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REVIEW ARTICLE

Lipidic Nanosystem as State-of-the-Art Nanovehicle for Biomedical Applications

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Abstract Lipids have tremendously transformed the biomedical field, especially in the last few decades. Nanosystems, especially Lipid nanocapsules (LNCs), have emerged as the most demanding nanovehicle systems for delivering drugs, genes, and other diagnostic agents. Unique attributes and characteristic features such as higher encapsulation efficiency, stealth effect, ability to solubilize a wide range of drugs, capability to inhibit P-gp efflux pumps, and higher stability play a vital role in engaging this nanosystem. LNCs are a lipid-based nano-drug delivery method that combines the most significant traits of liposomes with polymeric nanoparticles. Structurally, LNCs have an oily core consisting of medium and long triglycerides and an aqueous phase encased in an amphiphilic shell. This manuscript crosstalks LNCs for various biomedical applications. A detailed elaboration of the structural composition, methods of preparation, and quality control aspects has also been attained, with particular emphasis on application approaches, ongoing challenges, and their possible resolution. The manuscript also expounds the preclinical data and discusses the patents atlas of LNCs to assist biomedical scientists working in this area and foster additional research.

Keywords Nanomedicine · Nanotechnology · Lipid Nanocapsules · Biomedical applications

Abbreviations

AIDS	Acquired Immunodeficiency syndrome
AMPs	Antimicrobial peptides
AUC	Area under the curve
CNS	Central nervous system
DNA	Deoxyribonucleic acid
DSC	Differential scanning calorimetry
EPR	Enhanced permeation and retention
FDA	Food and drug administration
FTIR	Fourier-transform infrared spectroscopy
GRAS	Generally regarded as safe
HPLC	High-performance liquid chromatography
LNCs	LNCs
MAB	Monoclonal Antibodies
MSNs	Mesoporous silica nanoparticles
O/W	Oil in water
PBS	Phosphate bovine serum
PEG	Polyethylene glycol
PEO	Poly ethylene oxide
PIT	Phase inversion temperature
PIZ	Phase inversion zone
PK/PD	Pharmacokinetic/pharmacodynamic
PNIPAM	Poly (N-isopropyl acrylamide
PTX	Paclitaxel
RPV	Ropivacaine
SEM	Scanning electron microscope
si-RNA	Small interfering RNA
TEM	Transmission electron microscopy
W/O	Water in oil
XRD	X-Ray diffraction analysis

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Introduction to Nanomedicines and Nanotechnology

Nanotechnology and nanoscience have enormously impacted the advancement of the world. Nanotechnology has emerged as one of the most significant scientific breakthroughs of the twenty-first century [1, 2]. Nanotechnology is growing increasingly due to its versatile potential applications in material science, engineering, computing, biotechnology, and even the pharmaceutical realm [1, 3]. Nanoscience is an amalgamation of physics, material science, and biology. It focuses on manipulating materials at the molecular and atomic scales.

In contrast, nanotechnology is the capacity to understand, measure, customize, establish, govern, and fabricate a subject matter at the nanometer scale [4]. The National Nanotechnology Initiative (NNI) of the United States describes nanotechnology as "a science, engineering, and technology conducted at the nanoscale (1 to 100 nm), wherein special phenomena allow novel applications in a wide range of fields, from chemistry, physics, and biology to medicine, engineering, and electronics" [5]. Nanotechnology is no longer a new concept; it has existed since the fourth century AD [6]. The American physicist and Nobel prize laureate Richard Feynman introduced nanotechnology in 1959. Richard Feynman described the hypothesis and vision of creating smaller machines and forming molecular levels using devices and technology in that concept. For that idea and demonstration, he was recognized as the *Father of Nanotechnology* [1, 7].

In the pharmaceutical and biomedical realm, nanotechnology has gained more scientific attention than diagnosis, prevention, and treatment [8, 9]. Nanomedicine, a developing field formed by blending nanotechnology with medicine, is one of the most promising routes for developing efficient therapies [10]. At present, nanotechnology-based medicine is focused on improved and precise treatment, minimal adverse effects/toxicity, and unmet medical needs of patients [2]. As time moved on, nanomedicines were designed based on the need for such characteristics and functions to exhibit biodegradability, biocompatibility, no or minimal toxicity, non-immunogenic, easy preparation, and high drug loading efficiency [11]. Subsequently, nanomedicines were known by other names as well as nanocarriers, nanosystems, nanoghost, nano-vesicles, nano-composites, nano-complex, nanoscaffold, nano-matrix, nanosensors, nanoparticles and nanocapsules [12].

The transition of nanomedicines over conventional medicines is because of the outstanding benefits offered by nanomedicines, such as enhanced efficacy, safety, physicochemical characteristics, and pharmacokinetic/pharmacodynamic (PK/PD) profiles of pharmaceutical substances [13]. Increased frequent administration, toxicity, low solubility, and instability of drugs are significant concerns allied with conventional drug delivery that can be augmented using nanomedicines [2].

To date, countless nanomedicines have been developed and widely accepted in clinical and non-clinical applications [13]. Lipid-based nanosystems have presented new avenues to the pharmaceutical industry due to their characteristic structure and capability in drug delivery. The key factors that support their success in the field of superlative drug delivery over conventional drug delivery are ideal physicochemical characteristics, the ability to transport macromolecules (i.e., proteins, peptides, oligonucleotide, and DNA), offering high bioavailability, administered via numerous sites, built-in capacity to triumph over the blood–brain barrier, etc. [14]. Lipid-based nanosystems such as liposomes, solid lipid nanocarrier, lipoplex, nanostructured lipid carriers, and LNCs have shown noteworthy work by encapsulating small molecular weight phytochemical drugs and macromolecular likes identify RNA (iRNA), plasmid DNA, peptide and protein [15, 16]. In 1995, the first “nano-drug” Doxil (Liposome) received FDA approval for the treatment of AIDS-related Kaposi’s sarcoma and earned approximately $600 million in yearly sales across all of its markets [81].

By 2022, 21 lipid-based nanomedicines will be approved globally for different diseases, including vaccine delivery [11, 82]. However, significant drawbacks (i.e., use of organic solvents, instability in biological fluids) and the development of a non-toxic, solvent-free, low-energy process and stable vector that can encapsulate and deliver the drug as well genetic materials to the target cell have transformed towards LNCs [17–19].

This review will express the view of nanotechnology and nanoscience in the pharmaceutical research industry and the significance of nanosystems in advancing nanomedicines. Also, the importance and trends of LNCs in drug and gene delivery can be analyzed by exploiting their mechanism in efficient delivery. In addition, it will guide the merits of LNCs and possible approaches for drug delivery, gene delivery, and drug-gene hybrid delivery of various drugs and biomolecules. Here, we have summarised the functional superiority of LNCs over other nanocarrier systems.

Lipid Nanocapsules and their Biomedical Benefits

Lipid and polymeric nanoparticles have been controversial for a long time for the delivery of a vast range of drugs and biomolecules [12]. In a nutshell, nanocapsules can be defined as a vesicular system in a range of 50-300 nm, where a drug or active moiety is confined in the inner cavity (liquid/solid), which is layered by the polymeric coating [8, 20]. In general, LNCs have an oily core formed primarily of a medium chain triglyceride encompassing capric and caprylic

acids, which is encased by a surfactant shell composed of a pegylated surfactant and lecithin or other lipophilic co-surfactants [21]. LNCs are produced using generally regarded as safe (GRAS) and FDA-approved components that support inertness, non-toxic, non-immunogenic, biocompatible, biodegradable, and cost-effective [22–24] (Figs. 1 and 2).

In addition, they are smaller in size and possess large surface area, broad drug-loading features, and high specificity to the target site. These characteristics make them suitable nanocarriers for the transport of drugs and genes [25]. Owing to their structures and function, they are also known as bio-nanocapsules, nano-cargo, nano-vectors [26], and biomimetic nanocarriers [27, 83]. LNCs can be manufactured swiftly, without organic solvents, and at scale, and their characteristics can be tailored for the best possible form for drug delivery [24]. The salient feature of LNCs is mentioned in Fig. 3**.** LNCs consist of a lipid core and a tension-active shell, and the lipid core allows for the dispersion of drugs (hydrophilic, lipophilic, or amphiphilic) or adsorbed at the interface of capsules [28]. So far, several researchers have investigated LNCs as novel carriers for topical, transdermal [28, 29], intradermal [30], nose-to-brain [31] central nervous

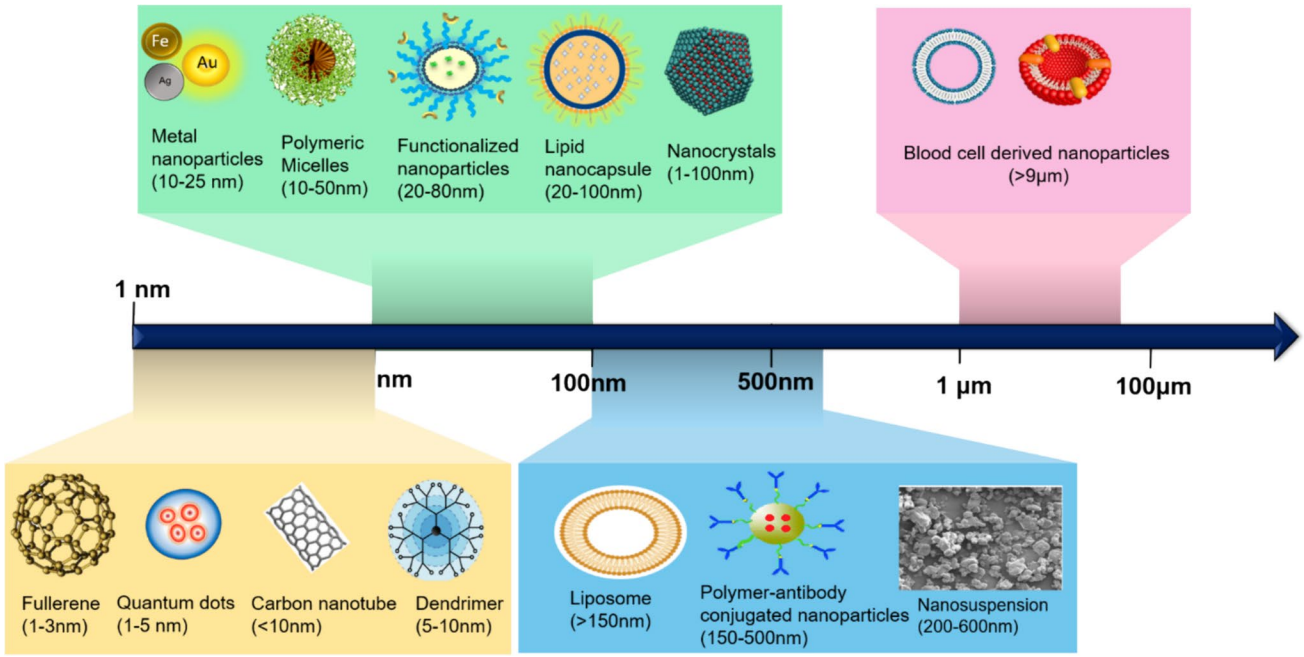

Fig. 1 Size comparison of nanomedicines

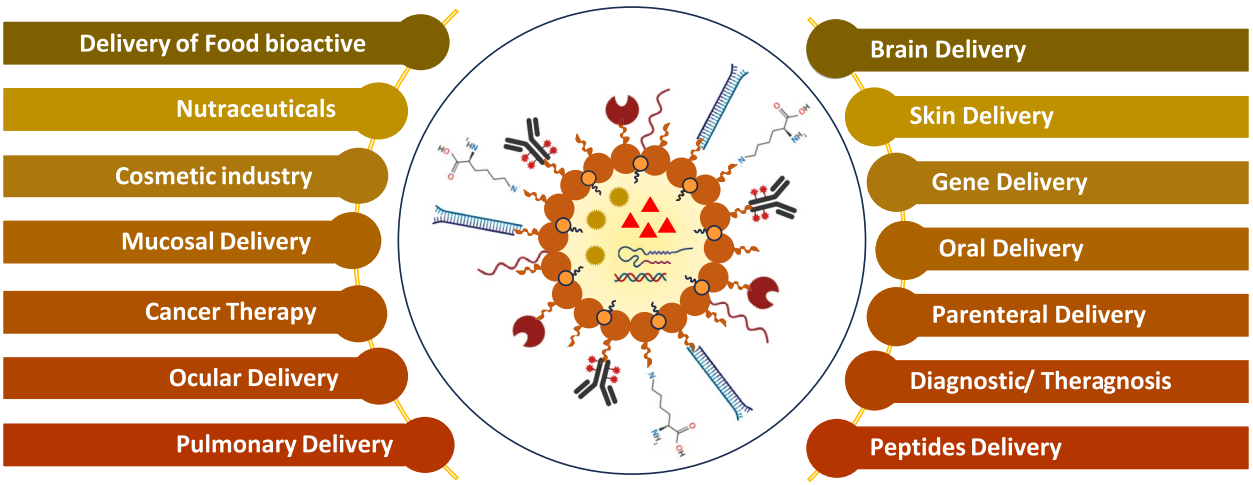

Fig. 2 LNCs as a novel system in biomedical applications

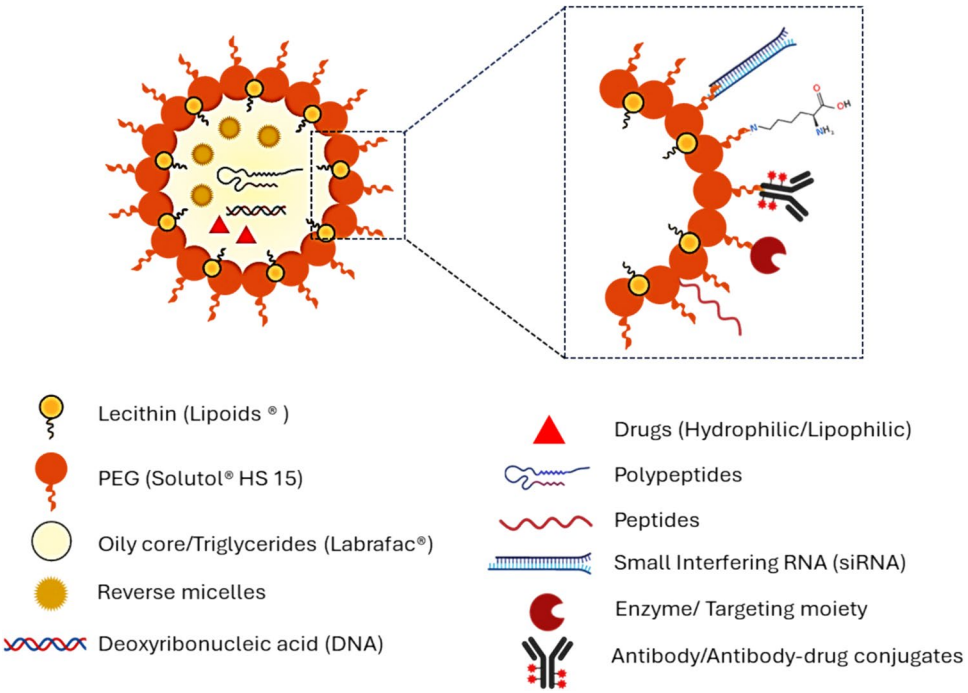

Fig. 3 Schematic representations of LNCs

system (CNS) delivery [24] for different disease by encapsulating drugs, peptides and DNA (Fig. 2).

Groo et al. utilized LNCs nanocarriers to deliver encapsulated antimicrobial peptide-loaded reverse micelle (RMs-AMP) against infectious diseases. The study's primary objective was preserving antimicrobial activity and efficient antimicrobial peptide (AMP) delivery of AMPs (AP138) through LNCs. RM-LNCs were developed using the phase inversion temperature method to incorporate AMP-loaded reverse micelles. Further, AMPs-loaded LNCs were evaluated for antimicrobial activity, and it was found that AP138 was efficiently encapsulated in the LNCs, which showed preserved antimicrobial activity and resistance against protease. LNCs not only shielded peptides from proteolytic enzymes but also enhanced the antimicrobial activity of peptides [27]. Zhai et al. developed the novel ropivacaine-loaded LNCs (RPV-LNCs) with a mean particle size of 62.1 ± 1.7 nm and evaluated RPV-LNCs, and transdermal delivery potential. Mice models were used for transdermal delivery of RPV-LNCs. Topical application of RPV-LNCs showed enhanced permeability as compared to propylene glycol. They proved that LNCs can modulate *stratum corneum*. Hence, LNCs could be used as promising nanocarriers for transdermal delivery [28].

The oral route is considered the most convenient route for drug administration and is widely accepted by patients. However, drugs with low bioavailability, solubility, low intestinal permeability, enzymatic degradation, and mucosal instability affect the drug's efficacy. For this reason, LNCs are considered promising techniques for enhancing oral drug delivery [32]. Mucus is a multifaceted biological fluid layered over the eyes, lungs, nasal tract, vagina, and gastrointestinal tract, that mainly lubricates and forms a protective layer on organ surfaces. However, the mucus layer has been noted as a barrier for the exogenous substances, including peptides and drugs like paclitaxel (PTX), affecting their physicochemical properties and hindering pharmacological responses. Groo et al. investigated the interaction between intestinal mucus and PTX/PTX-loaded LNCs for improved oral drug delivery. PTX-loaded LNCs were prepared following the original method, and DiI and DiD were also incorporated with PTX-LNCs for imaging tracking fluorescent substances.

Further, different formulations of Taxol® (Standard PTX formulation) and PTX-loaded LNCs were studied for transport using pig's intestinal mucus in the Transwell® diffusion system. They demonstrated that LNCs have considerably amended PTX transport in the mucus layer than Taxol® and also presented that PTX-loaded LNCs showed more extended stability under the mucus layer. These findings stated that LNCs are suitable nano vectors for oral delivery of biopharmaceutical classification system class IV (BCS-IV) drugs, including PTX [26]. Numerous advantages of LNCs loaded or coated with peptides and drugs are presented in Table 1.

Unique Architectural Features of Lipid Nanocapsules

LNCs are bio-inspired nanocarriers with an architecture that is a cross between polymeric nanoparticles and liposomes.

Table 1 Application of LNCs in drug and gene delivery

Formulations		Preparation method	Study design	Encapsulation rates	Advantages	References
Drug (s) / Biomolecule	Ingredients					
AP138 Antimicrobial Peptides (AMP)	Labrafac® WL 1349, Plurol® CC 497, Kolliphor® HS-15 (Sol),	Phase Inversion Technique	In vitro Microbial study against *Staphylococcus aureus* and *methicillin-resistant Staphylococcus aureus* (MRSA)	97.8 ± 1.2	Ability to encapsulate and deliver peptides, Protection against proteolytic enzymes, High loading efficiency,	[27]
Ropivacaine	Solutol HS15, Labrafac, Lipoid	Phase Inversion Technique	In vitro on Excised skin of Kunming mice, In vivo on Kunming mice through topical application,	92.6 ± 1.3%	Enhanced drug permeability through skin	[28]
NFL-TBS.40–63 Peptides	Solutol HS15, Lipoïd®, Labrafac®	Phase Inversion Temperature	**In vivo** Sprague Dawley female rats	–	Ability to adsorb peptide on the LNCs's surface	[22]
PTX	Captex® 8000, Lipoid® S75-3, Solutol® HS15	Phase Inversion Temperature	**Ex vivo** Pig intestinal mucus	–	LNCs Improved the transport of PTX through the mucus layer, LNCs showed excellent integrity with mucus	[26]
–	Captex® 8000, Kolliphor® HS-15, Lipoid® S75-3, DSPE-mP (2000), Stearylamine	Phase Inversion Temperature	**In vitro** *Caco-2* and *HMEC-1* Cells	–	Higher intestinal absorption	[32]
Prostaglandin D2-glycerol ester	–	Phase inversion temperature method	**In vitro In vivo** *Mice model*	–	Easily traceable, site-specific delivery, LNCs surface coating with cell-penetrating peptides, LNCs increased PGD2-G efficiency	[31]
Hypericin	Labrafac®, Kolliphor ® HS 15, Lipoid® S 100	Phase inversion method	**In vitro** *CT-26* (Mouse colon cell line) **In vivo** female Balb/C nude mice	99.67 ± 0.35	High encapsulation efficiency, Enhanced skin drug deposition (seven-fold), Safe, non-invasive, and site-specific delivery of Hy-LNCs	[30]
Indomethacin, Diclofenac, Caffeine	Phospholipon® 90G, Miglyol® 812N	Phase Inversion Temperature	Ex vivo porcine skin	> 98	Great encapsulation efficiency, LNCs showed superior effects on drug permeation through the skin	[29]

Table 1 (continued)

Formulations		Preparation method	Study design	Encapsulation rates	Advantages	References
Drug (s) / Biomolecule	Ingredients					
Doxorubicin HCl, Oxaliplatin, 5-Fluorouracil, Irinotecan, SN38, Regorafenib	Transcutol® HP, Labrafac WL 1349, Labrafil 1944 CS, Solutol, Lipoid	Phase Inversion Temperature	**In vitro** *HT29* & *SW480* (human colon cancer cells), *CT26* and *MCF-7* (human breast cancer cells) **In vivo** female Balb/C mice	**91.2 ± 1.1**, 23.0 ± 2.0, 62.4 ± 3.3, **78.6 ± 6.2**, **76.7 ± 7.2, 99.9 ± 0.4**	High drug loading efficiency, Reduction in tumour growth, SN38-loaded LNC showed reduced drug-induced haemolysis, LNCs are safer for intravenous administration	[58]
Benznidazole	Kolliphor® HS-1, Lipoid S-100, Labrafac® WL 1349	Phase Inversion Temperature	**In vitro** *Caco-2* Cell (human intestinal cell line)	**91.82 ± 1.03**	Improved drug permeability via intestinal epithelium (tenfold compared to free drug), provides a sustained release platform	[59]

Long or Medium-chain triglycerides make up the oily core of LNCs, which is encased in a surfactant shell formed of a PEGylated surfactant and lecithin or other co-surfactants (Fig. 2.) [10]. In a recent investigation, Urimi et al. used cryo-TEM, small-angle X-ray (SAXS), and neutron scattering (SANS) techniques to clarify the structure of LNCs developed using the phase-inversion approach. Further, combined analysis of Small-angle X-ray scattering (SAXS), Small-angle neutron scattering (SANS), and cryo-TEM data revealed the presence of a core–shell system in the LNCs. Additionally, the drug loading had an insignificant effect on the LNCs's overall core–shell structure. According to SANS data, the core size remained constant for unloaded LNCs and LNCs loaded with drugs (Fig. 3.).

LNCs comprise an oily phase, an aqueous phase, and a nonionic surfactant (Table 2). The oily phase is a blend of medium and long-chain triglycerides serving as the base for lipophilic substances, while aqueous and surfactant play a vital role in the stabilization and dispersion medium (Figs. 4 and 5).

Oily Phase

Most LNCs' components are liquid oils, whereas the shell comprises solid lipids with an oily phase that serves as a drug reservoir. The primary elements of this phase are medium-chain triglycerides, including capric acid, caprylic acid, and triglycerides (Labrafac® WR 1349). Compared to long-chain triglycerides, medium-chain triglycerides are distinguished by a more profound solvent capacity for hydrophilic molecules, GRAS status, good self-dispersibility, and higher stability [33]. On top of that, medium-chain triglycerides function as penetration boosters. There are lipids such as ethyl oleate, isopropyl myristate, Miglyol®, and Captex® 8000 (glyceryl tricaprylate), as well as some vegetable oils, notably castor oil, soybean, olive oil, and sesame oil are also used. [34].

Surfactants: Lipophilic and Nonionic

A prominent lipophilic surfactant used in LNCs is lecithin. It is a phosphatide that exists in both plants and animals. Lecithin is a complex compound made up of phosphatidyl esters such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine as well as additional elements like carbohydrates, fats, and triglycerides. Lecithin is derived from eggs, soy beans, sunflowers, and lysolecithin. It is also available commercially under brand names such as phospholipon® and Lipoid®. Phospholipon® is a blend of natural hydrogenated lecithin and phospholipids that serve as compound lipid and establishes the important elements of the plasma membrane [35]. Lipoid® S75-3 is the most widely utilized lipophilic surfactant, which includes

Table 2 Formulation Compositions and their role in LNCs

Excipients		Example	Effects	Percentage	References
Oil	Capric acid and Caprylic acids	Labrafac® WR 1349	Increase of LNC size, good self-dispersibility, penetration enhancers	10–25% (w/w)	[42, 60]
	Lipids	Ethyl oleate, isopropyl myristate, or Miglyol® and Captex® 8000			
	Vegetable oil	Castor oil, soybean, olive oil, and sesame oil			
Lipophilic surfactant	Lecithin	lipoid® and phospholipon®	Facilitating the freeze-drying process and preserving the LNCs hard shell	7–10% (w/w)	[24]
Nonionic surfactant	Polyethoxylated surfactant	Solutol® HS15 (Kolliphor™HS15), Cremophor RH40 and RH60,	A major influence on LNC formation and stability	10–40% (w/w)	[10]
Water	–	Mili-Q®	Dilution purpose	35–40%(w/w)	[10]
NaCl	–	–	Decrease the phase inversion temperature	1.75%(w/w)	[36]

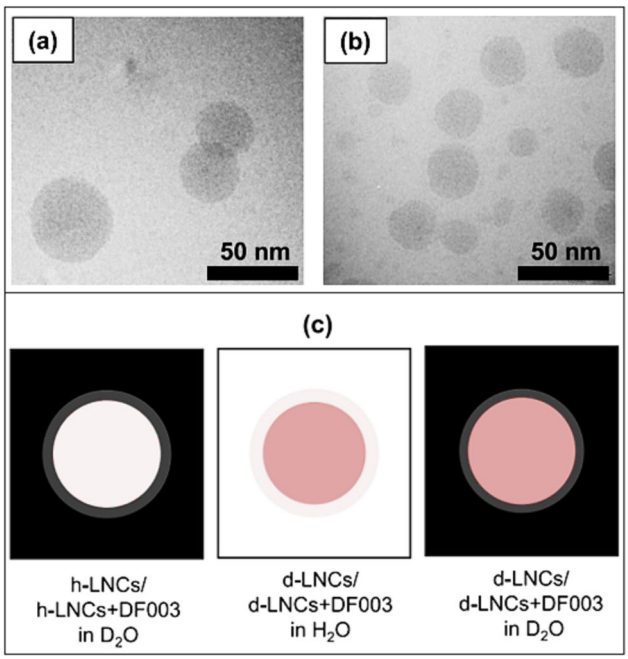

Fig. 4 Cryogenic transmission electron micrographs of **a** unloaded and **b** drug-loaded LNCs, **c** a representation showing various contrasts in the dispersion medium and the core. Adapted with permission from [57] under CC BY 4.0 DEED Attribution 4.0 International (https://creativecommons.org/licenses/by/4.0/) copyright © 2022 American Chemical Society

69% phosphatidylcholine soybean lecithin and functions as a co-surfactant. It is used in modest amounts to considerably boost LNCs stability, which is especially important in the case of lower-size (50-100 nm) LNCs. It is also known as amphiphilic co-surfactant. The amount of lecithin present in composition directly relates to the thickness and hardness of the outer shell. [36].

The phase inversion of an emulsion depends significantly on nonionic surfactants, polyethylene glycol (PEG) chain is likely to impact the temperature needed for the inversion. The most frequently used nonionic surfactant is Solutol® HS 15, which can be found under the brand term Kolliphor HS 15. It is a polyglycol ester of 12-hydroxystearic acid and PEG. Amphiphilic characteristics are added to Solutol® by the hydrophilic PEG combined with hydrophobic hydroxy stearate [37]. Consequently, it has considerable surface activity and affects the triglyceride/water interface. In LNCs, the ratio of Solutol® to total particle size is inversely proportional. Other nonionic surfactants from vegetable oils, especially hydrogenated Cremophor EL (ethoxylated castor oil), Cremophor RH40, and PH60 (ethoxylated hydrogenated castor oil), have been discovered for use in LNCs.

Water

Water is a commonly used solvent in the preparation of LNCs and has a significant role in forming, stabilizing, and functionalizing lipid nanocarriers [38]. The water ration in LNCs maintains hydration around the shell and the integrity of the hydrophilic shell. In addition, it acts as a continuous phase or dispersion medium for hydrophobic or lipid molecules during the emulsification process and prevents aggregation. The aqueous environment within LNCs makes it more biocompatible [37, 39].

Sodium Chloride (NaCl)

In developing LNCs, sodium chloride is often employed at a concentration of 1.75 percent. The phase inversion zone, where the W/O emulsion transforms into an O/W emulsion,

Fig. 5 Salient biomedical features of LNCs

shifts from higher to lower temperatures by adjusting the NaCl concentration (1–5%) before dilution [33].

Manufacturing Techniques and Quality Control Attributes of LNCs

Researchers have developed and utilized several approaches to prepare and characterize LNCs. The preparation approaches are solvent-free phase inversion method/ phase inversion method and emulsion-diffusion solvent evaporation. The phase inversion method/ phase inversion Technique is approach is based on the characteristic property that allows typical nonionic surfactants, such PEG surfactants, to change their affinity for oil and water depending on temperature [40]. As the temperature rises, heat agitation causes hydrogen bonds between water molecules and oxide groups to break, eventually leading the PEG moiety's ethylene oxide groups to dry, enabling the lipophilic property of nonionic surfactants to predominate. Accordingly, the system is a continuous micro-emulsion when the balance between lipophilicity and hydrophilicity appears in the phase inversion zone (PIZ). It is abruptly broken up by a rapid cooling step, promptly developing a nanoemulsion of small droplets constituting the LNCs [24, 41, 42].

In 1969, Shinoda and Saito introduced the Phase inversion method. This method is being adapted due to it is low-energy emulsification technique, which includes no use of

hazardous organic solvent (chloroform or dichloromethane) that needs to be removed after production and producing very small nanocapsules by thermally manipulating an oil/water mixture [42].

In this method, an aqueous phase comprising sodium chloride and distilled water was combined with the oily phase (i.e., Labrafac®). The hydrophilic surfactant, such as Solutol® HS 15 and Lipoid®, which contains 69% phosphatidylcholine soya bean lecithin in modest quantities to significantly improve LNCs stability, was added to the system and heated under magnetic stirring at 85 °C, ensuring that the phase inversion temperature is attained. W/O emulsion will form once the temperature reaches 85 °C, and O/W emulsion will follow when the temperature drops to 60 °C at a rate of 4 °C/min. Phase inversion is then achieved by applying three temperature cycles (85–60-85–60–85 C). To create an o/w emulsion, the created emulsion is next quickly chilled by dilution with cold water (0 °C) [41]. A rapid alteration in temperature causes an abrupt shock that breaks the micro-emulsion system. As a consequence, lipid content solidifies to form a shell, creating stable nanocapsules. In addition, surfactants are trapped and concentrated in the interfacial zone due to temperature cycling, creating a thick tension-active shell that serves as a barrier to oil diffusion [34].

The "emulsion-diffusion" method has been used successfully to create well-defined nanocapsules. This two-stage procedure starts with a typical emulsification step and removes certain portions of the oil phase in the next stage. The process begins with creating an oil-in-water emulsion with an "oil phase" that contains both the polymer and the oil in an organic solvent (i.e., ethanol, ethyl acetate). The polymer and the oil separate when the organic solvent in the oil phase is removed, which reduces particle size [43].

Zafar et al. followed a similar technique for loading Docetaxel (Dx) and thymoquinone (Tq) into LNCs simultaneously. DxTq-LNCs were obtained by blending appropriate proportions of liquid and solid lipids with lipophilic drugs in ethanol. Furthermore, the organic phase containing the drug-lipid complex was gradually injected into the aqueous phase containing sodium chloride (NaCl), followed by a continuous stirrer. This method has similar advantages as the phase inversion method, including high encapsulation yields, high repeatability, improved control over particle size, and ease in scaling-up while it differs as it uses organic solvent [40, 43].

Quality Control Approaches for Lipid Nanocapsules

Several characterization techniques have been implemented to assess features like hydrodynamic diameter size, polydispersity index (PDI), and morphological facets to demonstrate the development of LNCs. Other characteristics, such as encapsulation efficiency, stability, etc., can also be obtained using the technique mentioned in Table 3.

Biomedical Application of Lipid Nanocapsules

LNCs can deliver anti-inflammatory drugs and antimicrobial agents to target sites of inflammation or infection. This targeted approach enhances the drug's therapeutic effect and minimizes systemic side effects. LNCs can strengthen the effectiveness of vaccines by encapsulating antigens and adjuvants. This improves antigen presentation, leading to more robust and longer-lasting immune responses, potentially providing more effective vaccines against infectious diseases [44]. Since their development, LNCs have been used for various purposes, including the delivery of drugs

Table 3 Quality control strategies for LNCs

Techniques	Measurement	References
Zetasizer Nano	Average diameter (Hydrodynamic diameter), polydispersity index, and Zeta potential	[41, 60]
Dialysis bag method	*In-vitro* release study	[61]
Differential scanning calorimetry (DSC)	To compare the thermal properties of the drug-loaded LNC to those of the blank LNC, the physical mixture, and the raw ingredients	[41]
Stability study	Size distribution, polydispersity, zeta potential, and entrapment efficiency measurements are used to determine the stability of nanocapsule during storage at 4 °C and room temperature	[34, 60]
High-pressure liquid chromatography- ultra-violet (HPLC–UV) spectrometry	Encapsulation efficiency and drug loading capacity	[57]
Cryogenic Transmission Electron Microscopy (Cryo-TEM)	Morphology analysis (Shape) and Inner structure	[57]
X-ray Diffraction (XRD)	The crystallinity characteristics	[62]
Fourier Transform Infrared Spectroscopy (FTIR)	To evaluate possible interaction between the drug and different components of the nanosystems	[37, 63]
Atomic force microscopy (AFM)	To understand surface morphology (3D image)	[64]

and biomolecules, food and bioactive ingredients, nutraceuticals, cosmetics, diagnostics, and theranostics, among others.

Delivery of Therapeutic and Diagnostic Agents

LNCs have been explored to safely deliver medicinal drugs via oral, parenteral, cutaneous, inner ear, pulmonary, ophthalmic, and brain routes, enhancing their permeability and bioavailability. Although oral administration is the preferred mode, not all medications can be delivered via oral route. According to Draper et al., the presence of P-glycoprotein limits the oral bioavailability of several drugs. However, they discovered that encapsulating certain drugs in LNCs may overcome such a problem [45].

In an investigation by Ashour et al., a phytomedicine tanshinone IIA has been transformed into LNCs to improve its oral bioavailability. The prepared LNCs showed particle size, zeta potential, and entrapment efficiency of 70 nm, 13.5 mV, and 98%, respectively. In addition, around a four-fold increase in the area under the curve was observed compared to tanshinone IIA suspension. Additional evidence of LNCs showed an enhanced half-life and mean residence time of tanshinone IIA, indicating prolonged circulation. [46].

A comparable result was seen by Pensel et al. when albendazole LNCs were given orally to mice with echinococcus granulosus infection [47]. LNCs are believed to be ideal candidates for injectable nanocarriers due to their monodisperse nanometric size, biocompatible ingredients, GRAS surfactants, low macrophage uptake, and ability to suppress P-gp pump [48].

Gene Delivery

LNCs can also serve as carriers for gene delivery, allowing the transport of therapeutic nucleic acids (such as Small interfering RNA (siRNA) or Messenger RNA (mRNA)) to target cells, providing potential treatments for genetic disorders and other diseases. LNCs were initially created by mimicking lipoproteins and using biodegradable excipients for hydrophobic compounds. LNCs have found widespread use in the last five years for the delivery of nucleic acids and in treating various illnesses, particularly cancer [49]. Gene therapy using siRNA is beneficial for temporarily inhibiting protein expressions in cancer development or chemotherapy resistance. In contrast to intra-tumoral delivery, the development of nanomedicines for intravenous administration is prioritized. Additionally, it was noted that a significant accumulation of LNCs intra-tumorally was seen after siRNA LNCs were modified utilizing PEG [50].

Similarly, Skandrani et al., developed functionalized LNCs with poly(ethyleneimine) to deliver plasmid DNA. At the same dose of poly(ethyleneimine), the transfection efficiency increased by more than 2.8-fold compared to free pDNA. Additionally, it was shown that high drug efficacy was attained when LNCs were loaded with the hydrophobic medication PTX [51].

Diagnostic and Theranostic Application

LNCs are well demonstrated for therapeutic efficiency. However, their use in diagnosis is limited and has not been explored much. The lipidic core or the surface of LNCs could be loaded or functionalized with various radionuclides, such as 99mTc, 188Re, 111In, or 125I, and could be used for radiotherapy, imaging, and oxygen sensing. Magnetic resonance imaging can map hypoxia in oxygen-deficient areas of biological systems or oxygen in cancers with increased sensitivity to lipid signals. During a hyperoxia test, it was found that LNCs were responsive to oxygen shifts. Furthermore, the use of magnetic resonance imaging enabled the mapping of oxygen gradients and tumor heterogeneity. [45, 52, 53].

Nandwana et al. developed self-assembled lipid nanoconstruct magnetic LNCs for non-invasive theranostic application. As comparative data among traditional lipid nanocarriers, magnetic LNCs showed superior structure stability and theranostic (therapeutic and diagnostic) capabilities. Magnetic LNCs exhibit enhanced therapeutic efficacy (16 times) than the free drug due to a larger payload and triggered drug release. The magnetic resonance contrast enhancement of magnetic LNCs was investigated by measuring spin–spin relaxation time (T2) of the magnetic LNCs under 3 T-MRI, and it was shown to be nine times greater than that of ferumoxytol (FDA-approved T2 contrast agent), indicating the LNCs' diagnostic ability. As an outcome, LNCs will likely perform well in theranostic applications [54].

Targeted Delivery of Therapeutics

Surface modifications of LNCs with ligands, antibodies, or peptides can enable specific targeting to particular cells or tissues. This targeted drug delivery approach increases drug accumulation at the intended site, enhances therapeutic efficacy, and reduces off-target effects on healthy tissues. LNCs are considered versatile carriers for bypassing barriers such as the blood–brain barrier and blood-cerebrospinal fluid barrier. Hearing loss is challenging to cure because the temporal lobe has limited access to therapeutic medicines. LNCs proved to be suitable carriers for inner ear delivery and showed acceptable biocompatibility. The only nanocarriers that demonstrated genuine drug transport efficiency to the cochlea were LNCs. Low doses of hydrophobic medications can be linearly released into the inner ear using LNCs. There is no initial burst release with them.

Food Industry

Due to growing interest in health and life quality, natural foods and substances with functional characteristics have become more prevalent in the food sector. Owing to their antioxidant action, carotenoids-natural colorants linked to positive health effects, have drawn particular interest in this context. The carotenoid lutein is plentiful in crops like kale and spinach and is the second most common carotenoid in human blood plasma (carotene being the first). Flowers can also serve as a source of lutein to create nutritional supplements and vegetables. Only the marigold flowers (Tagetes) are commercially grown as a source of this carotenoid, and commercial production of lutein is limited to a few species. A powerful antioxidant that actively shields tissues against reactive species damage is lutein, a hydroxylated carotenoid. The consumption of lutein through diet has also been firmly linked to a decline in conditions, including arteriosclerosis, cataracts, diabetic retinopathy, cancer, and other disorders, in addition to reducing age-related macular degeneration. Despite having these significant biological functions, lutein is an unstable chemical with low bioavailability since it cannot dissolve in water. As a result of processing and storage, as well as variables including temperature, oxygen availability, light exposure, water activity, moisture, acidity, metals, and peroxides, lutein's stability varies greatly.

In an effort to boost the stability of carotenoids, new technologies are constantly being developed. One such technology is microencapsulation, which may disperse powders in water and prevent or lessen the oxidation of bioactive components found in meals. Application of delivery methods with sizes smaller than 1 μm, such as Nanoemulsions, nanoparticles, and nanocapsules, is another option for stabilizing the five carotenoids. The stability and likelihood of usage in the food business are improved by lutein-enriched LNC. Foods can be nano-encapsulated to alter their textures, flavors, colors, and stability. Nutraceuticals are ingredients added to food to improve nutrition. Depending on the size of the nanocarrier, these compounds have a higher bioavailability. The higher the transport capabilities and solubility of the nutraceuticals, the smaller the nanocarrier and the simpler it is for the nanocarrier to enter the bloodstream. For the encapsulation of nutraceuticals, LNCs are employed [55].

Cosmetic Industry

LNCs are well known for cutaneous and transdermal applications because of their small particle size, high encapsulation efficiency, biocompatibility, physical stability, and preservation of encapsulated compounds. According to Da Silva et al., LNCs are shown to be superior to polymeric nanocarriers for the treatment of skin problems. Using ibuprofen as the reference drug, they compared the characteristics of lipid and polymeric nanocarriers for dermal administration. Thus, LNCs improved drug penetration, which is desired for cutaneous application. LNCs are encapsulated with vitamin K1, and their skin penetration is checked using Franz-type diffusion cells. Permeability test revealed that LNCs had more Phytonadione (vitamin K1) in their dermis than controls [56]. 5.8 Biomedical applications.

Biomedical Applications

Researchers have describes the effects of hypoxia, or low oxygen levels, in solid tumors is a result of abnormal and twisted blood vessels that fail to supply enough oxygen to meet the tumor's needs. Magnetic resonance imaging (MRI), specifically the quantitative assessment of longitudinal relaxation time enhancement, has been shown to be capable of mapping oxygen levels in tumors. This method is more sensitive to lipids compared to water signal. Lipid nanocapsules (LNCs) are proposed as a therapeutic and diagnostic tool in this context. By utilizing the lipidic core of LNCs, researchers aim to develop a technique to increase the lipid content within tissues. This increase in lipid content is expected to enable the assessment and mapping of tissue oxygen levels (pO2). In vitro studies have shown that LNCs are permeable and responsive to oxygen, indicating their potential usefulness in this application. This suggests that LNCs could serve both as a therapeutic tool, possibly by delivering oxygen to hypoxic tumor regions, and as a diagnostic tool for mapping tissue oxygen levels ([53] #72).

Challenges Associated with Lipid Nanocapsules and their Possible Resolution

LNCs have distinct advantages in several aspects, including as drug delivery vehicles. However, they also have some drawbacks that hinder LNCs' functionality. Some of the issues that LNCs face and potential solutions have been presented in Table 4.

Patents Landscape in Arena of Lipid Nanocapsules

So far, different patent applications have been made globally concerning the development method of LNCs and the encapsulation of hydrophilic and lipophilic substances, such as active pharmaceutical ingredients, nucleic acids, and peptides. In addition, the ability to modify the surface is indicated by the successful filing of patents on stealth LNCs. The critical LNCs patent applications are summarized in Table 5.

Table 4 Overview of challenges and resolution concerned with LNCs

Challenges	Issues	Resolution	References
Stability	LNCs are susceptible to instability difficulties such as aggregation, coalescence, and Ostwald ripening, which can reduce their shelf life and drug delivery efficiency	LNCs stability can be improved by including stabilizing agents comprising surfactants, polymers, or antioxidants. Furthermore, solidification procedures such as lyophilization or spray drying are capable of helping improve long-term stability	[65]
Biological Compatibility	The potential toxicity of surfactants and lipids is a significant concern	Researchers have generally considered safe excipients and water as solvents in preparing LNCs to avoid biological incompatibility	[66]
Low entrapment efficiency	Lower encapsulation	Using Lipoid® in the LNC formula results in high encapsulation due to the shell stability-imparting effect of lecithin	[67]
In vivo Fate	Bio distribution of LNCs	To understand the In vivo fate of LNCs, researchers used fluorescent substance (DiO, DiI-Perchlorate, DiD- Perchlorate) coupling with LNCs formulation to examine the penetration at the molecular level	[68, 69]
Scalability	Oil molecule migration is hypothesized to cause a change in the structure of the PEG shell, thus increase in size and decrease in zeta potential	It is crucial to periodically inspect the long-term stability of the pilot-level batches because the scale-up process may influence the stability and strength of the nanocapsules; however, in that investigation, the active ingredient stabilized the oily core of nanocapsules	[34]
Regulatory approval	Safety, Efficacy, and Quality	**Safety:** Regulatory bodies demand rigorous toxicological evaluations to assure the safety of components **Quality**: Manufacturers are required by regulatory agencies to employ quality control procedures to minimize variability **Efficacy:** The regulatory requires detailed drug release studies and bioequivalence with the reference product	[70–72]

Conclusion and Future Prospect

The pharmaceutical, theranostic, cosmetic, and food industries have all undergone enormous transformations due to the successful development of LNCs delivery systems. LNCs boosted the delivery of medicines and biologics due to the improved bioavailability of numerous drugs. In addition, LNCs also improved integrity, loading efficiency, preserved activity, and stealth effect. Among the nanosystems, LNCs have considerably enhanced oral, dermal, and ocular drug delivery and gene therapy. Their unique features, such as surface functionalization, amphiphilic, and large molecule encapsulation efficiency, enable the delivery of brain, lung, and cancer therapy. Preclinical investigation has confirmed that LNCs have shown higher stability over 18 months and can be scalable with insignificant size and loading efficiency variations. Patents on LNCs support the utilization of LNCs in various drug delivery and gene delivery domains. However, regulatory concerns about using LNCs in drug delivery primarily matter safety, quality, and efficacy. Preclinical study on cell lines and animal data has revealed that LNCs are safe and effective nanocarrier systems for delivery or encapsulation of a wide range of drug and biomolecules, and they suggested that they can be used for theragnosis purposes also by encasing radio-labelled substances and dye over LNCs for In vivo investigation. In the near future, LNCs will continue to be explored as one of the most promising and widely used alternative nanocarrier systems for improved drug and gene delivery.

Table 5 Patent related to LNCs

Patent No	Patent	Status	Inventors	Filling year	Patent Granted	References
US9333180B2	Nanocapsules with a liquid lipid core charged with water soluble or water dispersible active agent	Expired—Fee-Related	Anton, N., Saulnier, P., Benoit, JP	2008	2016	[73]
US9005666B2	Process for preparing lipid nanoparticles	Expired—Fee-Related	Benoit, J.P., Anton, N., Saulnier, P	2008	2015	[74]
US8057823B2	LNCs, preparation method, and use as medicine	Active	Béatrice Heurtault, Angers, Patrick Saulnier, Les-Ponts-de-Ce, Jean-Pierre Benoit, Avrille, Jacques-Emile Proust, Saint-Leger-des-Bois, Brigitte Pech, Angers,Joel Richard, Longue,	2001	2011	[75]
US20050214378A1	Stealth LNCs, methods for the preparation thereof, and use thereof as a carrier for active principle(s)	Abandoned	Didier Hoarau Pascal Delmas Jean-Christophe Leroux	2003	2005	[76]
US20060073196A1	Use of P-glycoprotein inhibitor surfactants at the surface of a colloidal carrier	Abandoned	Jean-Pierre Benoit, Alf Lamprecht	2003	2006	[77]
US20220347111A1	LNCs charged with incretin mimetics	Pending	Ana Beloqui, Yining XU, Véronique Préat Patrice Cani	2020	2022	[78]
US20090238865A1	Lipidic nanocapsules, preparation, and use as a drug	Abandoned	Beatrice Heurtault, Patrick Saulnier, Jean-Pierre Benoit, Jacques-Emile Proust, Brigitte Pech SJoel Richard	2009	2009	[79]
US20130017239A1	Lipid nanoparticle capsules	Abandoned	Josep Lluis Viladot, Petit Raquel DELGADO GONZALEZ, Alfonso FERNÁNDEZ BOTELLO	2011	2013	[80]
US9522121B2	Aqueous-core LNCs for encapsulating hydrophilic and/or lipophilic molecules	Expired—Fee-Related	Anton, N., Saulnier, P., Benoit, J.P	2008	2016	[73]

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