the components of NLCs are derived from natural sources; hence, their *in vivo* use could only be permitted after strict toxicity tests [54].

Lipid-based nanoparticles, such as SLNs and liposomes, have several products that are available in the market [37, 247–249] though NLC entry in the market for delivering therapeutics is not available. NLCs hold significant promise as drug delivery carriers, yet available preclinical and clinical studies remain inadequate. Consequently, there is a pressing requirement to broaden their range of applications to encompass clinical trials conducted under appropriate ethical oversight. This inadequacy may be attributed to the absence of a comprehensive examination of the safety profile of NLCs as drug carriers. Oral lovastatin NLCs were reported to display improved stability and augmented clinical adequacy indicating NLC potential [250]. Topical NLCs were prepared for the delivery of acitretin (ACT) to treat psoriasis. The clinical study results revealed a decrease in erythema, followed by a significant reduction in scaling. These findings indicate a range of improvement in disease symptoms, ranging from moderate to excellent, as a result of using the ACT-NLC gel formulation [251].

NLCs have been used in the formulation of various cosmetic preparations that are currently marketed in different countries, for example, Cutanova Nanorepair Q10 cream, FloraGlo®, NanoLipid Restore CLR®, NLC deep effect eye serum, extra moist softener, and Cutanova Nanovital Q10 cream [61]. They have been involved in the delivery of functional foods to ensure their safety from degradation by environmental stresses such as pH, light, and oxygen [252]. NLCs have their share in the food and cosmetic market currently with regulatory approval; however, their explicit use is awaited in drug delivery applications.

3.12. Constraints of NLCs in Drug Delivery. While NLCs offer many advantages in drug delivery, it is important to know their potential drawbacks and limitations to ensure the fabrication of better formulations. Below are some limitations that must be taken into account while designing NLCs:

(i) Complex formulation: Developing NLCs can be technically challenging and time consuming. Achieving the desired properties, such as particle

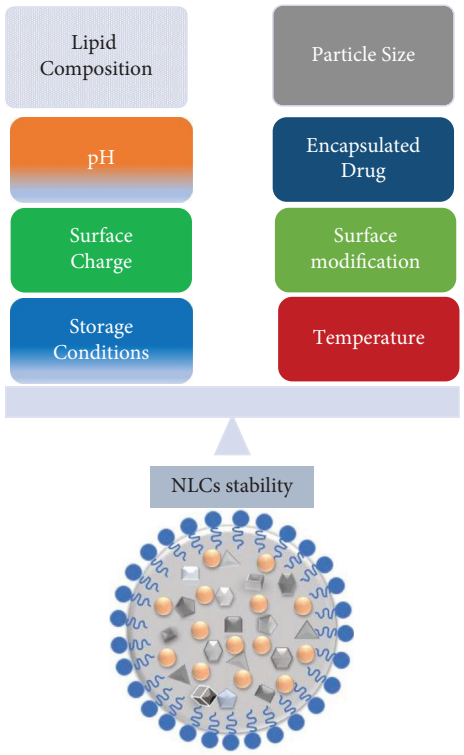

Figure 5: Influential factors on the stability of NLCs.

size, drug encapsulation efficiency, and controlled release, often requires careful formulation optimization.

(ii) Polymorphism: This hypothesis posits that the destabilization of the suspension occurs because of matrix lipid recrystallization, leading to the transformation of spherical particles into needle-shaped particles. The transition to a needle-like morphology is attributed to thermodynamic stability and the presence of a well-ordered β-structure within the lipid matrix. Furthermore, it is worth noting that this phenomenon can be influenced by surfactants, impurities, and stabilizers [253]. In the process of spray drying, the rapid evaporation of solvent leads to the formation of unstable polymorphic forms. A similar phenomenon has been documented in the spray-congealing method where the α-form of triglyceride can undergo a transformation into the more stable β-form, characterized by a higher melting point [254]. This transformation leads to the formation of crystalline aggregates and facilitates the release of drugs due to a reduction in the number of amorphous zones within the carrier matrix [255].

(iii) Phase separation: Particle aggregation can result in either irreversible processes, such as coalescence and sedimentation, or reversible processes such as flocculation, leading to phase separation. During storage, there is also a possibility of formulation gelling. To mitigate aggregation and gelling issues and to stabilize NLC suspensions, surfactants are employed. Depending on their characteristics, surfactants serve distinct roles in this stabilization process. Cationic or anionic surfactants can enhance the zeta potential value, whereas nonionic surfactants function as steric stabilizers. To address the storage stability challenges associated with liquid lipids, solid forms can be employed. This transformation can be achieved through processes such as lyophilization or spray drying of the suspension [256].

(iv) Lipid modification during storage: NLCs represent dynamic systems in which lipid molecules exhibit thermodynamic instability. This structural characteristic enhances their capacity to encapsulate drugs more effectively. However, the increased drug incorporation efficiency in these unstable configurations comes at the cost of enhanced drug mobility. Over time, during storage, the rearrangement of the crystal lattice may result in the formation of a thermodynamically stable configuration, ultimately leading to the expulsion of drug molecules.

(v) Storage conditions: NLCs are ideally stored at a temperature of 4°C. In this study, the physical stability of quercetin-loaded NLCs under various temperature conditions (4, 22, and 37°C) in the absence of light was assessed. Size, the polydispersity index, and zeta potential (ZP) were employed as stability indicators. The results revealed that quercetin-loaded NLCs exhibited stability when stored at a low temperature of 4°C for 28 days. However, exposure to higher temperatures, specifically 22°C for 10 days and 37°C for 24 hours, led to particle aggregation and a decline in the surface charge. This phenomenon was attributed to the disruption of hydrogen bonds between surfactant molecules at the lipid/water interface, which occurred as a consequence of the increasing temperature [257].

(vi) Manufacturing challenges: Scaling up the production of NLCs from a laboratory to a commercial scale can be challenging and costly, and maintaining consistent quality during large-scale manufacturing might be difficult.

(vii) Potential toxicity: Some lipid components used in NLCs may have toxic effects if administered in high doses or if they accumulate in the body over time. It is important to carefully select biocompatible lipids and conduct thorough toxicity studies.

(viii) Limited drug payload: NLCs may have limitations in terms of the amount of drug loaded into the lipid matrix. This limitation can be a constraint for drugs requiring high doses.

(ix) Drug release variability: Achieving precise control over drug release kinetics in NLCs can be challenging. Variability in drug release profiles may affect dosing regimens and therapeutic outcomes.

(x) Immune response: The body's immune system may recognize and clear NLCs, especially if they are administered repeatedly. Surface modifications may be necessary to reduce immunogenicity.

4. Conclusion and Future Perspectives

Designing and delivering therapeutics and biologicals via the oral route has always posed challenges to formulators. Issues such as poor solubility, permeability, drug degradation, first-pass metabolism, intraenterocyte metabolism, and enzymatic degradation have been persistent problems. Various approaches have been developed to address these issues, and NLCs offer a promising solution. NLCs represent a new generation of lipid-based nanoparticles with the capability to deliver medications effectively. They offer higher entrapment efficacy, protecting drugs from degradation in both in vitro and in vivo environments. NLCs are composed of biocompatible and biodegradable solid and liquid lipids that are FDA-approved. These NLCs also contain minimal amounts of surfactants and cosurfactants, making them suitable and safe for human use. The substantial amount of data available on PubMed reflects the significant interest among researchers in lipid-based nanoparticles.

The availability of NLC-based dermal products in the market has prompted scientists to explore NLC formulations for delivering therapeutics via other routes. In the context of oral drug delivery, NLCs can prolong GI transit time, enhance drug bioavailability, and reduce drug-induced toxicity. They offer adjustable release characteristics, facilitate the formation of micelles and chylomicrons, improve gastrointestinal permeation through intestinal lymphatic tropism, adapt at enterocyte layers, and enhance drug retention against P-gp pump efflux. This positions NLCs as promising candidates for drug delivery vehicles. However, one critical aspect to consider is the solubility of drugs in different lipids. This is crucial because many drugs exhibit limited solubility in various lipids, which can restrict achievable dosage levels. NLCs have been extensively studied for delivering both lipophobic and lipophilic drugs, brain delivery, proteins/peptides, theranostics, and other bioactives via oral administration. Addressing concerns related to lipid stability, cost-effectiveness, batch variability, and shelf life is essential.

We have highlighted the information about numerous patents that have been filed and granted for NLCs, underscoring their wide applicability and potential advantages over other carriers. Continuous advancements in NLC development, incorporating medications and biological substances, along with rigorous evaluation, promise to expand practical applications for formulators and researchers. Encouraging collaboration among academia, the industrial sector, and regulatory authorities is vital to ensure the safety and efficacy of NLCs. This collaboration may lead to the progression from research laboratories to well-defined clinical trials, ultimately paving the way for potential market entry of NLCs.

Data Availability

No data were used to support the findings of this study.

Conflicts of Interest

All the authors declare that there are no conflicts of interest.

Authors' Contributions

Kuldeep Kumar Bansal and Alok Kumar Mahor developed the concept of this review article. The literature survey was conducted by Priyanka Rathore, Ankita Kishore, and Jyoti Verma. Methodology and data collection were accomplished by Prem Prakash Singh, Rohit Goyal, Peeysuh Bhardwaj, Rishikesh Gupta, and Neeraj Sharma. Alok Kumar Mahor wrote the original draft. Kuldeep Kumar Bansal and Jessica M. Rosenholm performed a final review of the manuscript.

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