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REVIEW

Lipid Nanoparticles—From Liposomes to mRNA Vaccine Delivery, a Landscape of Research Diversity and Advancement

Rumiana Tenchov, Robert Bird, Allison E. Curtze, and Qiongqiong Zhou*

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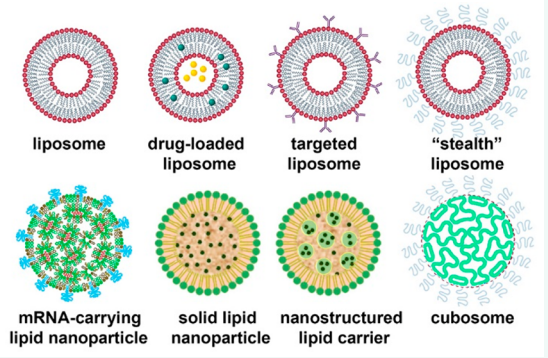
ABSTRACT: Lipid nanoparticles (LNPs) have emerged across the pharmaceutical industry as promising vehicles to deliver a variety of therapeutics. Currently in the spotlight as vital components of the COVID-19 mRNA vaccines, LNPs play a key role in effectively protecting and transporting mRNA to cells. Liposomes, an early version of LNPs, are a versatile nanomedicine delivery platform. A number of liposomal drugs have been approved and applied to medical practice. Subsequent generations of lipid nanocarriers, such as solid lipid nanoparticles, nanostructured lipid carriers, and cationic lipid−nucleic acid complexes, exhibit more complex architectures and enhanced physical stabilities. With their ability to encapsulate and deliver therapeutics to specific locations within the body and to release their contents at a desired time, LNPs provide a valuable platform for treatment of a variety of diseases. Here, we present a landscape of LNP-related scientific publications, including patents and journal articles, based on analysis of the CAS Content Collection, the largest human-curated collection of published scientific knowledge. Rising trends are identified, such as nanostructured lipid carriers and solid lipid nanoparticles becoming the preferred platforms for numerous formulations. Recent advancements in LNP formulations as drug delivery platforms, such as antitumor and nucleic acid therapeutics and vaccine delivery systems, are discussed. Challenges and growth opportunities are also evaluated in other areas, such as medical imaging, cosmetics, nutrition, and agrochemicals. This report is intended to serve as a useful resource for those interested in LNP nanotechnologies, their applications, and the global research effort for their development.



KEYWORDS: *lipid nanoparticle, liposome, cationic lipid, solid lipid nanoparticle, nanostructured lipid carrier, immunoliposome, "stealth" liposome, drug delivery*

Lipid nanoparticles (LNPs) have emerged across the pharmaceutical industry as promising vehicles to deliver a variety of therapeutic agents. The application of LNPs has also been extended to other fields, such as medical imaging, cosmetics, nutrition, agriculture, and other innovative areas such as nanoreactors. Currently in the spotlight as a vital component of the COVID-19 mRNA vaccines, LNPs play a key role in effectively protecting and transporting mRNA to cells.

Liposomes, an early version of LNPs, are an extremely versatile nanocarrier platform because they can transport hydrophobic or hydrophilic molecules, including small molecules, proteins, and nucleic acids. In fact, liposomes are the earliest nanomedicine delivery platform to successfully proceed from concept to clinical application. A number of liposomal drug formulations have been approved and successfully applied to medical practice.

The next generations of LNPs, including solid lipid nanoparticles, nanostructured lipid carriers, and cationic lipid−nucleic acid complexes, exhibit more complex internal architectures and enhanced physical stabilities. With their ability to control the location and timing of drug delivery in the body, LNPs can be used to deliver treatments for a variety of diseases. Increasingly, scientists are moving beyond traditional biopharmaceuticals to more complex and specialized therapies that can fight disease at the genetic level.

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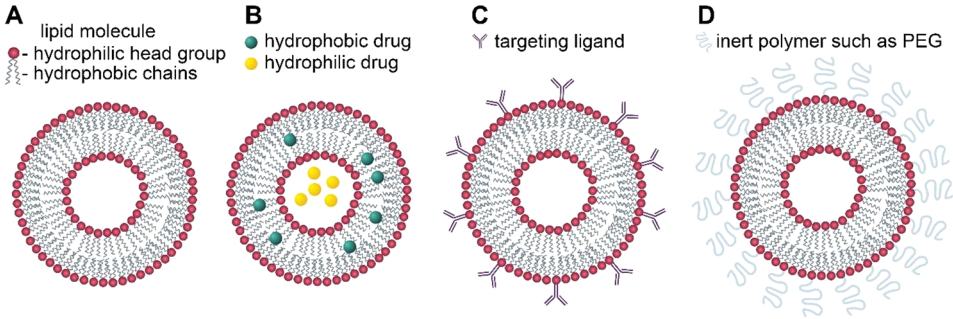

Figure 1. Schematic representation of (A) liposome, (B) liposome encapsulating hydrophobic and hydrophilic drugs, (C) immunoliposome functionalized with targeting ligands, and (D) sterically stabilized ("stealth") liposome functionalized with inert polymers such as PEG.

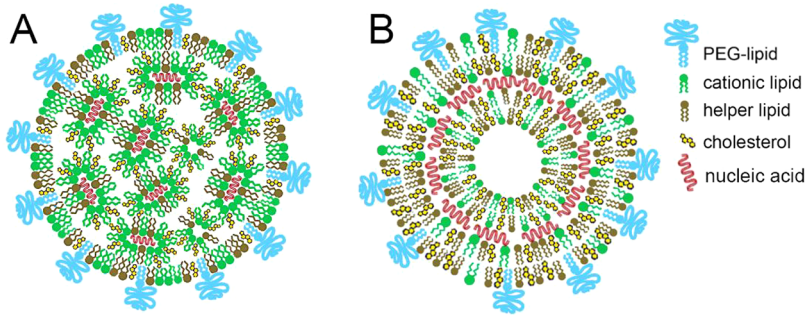

Figure 2. Suggested structures of lipid nanoparticle nucleic acid carriers: nucleic acids organized in inverse lipid micelles inside the nanoparticle (A); nucleic acids intercalated between the lipid bilayers (B).[26−29]

In this review, we provide an overview of the current knowledge regarding LNP structures and properties, primarily from the viewpoint of their pharmaceutical applications. We then discuss the multiple applications of LNPs, including drug delivery, medical imaging, cosmetics, and others. Furthermore, we present a landscape of LNP-related research based on a thorough analysis of the CAS Content Collection.[1,2] The CAS Content Collection is the largest human-curated collection of published scientific knowledge, proven useful for quantitative analysis of global scientific publications against variables such as time, research area, formulation, application, and chemical composition. The growth and diversity of LNP-related publications and their distribution among research areas and applications, as well as countries and organizations, are examined. Lists of the most widely used chemical substances involved in LNP formulations are provided, including phospholipids, PEG-lipids, and cationic lipids. We hope this report can serve as a useful resource for those interested in LNP nanotechnologies and the global research effort for their development.

LIPID NANOPARTICLE BASICS

Liposomes—The Earliest Generation of Lipid Nanoparticles. The term "liposome" was coined in the 1960s, shortly after it was found that closed lipid bilayer vesicles (Figure 1A) form spontaneously in water.[3−5] The term "lipid nanoparticle" came into use much later, in the early 1990s, with the beginning of the era of nanoscience and nanotechnology. Since liposomes are made of lipids and in most cases are nanosized, they are rightfully considered as the earliest generation of lipid nanoparticles.

The potential of liposomes as drug delivery systems was recognized almost immediately after their discovery. For example, it is known that over 40% of small-molecule drugs

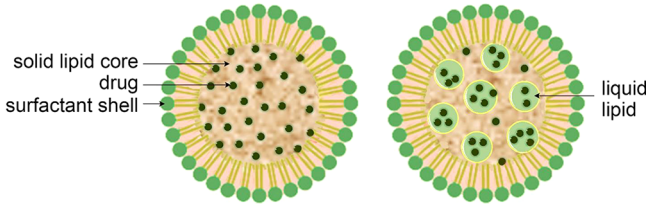

Figure 3. Schematic presentation of a solid lipid nanoparticle (left) and a nanostructured lipid carrier (right).

for cancer treatment exhibit low solubility in water, so the benefits of drug delivery systems capable of encapsulating these drugs and enhancing their aqueous solubilities was immediately appreciated. Liposomes were the earliest nanomedicine delivery platform to successfully proceed from concept to clinical application, with a number of approved pharmaceutical preparations. For example, the earliest approved liposomal drug was Doxil, a lipid nanoparticle formulation of the antitumor agent doxorubicin, which is used to treat ovarian cancer.[6] Another liposomal drug, Epaxal, is a lipid nanoparticle formulation of a protein antigen used as a hepatitis vaccine.[7] Many other liposomal formulations have been approved for use as drugs and vaccines, as shown in Table S1 in the Supporting Information. Liposomes have been used in numerous clinical trials to deliver anticancer, anti-inflammatory, antibiotic, antifungal, anesthetic, and other drugs and gene therapies.

Phospholipids such as phosphatidylcholines, phosphatidylethanolamines, phosphatidylserines, and phosphatidylglycerols, along with stabilizers such as cholesterol, are common liposome substituents. Liposomes consist of one or several lipid bilayers, ranging in size between 20 and ∼1000 nm. Hydrophilic drugs can be enclosed in the aqueous interior of liposomes, while hydrophobic drugs can be entrapped in the hydrocarbon chain

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Table 1. Common Ingredients Used for the Preparation of SLN and NLC[36,40−43]

Lipids	Emulsifiers/coemulsifiers
Triglycerides	Lecithin
Trimyristin (Dynasan 114)	Poloxamer 188
Tripalmitin (Dynasan 116)	Poloxamer 407
Tristearin (Dynasan 118)	Tyloxapol
Mono-, di-, and triglyceride mixtures	Polysorbate 20
Witeposol bases	Polysorbate 60
Glyceryl stearates (Imwitor 900)	Polysorbate 80
Glyceryl behenates (Compritol 888 ATO)	Sodium cholate
Glyceryl palmitostearates (Precirol ATO 5)	Sodium glycocholate
Waxes	Taurodeoxycholic acid sodium
Beeswax	Butanol and Butyric acid
Cetyl palmitate	Cetylpyridinium chloride
Hard fats	Sodium dodecyl sulfate
Stearic acid	Sodium oleate
Palmitic acid	Polyvinyl alcohol
Behenic acid	Cremophor EL
Other lipids	
Miglyol 812	
Paraffin	

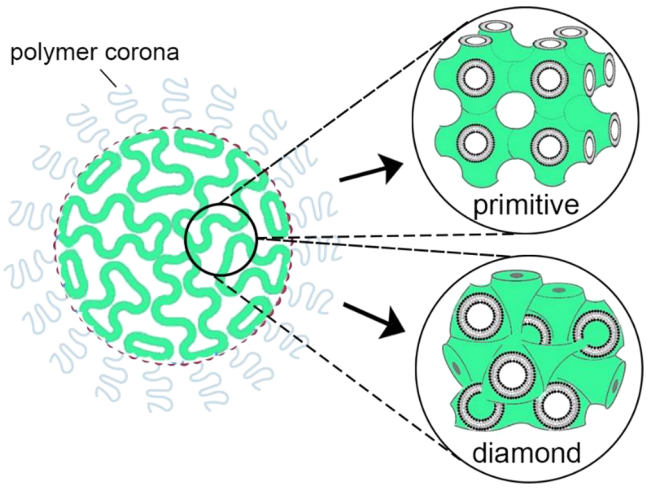

Figure 4. Cubosomes are nanoparticles comprising lipid in a bicontinuous bilayer cubic phase (either primitive or diamond type).

region of the lipid bilayer (Figure 1B), making liposomes a versatile drug delivery platform. The structures of liposomes depend strongly on how they are prepared. Liposomes may be either unilamellar (small unilamellar vesicles (SUV) with diameters of 20−100 nm, large unilamellar vesicles (LUV) with diameters of 100−1000 nm, or giant unilamellar vesicles (GUV) with diameters >1000 nm) or multilamellar vesicles (MLV), with diameters of >500 nm, in which concentric bilayers form an onion-like multilayer structure (Figure S1) in the Supporting Information.[8] Drug delivery systems primarily use SUV and smaller MLV, while GUV are used mainly as models for cells.

Size is a critical parameter in determining liposome drug encapsulation and half-life in circulation, with smaller liposomes having more chances of escaping phagocyte uptake.[9] It has been generally accepted that particles used for pharmaceutical purposes, and especially those for parenteral administration, need to be ≤100 nm.[10−12] The size of nanoparticles can be measured using a variety of techniques such as dynamic light scattering, size exclusion chromatography, nuclear magnetic resonance spectroscopy, and microscopy. The particle size distribution of LNPs can be controlled using manufacturing methods such as extrusion, sonication, and homogenization; more recently, microfluidic methods have been successfully used for LNP manufacture and size control.

The surface charges of LNPs are generally determined by the lipid head groups, which may be either positively or negatively charged or zwitterionic. The surface potential, which depends on the surface charge density, controls the interactions between particles and the adsorption of counterions and hence the stability of the nanoparticles. Uncharged particles or particles with low charge densities tend to aggregate over time, while more highly charged particles repel each other, preventing aggregation. The surface charge of nanoparticles is most often expressed by their zeta potentials, the electrical potential of a particle measured from a plane just outside the layer of fluid bound to the particle; it is commonly calculated from its electrophoretic mobility. Zeta potentials vary linearly with the fraction of ionic lipids incorporated into the liposomes; zeta potentials < −30 mV or >30 mV are generally sufficient to maintain interparticle repulsion and stable particle suspensions.[13−15]

Cationic Lipid Nanoparticles, Complexes with Nucleic Acids. Progress in understanding of the genetics of cellular pathogenesis has made possible therapeutic targeting of numerous genes involved in human diseases.[16] Nucleic acids have a variety of roles in medicine, including gene therapy agents and RNA therapeutics.[17] However, the development of nucleic acid therapeutics is hindered by difficulties in their cellular delivery. The negative charges and hydrophilicity of nucleic acids impedes their passive diffusion across plasma membranes. In addition, the association of nucleic acids with serum proteins, their uptake by phagocytes, and their degradation by endogenous nucleases interferes with their efficient delivery. As a result, nucleic acids require delivery vectors to protect them from degradation and to deliver them to the target cells for efficient uptake. Viral and nonviral vectors are used to deliver nucleic acids to cells. Cationic LNPs, comprising stable complexes between synthetic cationic lipids and anionic nucleic acids, are the most widely used nonviral delivery system for nucleic acid drugs.[18,19]

A large number of cationic lipid amphiphiles have been synthesized and tested for use as nucleic acid carriers. The molecular architecture of the cationic lipids is similar to that of natural lipids, except for the presence of an ionizable (cationic) head group instead of the zwitterionic or anionic head group of the natural lipids. They comprise a hydrophobic part with two alkyl chains or a cholesterol moiety, a positively charged polar head group, and a linker connecting the polar group with the hydrophobic moiety. Ionizable lipids which are positively charged only inside the cell and uncharged in the bloodstream due to a change in pH value are preferred because they are less toxic than nonionizable cationic lipids.[20] The structures of the most frequently used cationic lipids in LNP formulations according to the CAS Content Collection are presented further in this review (Table 12).

Complexation with positively charged lipids (Figure 2) stabilizes nucleic acids and increases their resistance to nuclease degradation, allowing them to be delivered to their desired target cells. Nucleic acids enter cells by adsorption of the LNPs to the

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Table 2. Example Ligands and Receptors Tested as LNP-Targeting Agents in Cancer Therapies[88−92]

Targeting ligand	Target receptor	Targeted cancer
Folate[93,94]	Folate receptor	Cancers overexpressing folate receptor
Transferrin[95,96]	Transferrin receptor	Cancers overexpressing transferrin receptor
Granulocyte-macrophage colony-stimulating factor (GM-CSF)[97]	GM-CSF receptor	Leukemic blasts
RGD (Arg-Gly-Asp tripeptide)[98]	Cellular adhesion molecules, such as integrins	Vasculature endothelial cells in solid tumors
NGR (Asn-Gly-Arg tripeptide)[99]	Aminopeptidase N (CD13)	Vasculature endothelial cells in solid tumors
Anti-VEGFR antibody[100]	Vasculature endothelial growth-factor receptor VEGFR (FLK1)	Vasculature endothelial cells in solid tumors
Anti-ERBB2 antibody (Trastuzumab)[101]	ERBB2 (erythroblastic oncogene B2) receptor	Cancers overexpressing ERBB2 receptor, such as in breast and ovarian cancers
Anti-CD20 antibody (Rituximab, Ibritumomab tiuxetan)[102]	CD20, B-cell surface antigen	Non-Hodgkin's lymphoma, B-cell lymphoproliferative diseases
Anti-CD22 antibody (Epratuzumab)[103,104]	CD22, B-cell surface antigen	Non-Hodgkin's lymphoma, B-cell lymphoproliferative diseases
Anti-CD33 antibody (Gemtuzumab)[105,106]	CD33, a sialo-adhesion molecule, leukocyte differentiation antigen	Acute myeloid leukemia
Anti-CD25 antibody (Denileukin diftitox)[107,108]	Interleukin-2 receptor	Cutaneous T-cell lymphoma
Antitenascin antibody[109]	Extracellular-matrix protein overexpressed in many tumors	Glial tumors, breast cancer
Anti-MUC1 antibody[110,111]	MUC1, an aberrantly glycosylated epithelial mucin	Breast and bladder cancer
Anti-TAG72 antibody[59,61]	TAG72, oncofetal antigen tumor-associated glycoprotein-72	Colorectal, ovarian and breast cancer
Anti-CEA antibody[110,112]	Carcinoembyonic antigen (CEA)	Colorectal, small-cell lung and ovarian cancers

Table 3. Examples of Stimuli-Responsive Liposomes for Enhanced Anticancer Drug Delivery

Stimuli	Anticancer Drug	Liposome Composition	Tumor
Temperature	Doxorubicin	DPPC:MSPC:DSPE-PEG2000 (86.5:9.7:3.8, mol %)[126]	Ovarian cancer
		DPPC:MSPC:DSPEmPEG2000 (21.6:2.6:1.0, molar ratio)[127]	Breast tumor
pH	Doxorubicin	DOPE, DSPE-PEG-$H_7K(R_2)_2$ (lipid−peptide conjugate with the pH-sensitive peptide $H_7K(R_2)_2$[128]	Glioma, Glioblastoma
Magnetic field	5-Fluorouracil	Phosphatidylcholine[129]	Colon carcinoma
Laser irradiation	AMD3100	Soybean phosphatidylcholine[130]	Osteosarcoma, Breast cancer

cell surface followed by their endocytosis and release of the nucleic acids into the cell. Adsorption of LNPs to and fusion with the cell membrane are electrostatically promoted because cell membranes commonly bear negative charges and the nanoparticle lipids for nucleic acid delivery bear positive charges; their attraction thus drives membrane fusion and endocytosis. Once the nucleic acids have entered the cell, release from their complexes with cationic lipids is necessary for nucleic acid delivery. The cell's anionic lipids likely help to release nucleic acids from LNPs by neutralizing the charge of their cationic lipid carriers, disrupting the electrostatic interactions between the lipid carriers and the nucleic acids. Binding of anionic lipids to the cationic lipids also disrupts the nanoparticle architecture, leading to formation of nonlamellar structures.[21,22] The efficacy of cationic lipid vectors in delivering nucleic acids has been proposed to correlate to their ability to promote the formation of nonlamellar lipid phases.[19,23] Short-lived nonlamellar structures are believed to mediate the processes of membrane fusion; the intermediates that form in membrane fusion are similar to those that form during lamellar−nonlamellar phase transformations.[24,25]

Solid Lipid Nanoparticles and Nanostructured Lipid Carriers. While liposomes are useful as drug carriers, they require complex production methods using organic solvents, exhibit low efficiency at entrapping drugs, and are difficult to perform on large scales. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) were developed to address some of these shortcomings (Figure 3). While conventional liposomes comprise liquid-crystalline lipid bilayers, SLN comprise solid lipids,[30,31] and NLC comprise mixtures of solid and liquid-crystalline lipids.[32,33] The particle sizes of SLN and NLC vary between 40 and ∼1000 nm. SLN and NLC exhibit enhanced physical stabilities, addressing one of the main limitations of liposome-based formulations. SLN and NLC also have higher loading capacities and higher bioavailabilities of their cargoes, are produced easily on large scale without the use of organic solvents, and are more stable to sterilization than other LNPs. In addition, the reduced mobility of molecules in the solid state allows SLN and NLC to control the release of their drug payloads more precisely. However, on long-term storage, crystallization of SLN can expel the incorporated drugs into the surrounding media.[34] NLC were then designed by introducing small amounts of lipids liquid at room temperature into SLN, reducing the degree of crystallinity of the lipid core. The reduced crystallinity of NLC suppresses expulsion of the drug from the matrix and enhances the drug-loading capacities and physical and chemical long-term stabilities of the nanoparticles.[35,36]

SLN and NLC are composed of lipids and stabilizing agents such as surfactants and other coating materials (Figure 3). Typical lipid constituents are shown in Table 1, including fatty acids, fatty alcohols, glycerides, and waxes. Surfactants, located at the lipid−water interface, reduce the interfacial tension between the lipid and the aqueous phases and improve the stabilities of the resultant formulations. A list of commonly used surfactants/emulsifiers in LNP preparation is also included in Table 1. SLN and NLC are usually produced using various organic solvent-free methods, such as high-pressure homoge-

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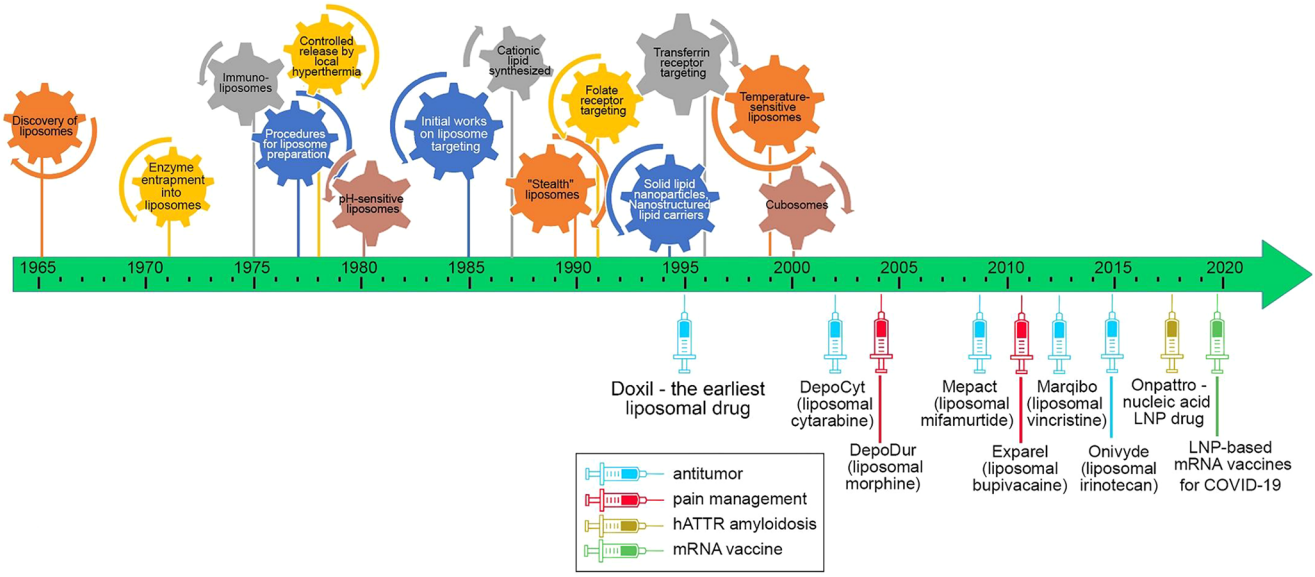

Figure 5. Timeline of liposome/LNP advancement. (Upper part) Technological advancement. Publications on LNPs, correlated to the timeline of LNP advancement: The discovery of liposomes;[3] Enzyme entrapment into liposomes;[136] Immunoliposomes;[80,137,138] Procedures for liposome formation;[139,140] Thermoresponsive liposomes to local hyperthermia;[124,141,142] pH-sensitive liposomes;[121] Liposome targeting;[77,143,144] Cationic lipids for gene delivery;[18,145,146] Long-circulating ("Stealth") liposomes;[115,116,147] Folate receptor targeting;[81,84,144,148,149] Solid lipid nanoparticles and nanostructures lipid carriers;[31,35,150,151] HER2 receptor targeting;[152−154] Transferrin receptor targeting;[155−158] Temperature-sensitive liposomes;[122,142,159,160] Stimuli-responsive liposomes;[123,149,161] Cubosomes.[46,47,50,162] (Lower part) Examples of FDA-approved LNP drugs. The earliest approved liposomal drug Doxil;[6] The earliest FDA-approved LNP-based nucleic acid (siRNA) drug Onpattro;[163] LNP-based mRNA vaccines for COVID-19 approved;[164,165] Useful general reviews.[166−173] For a full list of approved LNP-based drugs, see Table S1 in the Supporting Information.

nization, high-speed stirring, ultrasonication, emulsion/solvent evaporation, double emulsion, phase inversion, and solvent injection.[31,37−39]

Nonlamellar Lipid Nanoparticles. Other types of LNP structures have also been investigated for use in drug delivery. Technologies relating to the use of nonlamellar lipid phases in drug delivery and the use of inverted cubic and hexagonal liquid-crystalline phases in controlled release formulations for delivery of inhaled drugs were published in the 1980s.[44,45]

More recently, cubosomes, highly stable nanoparticles formed from lipid cubic phases (Figure 4) and stabilized by polymer-based outer coronas, were developed as lipid pharmaceutical nanocarriers.[46−50] Liquid-crystalline lipid cubic phases consist of single lipid bilayers that form a bicontinuous periodic lattice structure with pores formed by two interwoven water channels. Cubosomes are highly stable under physiological conditions. The composition of a cubosome can be tuned to customize its pore sizes and to include bioactive lipids; the polymeric outer corona can be used to control where the cubosome payload is released. Cubosomes provide a significantly higher membrane surface area for loading of membrane proteins and small-molecule drugs than do liposomes. This combination of properties allows cubosomes to be used in a variety of applications, such as drug delivery systems, membrane bioreactors, artificial cells, and biosensors.

Cubosomes are composed of amphiphilic lipids and a stabilizer. The amphiphilic lipid is the major component; upon hydration, the lipid spontaneously forms a cubic liquid-crystalline phase. The stabilizer is typically a polymer that prevents the reconstitution of the cubosome into a bulk cubic phase. The most frequent compositions of cubosomes use monoolein (glyceryl monooleate) as the lipid component with poloxamer 407 as a stabilizing surfactant; the monoglyceride/surfactant mixture makes up between 2.5% and 10% of the total weight of the dispersion. Polyvinyl alcohol is also used in addition to poloxamer 407 as a stabilizer for the dispersion.[51]

Hexosomes are another type of LNP, in which lipids form a nonlamellar phase—the inverted hexagonal phase H_{II}. Their compositions are similar to those of cubosomes, containing amphiphilic lipids, a polymeric stabilizer, and water.[52,53] Micelles are nonlamellar lipid nanosized particles with a hydrophobic core and hydrophilic shell; they have been used successfully to solubilize poorly water-soluble pharmaceuticals.[54,55] Reverse micelles, with a hydrophilic core and hydrophobic shell, have been used to encapsulate hydrophilic molecules such as nucleic acids in complex lipid carriers.[26,27,56,57]

Ethosomes. Ethosomes are phospholipid nanoparticles containing a high proportion (20−45%) of ethanol. The added ethanol increases the permeabilities and elasticities of the ethosomes, allowing them to perform transdermal delivery of drugs and cosmetics by squeezing through the pores of stratum corneum, the outermost layer of skin. This delivery route offers an alternative method to deliver liposomal formulations, avoiding the complications caused by the gastrointestinal tract in oral drug delivery.[58] Commercial products using ethosomal formulations include anticellulite (Cellutight EF, Noicellex, Skin Genuity, Osmotics Lipoduction) and antiaging (Decorin) agents, hair growth stimulants including Minoxidil (Nanominox) and Acyclovir (Supravir), and topical creams for the treatment of herpes virus infections.[59]

Echogenic Liposomes. Echogenic liposomes are acoustically active liposomes utilized as ultrasound contrast agents.[60] They have been developed following the discovery that microscopic bubbles of gas reflect diagnostic ultrasound waves. Gas−liquid interfaces provide a large discontinuity in

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density and reflect sound very efficiently. Encapsulated into liposomes, gas microbubbles provide improvements in medical acoustic imaging.[61] Echogenic liposomes also offer additional therapeutic applications, such as ultrasound-controlled drug delivery[60,62,63] and ultrasound-enhanced thrombolysis (sonothrombolysis).[64,65]

Procedures for LNP Formation. A wide variety of techniques are used to control the properties of LNPs, including their sizes, numbers of concentric bilayers (lamellarity), and their ability to encapsulate various compounds.[66−69]

The film hydration method represents the simplest and oldest method used for liposome preparation. Lipids are initially dissolved in an organic solvent and then dried down to yield a thin film at the bottom of a vial. The lipid film is hydrated to produce a liposomal dispersion. The hydration conditions affect the structure of the formed vesicles—giant unilamellar vesicles (GUV) are formed by gentle hydration, while multilamellar vesicles (MLV) with poor size homogeneity are formed upon intense agitation. Probe or bath sonication can be used to produce small unilamellar vesicles (SUV). Consecutive extrusion through polycarbonate filters of defined pore sizes can also be used to control liposome diameter; the number of extrusion cycles is important in determining the homogeneity of the liposomes formed.[70]

Another traditional liposome preparation technique is reverse phase evaporation, involving formation of a water-in-oil emulsion between an aqueous phase and an organic phase containing lipids. The mixture is briefly sonicated to homogenize it; removal of the organic phase under reduced pressure yields a gel and then a liposomal suspension.[71] The solvent injection technique for liposome formation involves the rapid injection of a lipid solution (in ethanol or diethyl ether) into an aqueous medium.[72] The detergent removal liposome preparation technique involves dissolution of phospholipids in an aqueous solution containing detergents at their critical micelle concentrations (CMC) followed by removal of the detergents by dialysis or other means. Dilution of the resultant suspension with water or aqueous solutions reconstitutes the formed micelles; over time, the micelles convert to liposomes.[70] In the heating method for liposome preparation, lipids are hydrated and then heated above the transition temperature of the phospholipids in the presence of a hydrating agent such as glycerin or propylene glycol. This method is attractive because it does not involve an organic solvent.[8,73]

A successful recent liposome production technique is microfluidic hydrodynamic focusing, in which a stream of lipid in alcohol solution is forced to flow in the central channel of a device, intersected, and sheathed by coaxial stream(s) of an aqueous phase. Reciprocal diffusion of alcohol and water across the focused alcohol/water interface causes the lipid to precipitate and self-assemble into liposomes.[69,72,74] Other recently developed techniques for producing liposomes include cross-flow injection[69] and methods using supercritical fluids.[68,72]

Similarly, preparation of other types of LNP, such as SLN, NLC, and cubosomes, includes various methods for homogenization (high-shear homogenization, hot or cold homogenization, high-speed homogenization), ultrasonication, and microfluidization.[75,76] Ultrasonication, extrusion, and microfluidic methods have been most often used to control LNP size, according to the CAS Content Collection.

Functional Modifications of LNPs. Despite their advantages, unmodified LNP drug delivery systems have significant limitations such as lack of targeting selectivity, short blood circulation time, and instability *in vivo*. Improved LNP formulations were designed to overcome each of these shortcomings.

Targeted Liposomes. Targeted liposomes were designed with surface-attached ligands (Figure 1C) to recognize and bind to specific receptors on cells.[77] Generally, targeted liposomes are prepared by conjugating small-molecule ligands, peptides or monoclonal antibodies to the surface of LNPs.[78,79] Antibodies were initially used to construct actively targeted liposomes (immunoliposomes). For example, the efficiency of liposomes modified with an IgM ligand was 100 times higher than that of unmodified liposomes.[80] Certain receptors, such as the folate receptor and the transferrin receptor, are overexpressed on many cancer cells, and their corresponding ligands have been used to direct liposomes to these types of cells or tissues.[81−84] Folate receptors bind strongly to their ligand, folic acid, allowing for specificity for tumor cells over noncancerous cells. The lack of immunogenicity of folic acid and the ability of its conjugates to be taken into cells nondestructively by endocytosis make folates preferable to protein-based targeting ligands. Folate receptors are also overexpressed on macrophages, which are present in inflammatory diseases such as psoriasis, Crohn's disease, atherosclerosis, and rheumatoid arthritis; thus, folate-mediated targeting can also be used to deliver antiinflammatory drugs.[85] Transferrin receptors are overexpressed in rapidly proliferating cancer cells to meet the increased iron demands of tumor cells, making possible the development of transferrin receptor-targeted anticancer therapies.[86] The epidermal growth factor receptor (EGFR), a tyrosine kinase receptor, is overexpressed in many solid tumors, including colorectal, nonsmall-cell lung cancer, squamous cell carcinoma, and breast cancer, making it an attractive target for therapeutic drug delivery.[87] Examples of ligands used in LNP targeting are shown in Table 2.

"Stealth" Liposomes. While immunoliposomes were highly selective for specific cell types, they were rapidly removed from the blood flow by phagocytes. To remedy this, liposomes were coated with biocompatible inert polymers, typically poly(ethylene glycol) (PEG), making them invisible to phagocytes ("stealth" liposomes) (Figure 1D). PEGylation (covalently attaching PEG to a compound) was initially invented to help protein drugs to avoid the body's immune response[113,114] but was later found to be also very effective at improving the surface properties of the liposomes by preventing access to their surface through steric hindrance.[115−117] The circulatory half-life of liposomes depends on the length and density of the polymer chains on the liposome surface, allowing stable, sterically stabilized liposomes to be prepared.[118] The increased circulation half-lives of sterically stabilized liposomes also increase their passive accumulation in cancer tissues by the enhanced permeation and retention (EPR) effect, further increasing their effectiveness.[119]

Stimuli-Responsive Liposomes. Another useful liposome modification includes formulations designed to release encapsulated drugs controllably when exposed to physicochemical or biochemical stimuli (stimuli-responsive liposomes). These drug delivery systems respond to specific triggers to release their cargo where needed, increasing drug efficacy and reducing adverse effects. Liposomes responsive to temperature, changes in pH, enzymes, light, magnetic and electrical fields, and ultrasound have been studied.[120] Among these stimuli, pH change is the most promising due to the existence of multiple pH gradients in the body.[121] When triggered by a stimulus, LNPs

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Figure 6. Approved LNP drugs and the diseases they target (more details in Table S1).

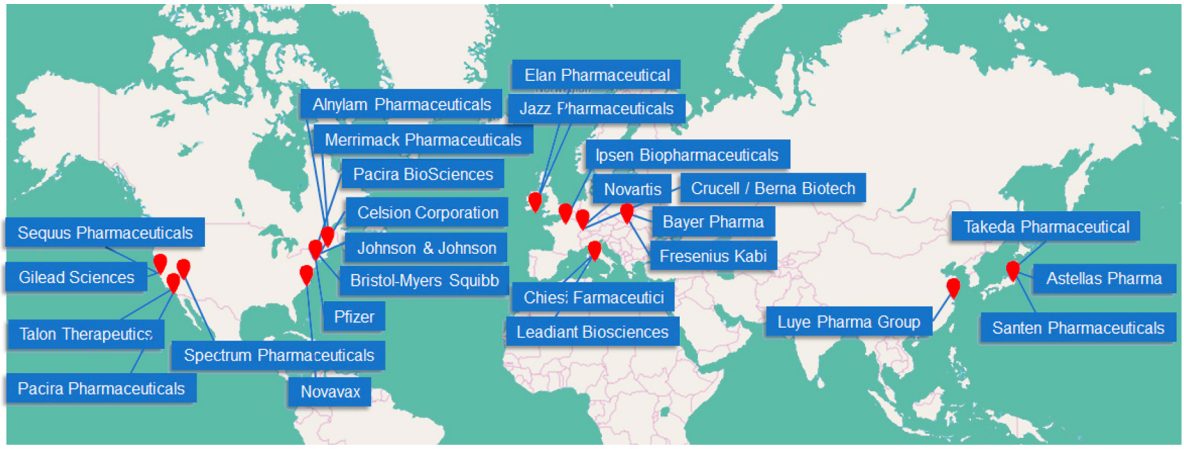

Figure 7. Key players operating in the global LNP drug delivery market according to a recent market analysis[180] and the summary of the LNP-based marketed drugs (Table S1).

undergo a phase transition (either between the gel and liquid-crystal phases or between lamellar and nonlamellar phases), increasing their membrane permeability.[122] Temperature-responsive systems have been studied extensively for anticancer drug delivery.[120,123] When exposed to mild local hyperthermia, the lipids approach their liquid-crystalline phase transition temperatures, creating disorder between their solid and fluid domains and becoming more permeable to water-soluble molecules. This results in burst release of the entrapped drug within the tumor.[124,125] Table 3 provides examples of stimuli-responsive liposomes.

Toxicity of Lipids Used in LNP Formulations. Since LNPs are mainly composed of natural lipids, they have been considered pharmacologically inactive and minimally toxic. However, in some cases, LNPs are not immunologically inert[131] while LNP constituents are unnatural compounds which may be toxic to human cells.[132] For example, while cationic lipids offer great promise as carriers for the delivery of fragile compounds such as nucleic acids, some cationic lipids cause cytotoxicity.[133] In some cases, cationic lipids reduce mitosis in cells, form vacuoles in the cytoplasms of cells, and cause detrimental effects on key cellular proteins such as protein kinase C.[134] The cytotoxicity of cationic lipids depends on the structures of their

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Table 4. Lipid Constituents of the LNP Carriers of the COVID-19 mRNA Vaccines[164,165,195−197]

Lipid Name	Role	Abbreviation or Lab Code	CAS Registry Number
BNT162b2 vaccine (Pfizer/BioNTech)			
(4-hydroxybutyl)azanediyl bis(hexane-6,1-diyl)bis(2-hexyldecanoate)	ionizable cationic lipid	ALC-0315	2036272-55-4
(2-hexyldecanoate), 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide	PEG-lipid	ALC-0159	1849616-42-7
1,2-distearoyl-*sn*-glycero-3-phosphocholine	helper lipid	DSPC	816-94-4
cholesterol	helper lipid	Chol	57-88-5
mRNA-1273 vaccine (Moderna)			
heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate	ionizable cationic lipid	SM-102	2089251-47-6
1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000	PEG-lipid	PEG2000-DMG	160743-62-4
1,2-distearoyl-*sn*-glycero-3-phosphocholine	helper lipid	DSPC	816-94-4
cholesterol	helper lipid	Chol	57-88-5

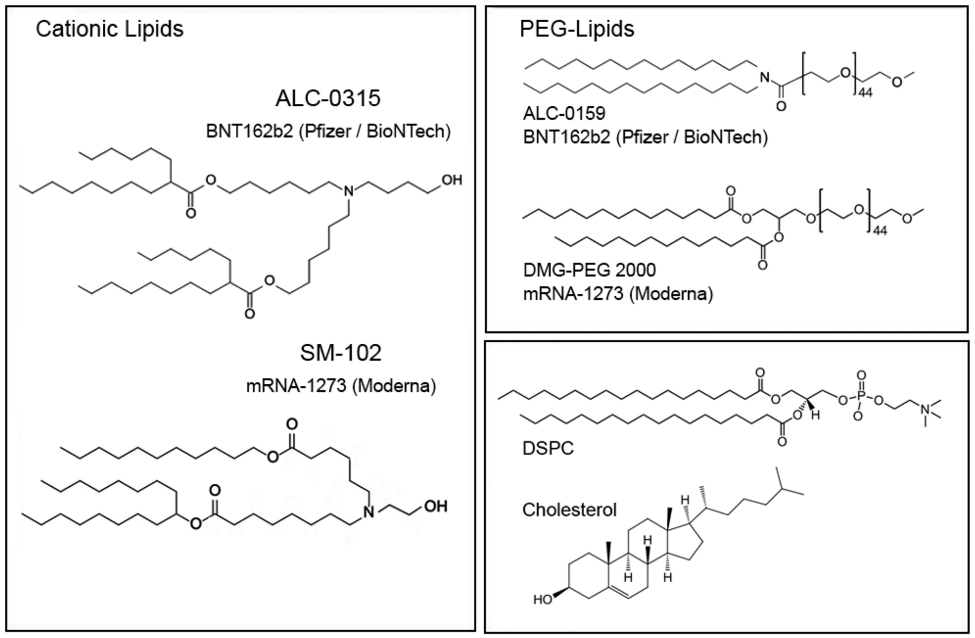

Figure 8. Structures of the lipid constituents of the LNPs of the COVID-19 mRNA vaccines[164,165,195−197]

hydrophilic head groups; amphiphiles with quaternary ammonium head groups are more toxic than those with tertiary amine head groups.[134] The effect of the hydrophobic chains on the toxicity of lipids is not well studied, hindering the design of less toxic lipids. The hydrophobic portions of lipid molecules strongly modulate their phase behavior and their usefulness for LNP, but the presence of certain lipid phases also correlates to membrane damage and cytotoxicity.[25] PEG−lipid conjugates may also cause undesired toxicity, while LNPs containing PEG−lipid conjugates are known to interact with immune cells to generate undesired antibodies against some PEGylated lipids.[135]

Timeline of Liposome/LNP Advancement. A timeline of liposome/LNP advancement is shown in Figure 5.

APPLICATIONS OF LIPID NANOPARTICLES

Drug and Vaccine Delivery. *Clinically Approved LNP-Based Pharmaceuticals.* Liposomes have been recognized as a powerful tool in medicine for over 50 years. Their ability to encapsulate and deliver therapeutics controllably to specific locations within the body makes them useful for treating a variety of diseases. A number of LNP drug formulations have been approved and used in medical practice (Figure 6).[88,174−179] More information on these formulations is included in Table S1 in the Supporting Information. A selection of key players operating in the global liposome drug delivery market according to a recent market analysis,[180] as well as from the summary of the LNP-based marketed drugs (Table S1), is shown in Figure 7.

The largest single application of LNPs in drug delivery is in cancer treatment (Figure 6), because of the improved bioavailability and selectivity of LNP-encapsulated antitumor agents over the free drugs. Lipid-based nanocarriers reduce the toxicity of anticancer drugs to normal tissues, increase the water solubilities of hydrophobic drugs, extend the drug residence time, and improve control over drug release.[92,181,182]

LNPs also improve the efficacy of cancer therapies through the enhanced permeability and retention (EPR) effect.[183] Rapid but defective angiogenesis in tumors leads to blood vessels that possess large fenestrations (>100 nm in size) through which LNP can readily pass. The tumor blood vessels are thus much more permeable to LNPs, allowing their selective accumulation in tumors when administered intravenously. In addition, dysfunctional lymphatic drainage in tumors reduces the rate at which LNPs leave tumors and thus improves their retention. The accumulation of LNPs in tumors as a result of the EPR effect allows the nanoparticles to release the antitumor agents selectively in the vicinity of tumor cells.

Doxil was the earliest approved anticancer nanoformulation and the earliest approved liposomal drug. The formulation was designed to improve the pharmacokinetics and biodistribution of the anthracycline drug doxorubicin, which is a potent

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Table 5. Clinical Trials of LNP-Formulated mRNA Drugs and Vaccines[208,210]

Disease	mRNA/encoding sequence	NCT Number/Phase
	Infectious disease vaccines	
Rabies	mRNA/Rabies virus glycoprotein (RABV-G)	NCT03713086/Phase I
Zika Virus	mRNA-1893/Structural proteins of Zika virus	NCT04064905/Phase I
	mRNA-1325/Zika virus antigen	NCT03014089/Phase I
Cytomegalovirus (CMV)	mRNA-1647 and mRNA-1443/Pentamer complex and full-length membrane-bound glycoprotein B and pp65 T cell antigen of CMV	NCT03382405/Phase I
hMPV and PIV3	mRNA-1653: Fusion proteins of hMPV and PIV3	NCT03392389/Phase I
Tuberculosis	GSK 692,342/Immunogenic fusion protein (M72) derived from *Mycobacterium tuberculosis*	NCT01669096/Phase II
Influenza	VAL-506440/H10N8 antigen	NCT03076385/Phase I
	VAL-339851/H7N9 antigen	NCT03345043/Phase I
COVID-19	ChulaCov19 mRNA/SARS-Cov2-spike protein-binding IgG antibody	NCT04566276/Phase I/II
	self-amplifying mRNA (SAM) platform/anti-Spike IgG antibodies GMCs	NCT04758962/Phase I
	Chimpanzee Adenovirus serotype 68 (ChAd) and self-amplifying mRNA (SAM) vectors/Spike (ChAdV68-S)	NCT04776317/Phase I
	Cancer immunotherapy	
Melanoma	mRNA-4157/personalized cancer vaccine targeting 20 tumor-associated antigens	NCT03897881/Phase II
	RBL001.1; RBL002.2; RBL003.1; RBL004.1/malignant melanoma-associated antigens	NCT02410733/Phase I
Ovarian Cancer	W_ova1 vaccine: Three ovarian cancer tumor associated antigens mRNAs	NCT04163094/Phase I
Triple-negative breast cancer	IVAC_WAREHOUSE_bre1_uID; IVAC MUTANOME_uID/personalized cancer vaccine targeting tumor-associated antigens	NCT02316457/Phase I
Solid tumors	mRNA-4157/personalized cancer vaccine targeting 20 tumor-associated antigens	NCT03313778/Phase I
Melanoma, Colon cancer, Gastrointestinal cancer, Genitourinary cancer, hepatocellular cancer	NCI-4650/mRNA-based, Personalized Cancer Vaccine	NCT03480152/Phase I/II
Melanoma, NSCLC, Bladder Cancer, Colorectal Cancer, Triple Negative Breast Cancer, Renal Cancer, Head	RO7198457/personalized cancer vaccine targeting tumor-associated antigens	NCT03289962/Phase I
Relapsed/Refractory Solid Tumor Malignancies or Lymphoma, Ovarian Cancer	mRNA-2416/OX40L	NCT03323398/Phase I and II
Solid Tumor Malignancies, Lymphoma, Triple Negative Breast Cancer, Head and Neck Squamous Cell Carcinoma, Non-Hodgkin Lymphoma, Urothelial Cancer	mRNA-2752/Human OX40L, IL-23, and IL-36γ	NCT03739931/Phase I
Adult Glioblastoma	Autologous total tumor mRNA and pp65 full length lysosomal associated membrane protein (LAMP) mRNA loaded DOTAP liposome vaccine	NCT04573140/Phase I
	Protein-replacement therapies	
Propionic Acidemia	mRNA-3927/α and β subunits of the mitochondrial enzyme propionyl-CoA carboxylase	NCT04159103/Phase I and II
Isolated Methylmalonic Acidemia	mRNA-3704/methylmalonyl-coenzyme A mutase (MUT)	NCT03810690/Phase I and II
Ornithine Transcarbamylase Deficiency	MRT5201/Ornithine transcarbamylase	NCT03767270/Phase I and II
Cystic Fibrosis	MRT5005/Human Cystic Fibrosis Transmembrane Regulator protein (CFTR)	NCT03375047/Phase I and II
Carnitine Palmitoyl Transferase 2 Deficiency	CPT2 mRNA/Carnitine Palmitoyl Transferase 2	NCT00336167/Phase I
Hereditary Transthyretin Amyloidosis with Polyneuropathy	Cas9 mRNA/NTLA-2001 (CRISPR/Cas9 technology)	NCT04601051/Phase I

anticancer agent but is cardiotoxic.[184] Doxil takes advantage of EPR, using sterically stabilized nanoparticles (~100 nm) to extend the circulation time in human plasma while reducing doxorubicin's cardiotoxicity. It was developed as an intravenous injection for the management of advanced ovarian cancer, multiple myeloma, and HIV-associated Kaposi's sarcoma.[6] The LNPs used for Doxil are composed of hydrogenated soy phosphatidylcholine, cholesterol, and DSPE-PEG2000.[185]

The second largest group of liposomal dugs comprises fungicides (Figure 6). Amphotericin B, a broad-spectrum polyene antibiotic, has been in medical use for decades and is considered the gold standard for treating invasive fungal infections. It targets cell membranes, exhibiting higher affinity for ergosterol-containing membranes typical of fungal cells than for cholesterol-containing mammalian cell membranes.[186] While it has high antifungal activity, amphotericin B also has severe side effects, particularly nephrotoxicity. It is amphipathic and characterized by complicated self-association behavior, with different types of aggregates displaying different solubilities and toxicities; the aggregation state also correlates to drug efficacy.[187] Thus, controlling the aggregation state of the drug may enhance its therapeutic effect and lower its toxicity. Such aggregation control has been achieved via lipid nanoformulations.[188,189] Several lipid-based nanoparticle preparations of

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Table 6. Notable Patents from the CAS Content Collection Related to the Use of LNP in Theranostic Formulations

Patent #	Title	Key Feature
WO2016024281	Theranostic compositions and methods for therapeutics prescreening	A theranostic composition and method for determining the cell-specific potency of drugs is provided. Lipid nanoparticles are fabricated using a microfluidic apparatus.
WO2013012891	Intraperitoneally administered nanocarriers that release their therapeutic load based on the inflammatory environment of cancers	Nanocarrier compositions that release their therapeutic load specifically at the site of intraperitoneal cancers. These nanocarriers comprise a plurality of porous nanoparticles loaded with pharmaceutically active agents in combination with imaging agents, thus providing a theranostic value, and are encapsulated by a lipid bilayer.
WO2019083365	Delivery vectors	Liposome compositions that selectively deliver a cargo such as an active pharmaceutical ingredient or an imaging agent to the blood brain barrier (BBB) of a subject. The liposomes may be used for therapeutic, diagnostic, or theranostic purposes.
WO2018218052	Nanoparticle-lipid composite carriers as theranostic agents	Nanoparticle−lipid composite carriers comprising a lipid core and an outer shell of functionalized nanoparticles for use as theranostic agents, particularly for diagnosis and/or treatment of cancers and related diseases.
WO2018185290	Use of a liposome encapsulating a sugar compound in theranostic CEST imaging	Agents comprising a liposome encapsulating a suitable sugar compound, for use in Chem. Exchange Saturation Transfer (CEST) imaging for diagnostic and theranostic purposes. The liposomes can be shielded and\or targeted to a sugar uptake site such as a tumor. The invention has particular utility in modulating the glycemic response in the subject.
WO2018146700	A biodegradable nano-theranostic composite and process of preparation thereof	A biodegradable nanotheranostic composite in which graphene oxide is coated as a film on the inner side and outer side of the liposome. The composite can perform targeted combined chemo- and photothermal therapy. The nanotheranostic nanocomposite is designed to collapse and biodegrade after use.
US20180178043	Focused ultrasound hyperthermia	Focused ultrasound hyperthermia method is applied repeatedly using image guidance. Hyperthermia is applied after a drug or biopharmaceutical and/or their labeled equivalents (theranostics) have been administered to cause the enhanced tissue distribution and/or controlled release of the drug encapsulated in thermosensitive lipid nanoparticles. The drug and/or the drug delivery system are labeled for imaging to allow real time monitoring and modulation.
WO2017173089	Systems and methods for enhancing delivery of diagnostic and/or therapeutic compositions *in vivo* using electric pulses	Systems and methods for manipulation of tumors with pulsed elec. fields. These systems and methods can be used for theranostic applications in oncological diagnosis and treatment, especially when combined with liposome-delivered drugs.
WO2016198859	Precision therapeutics	Pharmaceutical composition comprising a combination of imaging lipid nanoparticles and therapeutic agent(s). Imaging LNPs may have receptor-targeting ligands. Image-guided hyperthermia applied to target sites enables the imaging LNPs and therapeutic agent(s) to partition from the blood into target tissues for therapy by means of hyperpermeability and retention (HPR). Therapeutic outcomes can be followed by clinically relevant imaging modalities such as MRI.
WO2012040710	Stabilized nanobubbles comprising lipid membranes for diagnostic and therapeutic applications	Stabilized echogenic nanobubbles for diagnostic, therapeutic, and theranostic applications. The stabilized nanobubble includes a membrane-defining internal void, containing gas. The membrane comprises a lipid and nonionic triblock copolymer effective to control the size of the nanobubble. The gas has a low solubility in water and includes a perfluorocarbon.

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Table 7. Notable Patents from the CAS Content Collection Related to the Use of LNPs in Food and Nutrition Formulations

Patent #	Title	Key Feature
EP3417846	Food and/or nutraceutical composition, in the form of liposomes, comprising endocannabinoid	A food and/or nutraceutical composition, for example in the form of liposomes, which comprises an endocannabinoid, an active ingredient and an excipient.
WO2014140268	Solid lipid nanoparticles	Food-grade SLN comprise a solid lipid phase core comprising lipophilic and/or amphiphilic active ingredient and an emulsifier comprising mono- and diglyceride citric acid ester.
WO2017095138	Curcumin-containing lipid nanoparticle complex comprising ginsenosides	Curcumin-containing lipid nanoparticles containing ginsenosides exhibit improved stabilities, dispersibilities, and bioavailabilities. They can be used in curcumin-containing products such as antioxidant food compositions.
WO2004064805	Lipid-based cochleate preparations of fragile nutrients for the food, cosmetic and pharmaceutical industries	Cochleate-containing nanoparticles including one or more cochleates of fragile nutrients such as β-carotene are disclosed.
US20100196543	Microencapsulated citrus phytochemicals and application to beverages	A beverage includes citrus juice, microencapsulated citrus phytochem, $\leq$90 mg unencapsulated hesperidin, $\leq$150 mg unencapsulated naringin, and $\leq$0.9 mg unencapsulated limonin. Phytochemicals (such as limonoids and flavonoids) are microencapsulated to conceal their bitter taste.
EP1894477	Food protein and charged emulsifier interaction	A coated denatured supramolecular protein core with food applications comprises an electrostatically bound lipid monolayer. Thus, heat-denatured whey protein aggregates may be coated with sulfated Bu oleate to form liposome-like structures.
WO2013008261	Food product of the type to be stored and consumed refrigerated or frozen	A food product of the type to be stored and consumed refrigerated or frozen comprises an active ingredient encapsulated in liposomes, thus increasing its stability and its antioxidant capacity. Liposomes containing vitamin A are formulated with α-tocopherol, rosmarinic acid, and lecithin.
WO2011119953	Orally bioavailable lipid-based constructs for delivery of biotin derivatives	Lipid-based compositions facilitate efficient oral absorption of biotin compounds for inducing weight loss. An orally bioavailable composition comprises gelatin, liposomes, and lipid particles and a biotin-derived targeting agent.
US20170127712	Dietary supplement compositions with enhanced delivery matrix, gummies, chocolates, atomizers and powders containing same, and methods of making same	A dietary supplement composition includes liposomal vesicles, an active ingredient, a phospholipid contained in the liposomal vesicles, and a coating material. The liposomal vesicles have a barrier coating made of a biopolymer, polyethylene glycol, and/or chitosan. The dietary supplement composition may be incorporated in gummies, chocolates, atomizers, or powders.
WO9922601	Enhanced infant formula containing liposome encapsulated nutrients and agents	Liposomes are used in an infant formula to improve the delivery and stability of nutrients, and they enhance their bioavailability. The formula more closely resembles the ultrastructure and infrastructure of natural human milk due to the presence of liposomes. The phospholipid concentration is the same as that in human milk.

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Table 8. Notable Patents from the CAS Content Collection Related to Use of LNPs in Agrochemical Formulations

Patent #	Title	Key Feature
US20190104734	Cure and prevent diseases in plants, bushes and trees using rhamnolipid liposomes	A fertilizer using rhamnolipid-containing liposomes eliminates disease in plants, bushes, and trees by breaking down the cell walls of disease-causing bacteria.
US20150150245	Liposome formulations	Liposomal formulations comprise pesticides, nematicides, or herbicides for control of pests and weeds. The formulations can be applied to pre- or postemergent crops and to soil, plant media, plants, plant tissues, and seeds.
WO9817110	Formulations for enhancing the efficacy of pesticides, especially herbicides	Formulations comprise the active ingredient and a liposome-forming excipient such as a diacylphosphatidylcholine or diacylphosphatidylethanolamine having a cationic hydrophilic moiety and a hydrophobic moiety comprising two hydrocarbon acyl chains. The formulations also comprise an amphiphilic quaternary ammonium ingredient.
WO9821945	Lecithin-microencapsulated boron pesticides	Liposomal microencapsulated boron-containing products are disclosed to be used in agricultural formulations. Boron-containing materials formulated according to the invention may be applied to agricultural field crops and fruits.
WO2002052939	Controlled-release formulations of anionic herbicides	An herbicidal formulation comprising an herbicide incorporated in a micelle using a quaternary amine cation or a lipid vesicle, adsorbed on a clay mineral. Suitable in particular for negatively charged herbicides at pH above 6. The formulation provides slow release and reduced leaching of the herbicide to deep soil layers, thus reducing contamination of underground water and soil. Furthermore, because the herbicide stays near the target, efficiency is enhanced and smaller dosage may be used.
US20100233224	Photolytic release of biocides for high efficiency decontamination through phospholipid nanoparticles	Biocide-filled liposome vesicles contain photosensitizers. Irradiation of the liposome vesicles with light causes the vesicle membranes to break, releasing the biocidal agents. Preferred biocidal agents include hydrogen peroxide, benzalkonium chloride, and photooxidizing nanoparticles such as titanium dioxide, iron oxide, and biocides such as Ucarcide 25 and Ucarcide 50 (Dow Chem. Co).

amphotericin B have been developed (Figure 6; Table S1), which exhibit favorable pharmacokinetic profiles and significantly reduce the side effects of this drug.[188]

Nucleic acid therapeutics are an emerging class of drugs showing potential for treating various diseases. However, since nucleic acids are polyvalent anionic and highly hydrophilic molecules, they are hardly taken up into cells. They are also easily degraded by nucleases in the blood. Therefore, they require a delivery vector in order to enter cells and to be effective. LNP carriers are one of the successful methods for delivering nucleic acid drugs.[190,191] The nucleic acid drug Patisiran (ONPATTRO), an siRNA formulated in LNPs to reduce transthyretin protein formation in the liver, recently received FDA approval for the treatment of hereditary transthyretin-mediated amyloidosis. It is the earliest approved siRNA drug and the earliest LNP-formulated nucleic acid drug, marking an important milestone in nucleic acid therapeutics development.[163,192]

LNPs in the COVID-19 mRNA Vaccines. The latest successful use of LNPs is as the delivery vehicle in the two recently approved COVID-19 messenger RNA (mRNA) vaccines by Pfizer/BioNTech and Moderna, which have been developed with unparalleled speed and have shown notable effectiveness in disease prevention.[29,164,165,193,194] The vaccines deliver mRNA encoding for the SARS-CoV-2 spike protein into the cytoplasm of host cells; the mRNA is translated into the spike protein, which acts as an antigen and leads to development of an immune response to the virus. The mechanism of action of the mRNA mediated vaccines is depicted in Figure S2 in the Supporting Information.

The compositions of the lipid nanoparticles of the two mRNA vaccines are very similar. Both contain an ionizable lipid which is positively charged at low pH (enabling RNA complexation) and neutral at physiological pH (reducing the potential toxic effects and facilitating payload release). They also contain a PEGylated lipid to reduce antibody association (opsonization) by serum proteins and clearance by phagocytes thus conferring longer systemic circulation. The phospholipid distearoylphosphatidylcholine (DSPC) and cholesterol help to pack the cargo into the LNPs (Table 4).[164,165,195−198] The molar ratios of the cationic lipid:PEG-lipid:cholesterol:DSPC are (46.3:1.6:42.7:9.4) for the Pfizer and (50:1.5:38.5:10) for the Moderna vaccine.[199] Those nanoparticles are 80−100 nm in diameter[200] and contain approximately 100 mRNA molecules per lipid nanoparticle.[201]

Proprietary cationic lipids—ALC-0315 (Pfizer) and SM-102 (Moderna) (Figure 8)—are used in the COVID-19 vaccine nanoparticles; both lipids are tertiary amines which are protonated (and thus positively charged) at low pH. Their hydrocarbon chains are connected through biodegradable ester groups, enabling safe clearance after mRNA delivery. The cationic lipids used in the mRNA vaccines contain branched hydrocarbon chains (Figure 8), which optimize the formation of nonlamellar phases and the mRNA delivery efficiency. The PEG-lipids are both PEG-2000 conjugates. The LNPs are prepared at low pH (pH 4.0), at which the ionizable lipid is positively charged, so that it can easily form complexes with mRNA.[202] A microfluidic device is used to mix a stream containing mRNA in water with a stream containing a lipid mixture in ethanol. When rapidly mixed, the constituents of these two streams form nanoparticles which entrap the negatively charged mRNA.[203−205]

LNP-Based mRNA Vaccines and Therapeutics in Clinical Trials. mRNA vaccines and therapeutics hold great promise in

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prevention and treatment of diseases. LNP-enabled intracellular delivery of mRNA allows the expression of virtually any desired protein inside the host cells.[206] An important feature of mRNA-based therapeutics is the low risk of insertional mutagenesis.[207] Unlike DNA therapies, mRNA does not need the machinery of the nucleus to perform its task. Because mRNA does not integrate into the host genome, the risk of carcinogenesis and mutagenesis from mRNA-based therapeutics is reduced, improving their safety. Lastly, the manufacture of mRNA is more readily standardized than the production of DNA and affords much better reproducibility.[208]

mRNA vaccines have revolutionized vaccine development because of their high efficacies, accelerated development cycles, and potential for low-cost manufacture.[209] The rapid development of mRNA vaccines would not have been possible without advances in LNP technologies to deliver nucleic acids. LNP-based mRNA vaccines have entered clinical trials against a variety of infectious diseases, such as nucleoside-modified mRNA vaccines for Zika virus, cytomegalovirus, tuberculosis, and influenza (Table 5).[210] mRNA therapeutic vaccines have great potential in cancer immunotherapy against melanoma, ovarian cancer, breast cancer, and other solid tumors (Table 5).[208,209] LNP vectors are crucial for the successful intracellular delivery of mRNA to the cytosol of immune cells, particularly antigen-presenting immune cells, which are responsible for triggering the desired immune responses.

The use of mRNA for the expression of therapeutic proteins bears promise in treating a wide range of diseases. Protein replacement therapy is a medical therapy that replaces or supplements a protein which is deficient or missing in a patient.[211] It is achieved by engineering mRNA to code for the protein of interest.[212] LNPs are the preferred vehicle to deliver mRNA to cells, but LNP-based mRNA drugs typically require repetitive dosing through prolonged time-periods and thus need careful safety analyses and tests. The earliest study using LNP-formulated mRNA for protein replacement therapy was published only in 2016, using LNP-entrapped mRNA encoding human frataxin as a potential therapeutic agent against Friedreich's ataxia.[213]

Medical Imaging. Medical imaging plays an essential role in modern precision medicine. Medical imaging is used to improve disease diagnosis, monitor drug delivery, verify response to therapy, and guide minimally invasive procedures. Traditional imaging methods such as magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), and single photon emission computed tomography (SPECT) have limited resolution and specificity. Nanoparticle delivery systems such as LNPs and their versatile surface functionalization provide opportunities to enhance the resolution and specificity of those imaging methods.[214]

Due to the EPR effect, liposomes are more likely to accumulate in tumor tissue than normal tissues. Radiolabeled liposomes have been applied for imaging of various cancers. A recent application of the radiolabeled liposomes is in early detection of cancer metastases, by localizing the sentinel lymph node, the initial lymph node receiving metastatic tumor cells.[215] Various PET and SPECT radioisotopes have been conjugated to liposomes for use as imaging agents.[216] The most common radionuclides for radiolabeling liposomes are technetium-99m (^{99m}Tc), indium-111 (^{111}In), and gallium-67 (^{67}Ga).[217−223] These radionuclides have different half-lives and photon energies, so they may be applied to meet the requirements of a particular application. For example, ^{99m}Tc has a half-life of 6 h and allows imaging up to 24 h after injection, while ^{111}In has a longer half-life of 68 h and is useful when delayed imaging of a slow physiological process is needed. There are various methods for liposome radiolabeling. Radiolabels may be encapsulated in the aqueous core of the liposome during the manufacturing process or nonspecifically attached to the liposome surface. A chelator with high affinity for the radionuclide may be covalently attached to the head group of a lipid and the lipid−chelator conjugate added to the liposome formulation during production, this way enhancing its stability.[216]

Liposomes can also provide a suitable biocompatible nanocarrier platform for developing MRI diagnostics.[224] For example, liposomes comprising a gadolinium-chelating lipid, such as 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-diethylenetriamine pentaacetic acid (PE-DTPA (Gd)) have been administered intravenously to visualize thrombi or obstructions in blood vessels.[225] One of the important advantages of entrapment of MRI contrast agents into liposomes is the reduced toxicity of the formulations.[226]

The term theranostics was recently coined as a portmanteau of therapeutics and diagnostics. Theranostics combine pharmaceutical and diagnostic techniques to simultaneously or sequentially diagnose and treat diseases at their earliest stages.[227] LNPs incorporating diagnostic and pharmaceutical agents are called hybrid LNPs. Diagnostic probes such as fluorescent dyes or quantum dots can be encapsulated into liposomes;[228] at the same time, pharmaceutical agents such as doxorubicin, docetaxel, cisplatin, asanginex, or endostatin can be entrapped in LNPs.[229,230] For example, liposome−quantum dot hybrids loaded with the cytotoxic drug doxorubicin have been developed as theranostics. Encapsulation of quantum dots into the lipid bilayers of LNPs makes the quantum dots soluble under physiological conditions while liposomes loaded with doxorubicin are retained by tumors and more selective for cancer cells than the free drug, resulting in LNPs capable of both labeling and killing cancer cells.[229] Notable examples of patents from the CAS Content Collection related to the use of LNPs in theranostic formulations are listed in Table 6.

Cosmetics. The cosmetics industry was among the earliest to recognize and employ nanotechnology advances in various product development. Anticipated advantages of liposomal cosmetic formulations include enhanced stability and efficacy of these formulations, as well as successful penetration of the ingredients into the skin. A variety of marketed liposomal cosmetics are currently in use. The earliest product incorporating liposomes, Capture, was introduced by C. Dior in 1986. It contains thymus extract, collagen and elastin peptides, and hyaluronic acid in soya lecithin liposomes.[231] Another product containing hyaluronic acid in a liposomal delivery preparation is the Advanced Night Repair Protective Recovery Complex introduced by Estée Lauder. The formulation neutralizes and repairs the damage caused by UV-generated free radicals and moisturizes as well. L'Oréal has introduced an antiwrinkle liposomal product, Revitalift Double Lifting, containing pro retinol A.[232] Royal Jelly Lift Concentrate of Jafra Cosmetics International includes liposomes with a complex mixture of amino acids, vitamins, and minerals, to stimulate cell renewal and prevent skin wrinkles.[232] Liposomes are also formulated in commercial products with various extracts, moisturizers, antibiotics, and proteins, for uses such as wound healing, sunburn relief, hair conditioners, antiaging products, and long-lasting perfumes. A summary of marketed LNP cosmetic products is included as Table S2 in the Supporting Information.

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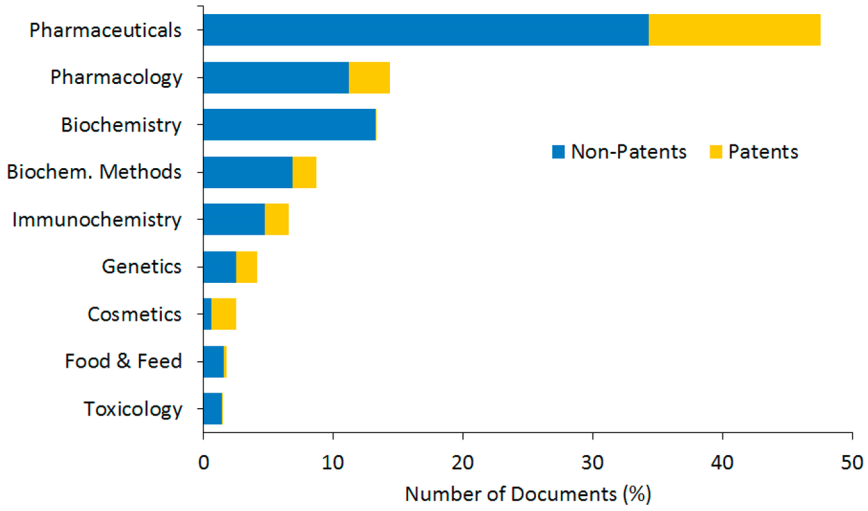

Figure 9. Distribution of LNP-related documents among top research areas in the CAS Content Collection in the years 2000−2021, presented as percentage of all LNP documents.

Nutrition. LNPs are increasingly prominent in the food industry and in nutrition.[233−235] LNPs have been used to control the delivery of functional components such as proteins, enzymes, vitamins, and flavors in various food and nutritional applications. The term "nutraceutical" is used to describe formulations potentially providing both pharmaceutical and nutritional benefits.[236−238] These formulations may involve nutrients, dietary supplements, herbal preparations, and genetically engineered and processed foods. Recently, the use of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in food and dietary supplements has markedly increased due to their advantages in higher loading capacities, higher bioavailabilities of their cargoes, and easier large-scale production. For example, SLN are used to incorporate food-related bioactive compounds including essential oils such as peppermint oil, vitamins such as vitamins A, B_2, B_{12}, D_2, and E, palm oil, coconut oil, copaiba oil, rosmarinic acid, resveratrol, and hesperidine.[239] NLC have been used to encapsulate food-related ingredients such as rutin, curcumin, quercetin, astaxanthin, vitamin C, vitamin A palmitate, α-lipoic acid, and green tea extract.[239] Notable examples of patents from the CAS Content Collection related to LNP formulations for use in nutrient and nutraceutical encapsulation and delivery are presented in Table 7.

Agriculture. LNPs have been studied in agriculture as delivery systems for agrochemicals and as model membrane systems. A list of notable patents from the CAS Content Collection related to LNPs in agrochemical formulations is presented in Table 8.

Nanoreactors. A recent application of LNPs is as nanoreactors, nanoscale chemical reactors applied to nanotechnology and nanobiotechnology. For example, LNPs have been used as nanoreactors for the size-controlled synthesis of metal nanoparticles.[240−242] Metal nanoparticles are used in electronics, biosensors, and catalysis and are also used in biomedical applications such as imaging, drug delivery, and photothermal therapy. The sizes of nanoparticles determine many of their properties; thus, control over metal nanoparticle size is important in controlling their properties and in determining their suitability for use. For instance, nanosized liposomes encapsulating tetrachloroauric acid were used to prepare 2−5 nm gold nanoparticles.[240] The controlled diffusion of the reducing agent—sodium borohydride—through the liposomal membrane regulated the particle formation kinetics and resulted in ultrasmall nanoparticles with a narrow size distribution. In another example, stable palladium nanoparticles with sizes between 1−3 nm were prepared by reduction of a palladium precursor within liposomal nanoreactors using glycerol as both the reducing agent and stabilizer.[242] Palladium nanoparticles ~5 nm in diameter were prepared in the aqueous mesophase channels of lipidic cubic phases by reduction of Pd^{2+} salts and used as supported catalysts for Suzuki−Miyaura cross-coupling reactions.[243] Similar methods using nanoreactors have been used for the synthesis of nonmetallic nanoparticles. For example, monodisperse nanocrystals of CdS, ZnCdS, and HgCdS have been synthesized in the cores of liposomes, using them as nanoreactors for precipitation or crystallization.[244]

Nanoreactors have also been proposed as tools for treatment of disease and eliminating harmful substances by allowing the production of therapeutic agents *in situ*. For example, the antioxidant enzyme catalase has been encapsulated inside liposomes comprising a cisplatin prodrug-conjugated phospholipid, for enhanced chemo-radiotherapy of cancer.[245] The liposomes protect the enzyme from proteolysis and enhance its stability. The enzyme has been able to trigger decomposition of hydrogen peroxide produced by tumor cells thus producing oxygen in order to overcome hypoxia-induced treatment resistance of the tumor. At the same time, the entrapped cisplatin prodrug is oxidized, releasing cisplatin, and subsequent radiation therapy results in successful tumor growth inhibition.[246,247] Polymeric dots (Pdots) loaded in liposomes have been used to reduce inflammation through *in situ* photocatalytic hydrogen generation.[248] Pdots containing π-conjugated polymers generate hydrogen when exposed to light, while liposomes hold the reagents and Pdots together. As hydrogen is formed in the liposomes, it diffuses across the lipid bilayer to reduce reactive oxygen species (ROS) abundant in diseased and damaged tissue.[248] In addition, liposome-based nanoreactors may also be useful for delivering enzymes for eliminating harmful substances. For example, the ability of the exogenous cholinesterase enzymes to act as scavengers of organophosphate toxins has been explored. Butyrylcholinesterase has been encapsulated in liposomes, which protect the enzyme from proteolysis. The organophosphate toxins can diffuse through the

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liposomal membrane and be neutralized by the encapsulated enzyme.[249,250]

Membrane Models in Basic Science. Lipid models have been used for decades to investigate membrane-related processes and characteristics. While biomembranes are heterogeneous multicomponent structures with sophisticated molecular organization, model LNP systems are much simpler and more stable, and therefore amenable to study of the structure and function of biological membranes. Virtually all of our current understanding of membrane lipid phase behavior results from the use of lipid membranes as model systems.[251−260]

INSIGHTS ON LIPID NANOPARTICLES FROM THE CAS CONTENT COLLECTION

In what follows, we used the CAS Content Collection to get an overview of the current LNP research landscape, classifying and quantifying all documents related to LNPs from the years 2000−2020. As the largest human-curated collection of published scientific knowledge, this data collection curated by CAS scientists is particularly useful for quantitative analysis of publications with respect to variables such as time, research area, formulation, and application, as well as the details of chemical compositions.

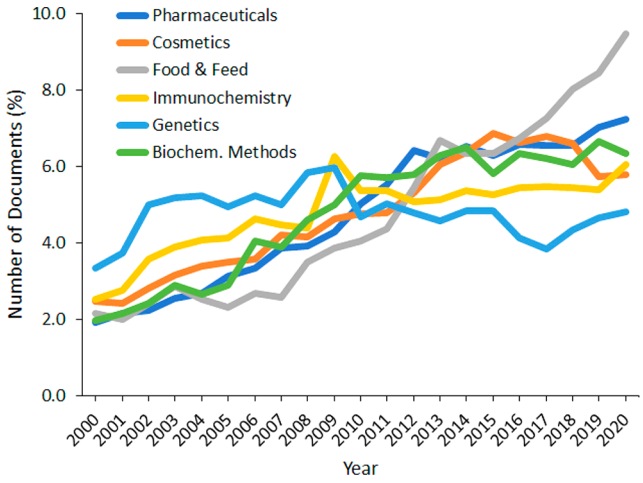

Figure 10. LNP-related patents (%) in the top research areas over time in the years 2000−2020. The percentages are calculated within the given research area.

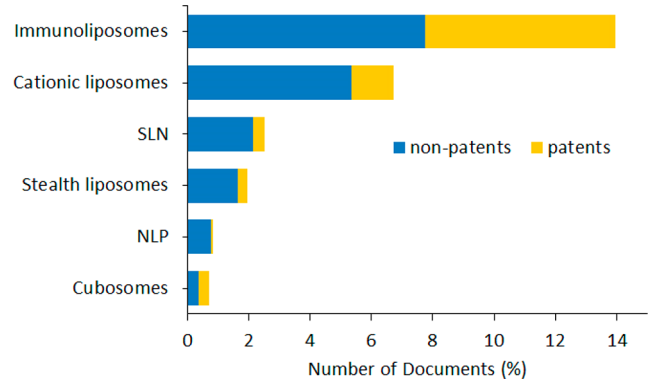

Figure 12. Number of LNP-related documents (patents and nonpatents) in the CAS Content Collection in the years 2000−2020, with respect to different types of LNPs.

Landscape of LNP Research Publications. Currently, there are over 216,000 LNP-related scientific publications in the CAS Content Collection, including patents and nonpatents (journal articles, books, dissertations, meeting abstracts, *etc.*), of which over 170,000 are from the period 2000−2020. The distribution of these documents among the top research areas is presented in Figure 9. LNP-related studies are dominated by pharmaceutical research in both patents and nonpatents. The research areas of cosmetics, genetics, and immunochemistry have the highest percentages of patent publications (Figure 9).

The evolving distribution of documents within these research areas over the past 20 years is shown in Figure 10. The research areas with the fastest growth are pharmaceuticals, food and feed, and cosmetics. The decrease in the number of documents in genetic research in the past decade may be due to the limited success of delivering DNA for gene therapy using lipid vectors (lipofection). A review of gene therapy clinical trials performed worldwide before 2017[261] reported that only 4.4% of the trials used lipid vectors in gene delivery, while most trials used viral vectors. Although LNPs have many advantages in gene delivery (low immunogenicities, facile production on a large scale, and

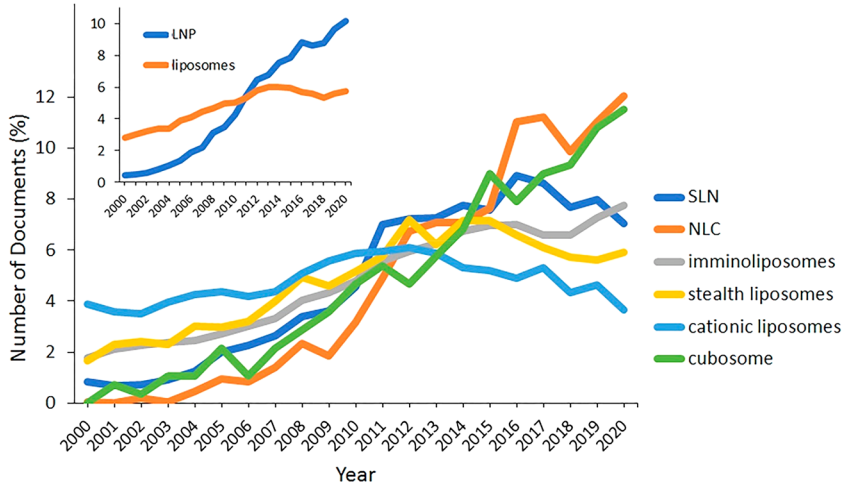

Figure 11. Number of LNP-related documents per year (%) in the CAS Content Collection in the years 2000−2020, with respect to different types of LNPs. The percentages are calculated within the given type. The inset shows the LNP vs liposome documents (%) per year.

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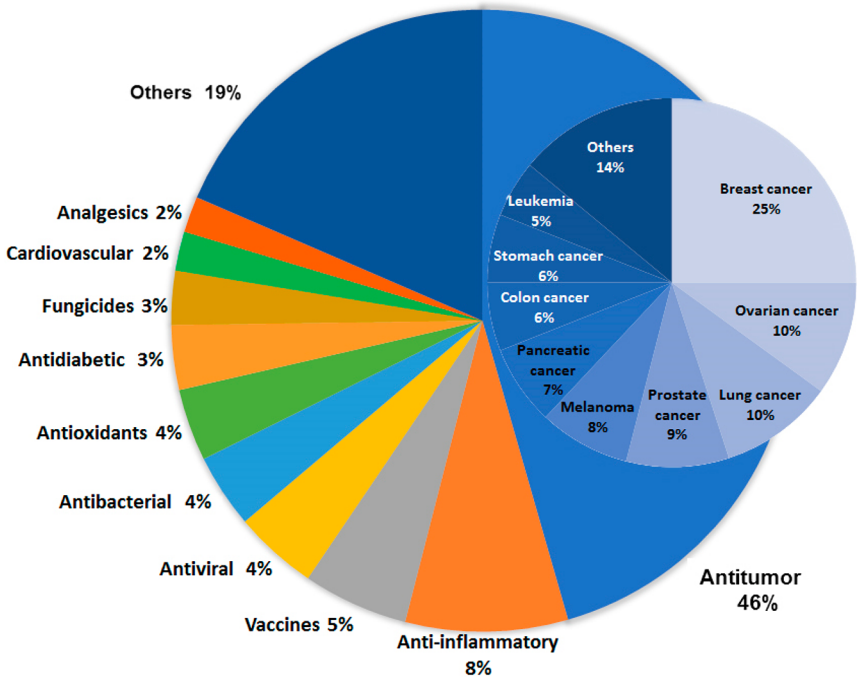

Figure 13. Distribution of documents in the CAS Content Collection related to LNP pharmaceutical formulations with respect to their target diseases.

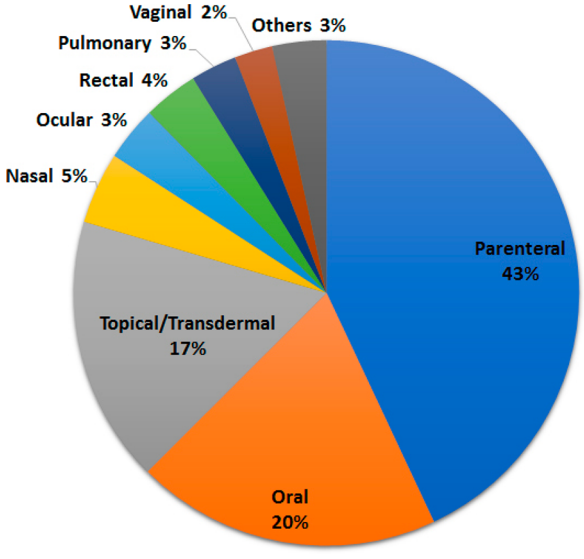

Figure 14. Distribution of documents related to LNP drug delivery with respect to their delivery route.

the ability to deliver large genes), viral vectors have been used more often because current LNPs are not as efficient as viral vectors in delivering genes.[262] The recent rise in interest in RNA therapeutics may hopefully change that perception. While gene therapy requires delivery of DNA into the cell nucleus through the nuclear membrane, entry into the cytoplasm is sufficient for RNA drugs and vaccines, enhancing their chances for success. The effectiveness of the recently developed mRNA vaccines using LNPs as delivery agents may reawaken interest in LNPs for nucleic acid delivery. Indeed, the number of nucleic acid delivery-related LNP patents filed during the first quarter of 2021 is more than half of the number of such patents published in all of 2020.

Table 9. Advantages and Disadvantages of the Three Major LNP Administration Routes[175]

Administration route	Advantages	Disadvantages
Parenteral	Good bioavailability	Painful, causing discomfort
	Appropriate for all LNP types	May require administration at medical facilities
	No liver toxicity	
	Good reproducibility	
Oral	Comfort of use	Low bioavailability
	Acceptable by patients	Hepatic toxicity
		Inconsistent reproducibility
Topical/ transdermal	Comfort of use	Limited penetration
	Acceptable by patients	Lag-time delay
	Satisfactory reproducibility	

Distribution of Research Documents with Regard to the LNP Type. As discussed above, there are various types of LNPs, with different properties and applications; their usage has changed over time and with improvements in the understanding of LNP properties and technologies. The distribution of the types of LNPs in related documents published between 2000 and 2020 is shown in Figure 11.

The terminology used for lipid nanoparticles has changed over time. Many more publication records in the CAS Content Collection contain the term "liposome" (~147,000 for the period 2000–2020) than "lipid nanoparticle" (~26,000 for the same period), even though "lipid nanoparticles" forms a broader class of nanoparticles than "liposomes", including also formulations such as solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), cubosomes, *etc.* While "lipid nanoparticle" is a more general term than "liposome", the term "liposome" was invented earlier, when these lipid vesicles were

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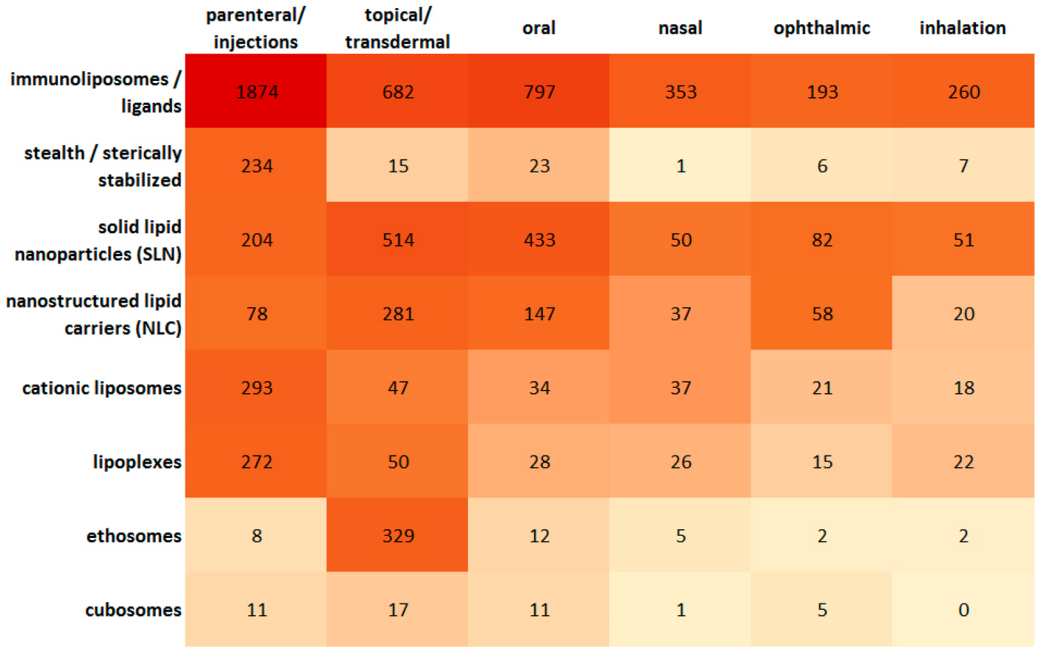

Figure 15. Correlation of the number of documents for the various LNP types with their delivery routes.

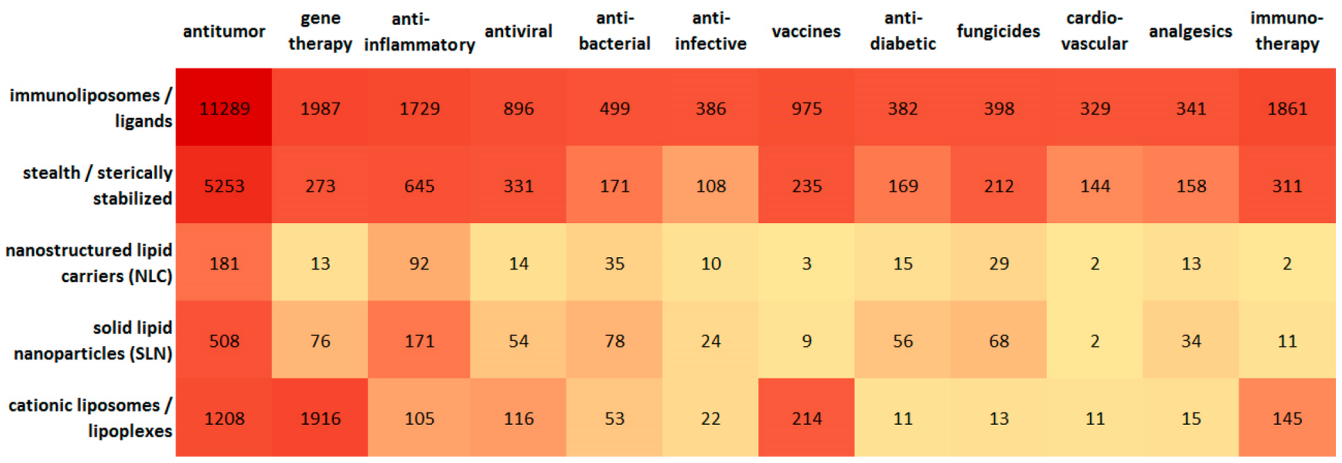

Figure 16. Correlation of the number of documents for the various LNP types and therapies they have been applied to.

discovered in the 1960s. The term "lipid nanoparticle" was coined only in the early 1990s (the earliest document in the CAS Content Collection referring to lipid nanoparticles is from 1992), at the beginning of the era of nanoscience and nanotechnology. The more rapid increase in the number of documents using the term "lipid nanoparticles" than in documents using "liposome" (Figure 11, inset) may arise from its more recent coinage.

In the LNP subcategories, immunoliposomes and cationic liposomes are reported in the largest numbers of documents (Figure 12), while the fastest growth in publication is observed in the most recent areas—solid lipid nanoparticles (SLN), cubosomes, and especially nanostructured lipid carriers (NLC), which are steadily becoming the preferred formulation type for many applications (Figure 11) due to their advantages including higher drug-loading capacity, long-term colloidal stability, enhanced oral bioavailability of hydrophobic drugs, and improved drug release properties.[263]

LNP-Based Drug Delivery Systems. *Distribution of Documents in the CAS Content Collection Related to Pharmaceutical Formulations with Respect to Target Diseases.* As seen above, LNP-related research is dominated by scientific areas related to drug delivery——pharmaceuticals, pharmacology, and also biochemistry, biochemical methods, immunochemistry, and genetics (Figure 9). Documents using LNPs in pharmaceutical formulations were classified by their target diseases to understand how different LNP types are used in practice. The distribution of treatment areas using LNP formulations in drug delivery-related documents in the CAS Content Collection is presented in Figure 13. The use of LNPs in antitumor drug formulations dominates the use of LNPs. Antitumor LNP formulations are used to treat a wide range of cancers; the largest single use (>25%) was in treating breast cancer, with more than 10% of antitumor formulations used for ovarian and lung cancers and significant proportions used for melanoma, leukemia, and prostate, pancreatic, colon, and stomach cancers (Figure 13, inset).

Distribution of Documents Related to Drug Delivery with Respect to Their Delivery Route. Most of the LNP pharmaceutical formulations are for parenteral, oral, or dermal

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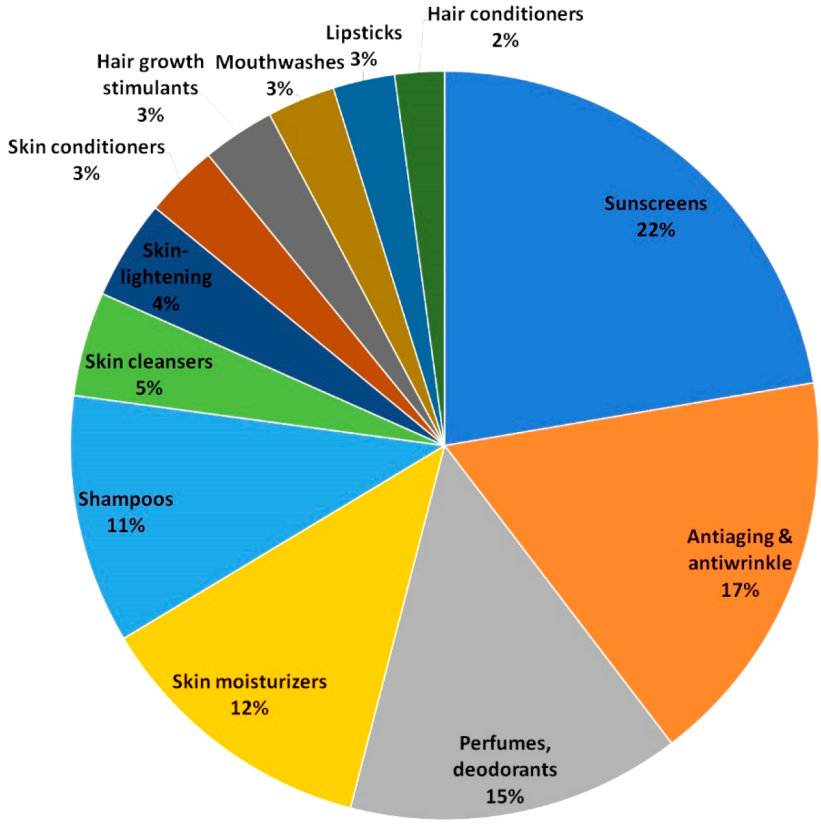

Figure 17. Distribution of LNP-related documents with respect to cosmetic product types.

Country	
34.6	USA
24.9	China
7.2	Japan
4.6	S. Korea
4.3	Germany
3.0	France
2.6	Canada
1.7	India
1.6	UK
1.4	Israel
1.4	Switzerland
1.2	Russia
1.2	Taiwan
1.1	Italy
1.1	Denmark
1.0	Spain
0.9	Brazil
0.8	Netherlands
0.8	Belgium
0.5	Australia
0.3	Norway
0.3	Hungary
0.2	Turkey
0.2	Mexico
0.2	Poland

Organization		
1.10	University of California	USA
0.56	Massachusetts Institute of Technology	USA
0.55	China Pharmaceutical University	China
0.49	Fudan University	China
0.45	Shenyang Pharmaceutical University	China
0.37	Zhejiang University	China
0.34	Sichuan University	China
0.33	Suzhou Zhiweitang Biotechnology Co., Ltd.	China
0.33	Shanghai Jiao Tong University	China
0.31	Boston Scientific Scimed, Inc.	USA
0.31	Peking University	China
0.29	Genentech, Inc.	USA
0.29	University of Texas	USA
0.29	United States Dept. of Health and Human Services	USA
0.27	Abbott Cardiovascular Systems Inc.	USA
0.26	The Johns Hopkins University	USA
0.26	Harvard University	USA
0.25	Novartis AG	Switzerland
0.23	Schering Corporation	Germany
0.23	Centre National de la Recherche Scientifique	France
0.22	Immunomedics, Inc.	USA
0.22	Bristol-Myers Squibb Company	USA
0.22	Massachusetts General Hospital	USA
0.20	Yale University	USA
0.20	Duke University	USA
0.20	Merck & Co., Inc.	USA

Figure 18. LNP-related patents classified by the top countries and organizations, presented as percent of the total number of LNP-related patents in the years 2000−2020.

administration (Figure 14). The major advantages and disadvantages of these routes are summarized in Table 9.

Correlation between Various LNP Types and Their Delivery Routes. The correlation between the various kinds of LNP preparations and their delivery routes is illustrated in Figure 15. The strongest correlation was in the use of immunoliposomes for parenteral applications. Some formulations such as ethosomes are designed mainly for topical administration, while solid lipid nanoparticles and nanostructured lipid carriers can be applied topically, orally, and parenterally.

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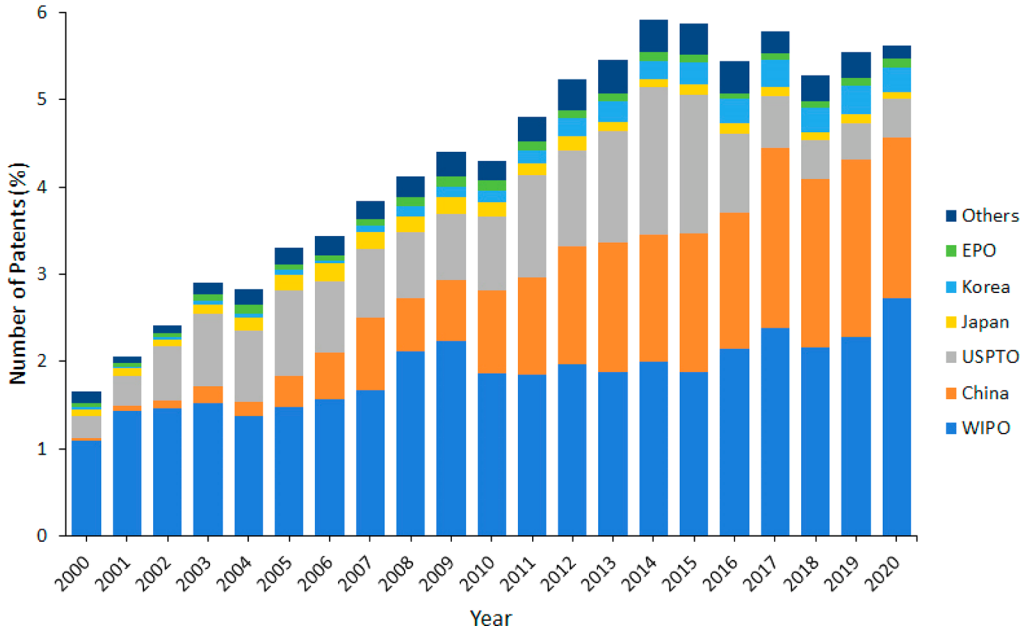

Figure 19. LNP-related patents per year for the top patent offices presented as percent of the total number of LNP-related patents in the years 2000−2020.

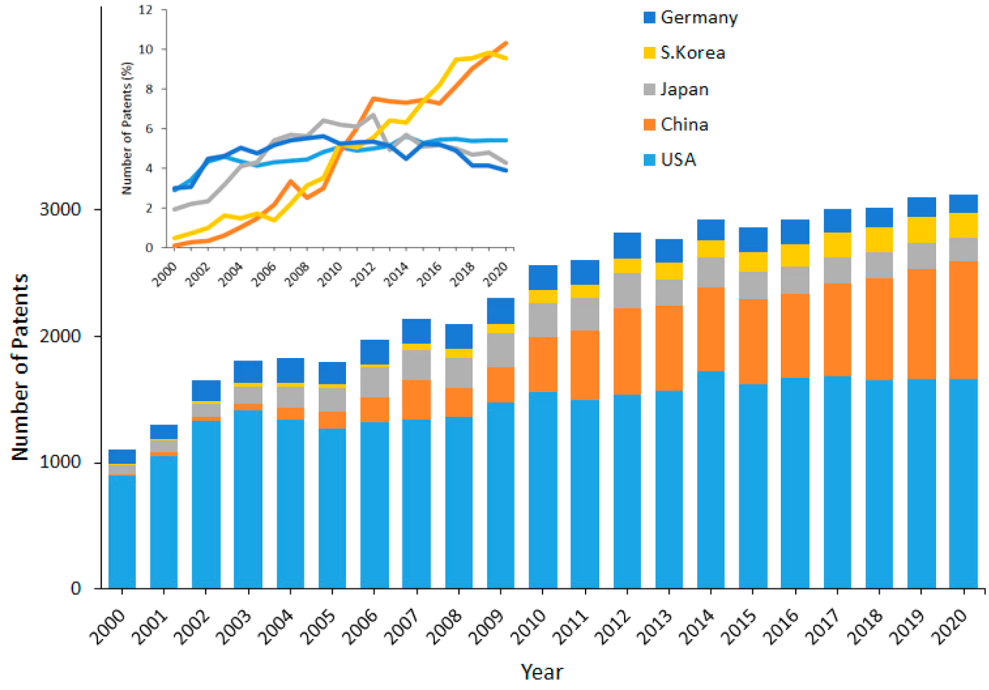

Figure 20. LNP-related patents per year in the years 2000−2020 for the top five countries presented as the total number of LNP-related patents. Inset: Percentage of the total number of LNP patents for the given country.

Correlation of the Various LNP Types and Therapies They Have Been Applied to. Different types of LNPs have different advantages and disadvantages and thus are appropriate for different therapies. Figure 16 illustrates the correlations between LNP types and the therapies to which they have been applied. Immunoliposomes, sterically stabilized liposomes, and cationic liposomes are the most commonly used LNPs for antitumor therapy. Understandably, cationic liposomes are the preferred formulation for gene therapy, and immunoliposomes are preferred as delivery vehicles for immunotherapies, including cancer immunotherapy.

LNP-Based Cosmetics. In cosmetics, LNP formulations are most prevalent in patents for sunscreens, antiaging preparations, and perfumes (Figure 17). Nanostructured lipid carriers (NLC) are the preferred carriers for sunscreens because of their ability to enhance the photostability of normally photolabile UV absorbers and to allow their sustained release over time, reducing skin irritation.[264] Various active ingredients are used to prevent, delay, and treat skin aging, such as antioxidants, biological growth factors, herbal ingredients, and retinoids. Such preparations have been termed cosmeceuticals, because they are intended to have both cosmetic and pharmaceutical benefits.

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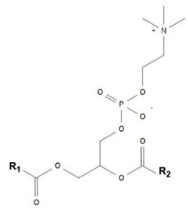
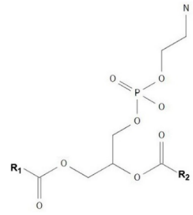
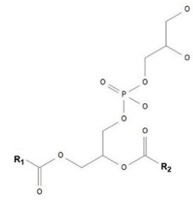
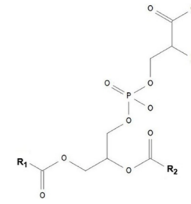
Table 10. Number of Patents for the Four Most Widely Used Phospholipid Classes in LNP Formulations[a]

R1 / R2	Phosphatidylcholines (PCs)	Phosphatidyl-ethanolamines (PEs)	Phosphatidylglycerols (PGs)	Phosphatidylserines (PSs)
				
6:0 / 6:0	37	19	7	3
7:0 / 7:0	14	8		
8:0 / 8:0	30	5	5	5
11:0 / 11:0	29			
10:0 / 10:0	82	16	8	5
12:0 / 12:0	406	104	154	18
13:0 / 13:0	12		1	
14:1c9 / 14:1c9	23			
14:0 / 16:0	95			
14:0 / 14:0	1407	445	612	176
14:0 / 18:0	41			
15:0 / 15:0	41	5		
16:0 / 2:0	10			
16:0 / 14:0	88		4	2
16:0 / 16:0	2507	827	755	314
16:1c9 / 16:1c9	45	21		
16:4me3,7,11,15 / 16:4me3,7,11,15	47	116	3	
16:0 / 18:0	130			
16:0 / 18:2c9,12		11		
16:0 / 18:1c9	660	217	155	68
16:0 / 20:4c5,8,11,14		6		
17:0 / 17:0	18	3	2	
18:0 / 14:0	21	20		
18:0 / 16:0	99		15	
18:0 / 18:0	1930	841	417	134
18:0 / 18:1c9	74	65	6	7
18:0 / 18:2c9,12		10	2	
18:0 / 22:6c4,7,10,13,16,19		7		
18:1c9 / 14:0	9	4		
18:1c9 / 18:1c9	1391	1537	430	271
18:1c9 / 16:0	40		9	
18:1c9 / 18:0	11			
18:1t9 / 18:1t9	139	50	6	2
18:1y17 / 18:1y17				2
18:2c9,12 / 18:2c9,12	77	42	8	11
18:2c9,12 / 16:0			2	3
18:3c9,12,15 / 18:3c9,12,15	6	27	5	
19:0 / 19:0	12			
20:4c5,8,11,14 / 16:0			2	2
20:0 / 20:0	83	18	8	9
20:4c5,8,11,14 / 18:0			2	
20:4c5,8,11,14 / 20:4c5,8,11,14		8	13	
21:0 / 21:0	10			
22:1c13 / 22:1c13		10	2	2
22:0 / 22:0	53			
24:0 / 24:0	34			
22:6c4,7,10,13,16,19 / 16:0		5	2	2
22:6c4,7,10,13,16,19 / 18:0			2	3
22:6c4,7,10,13,16,19 / 22:6c4,7,10,13,16,19	33	28	5	2

[a]The structures of these phospholipids are shown at the top of the table. Designation nomenclature: All acyl chain residues are fully specified, using a systematic nomenclature, as follows. The two chain lengths, in units of carbon atoms (and with the first carbon of the chain defined as the one bonded through an oxygen atom to the glycerol backbone), are given, each to the left of a colon (:). The two chain descriptors are separated from each other by a backslash. In the default configuration the hydrocarbon chains are saturated. Modifications to each chain are indicated to the right of the colon and are listed according to number, kind, and location. First, to the right of the colon appears the number of modifications on that

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Table 10. continued

chain. A zero (0) indicates that the chain is in the default configuration, with no modifications. Following the number of modifications, the modifications themselves are listed. Following each modification is a number indicating the carbon atom position on the chain where the modification is located. The letters "c" and "t" denote the cis and trans configuration, respectively, of the double bond, followed by a number or set of numbers identifying double bond position; "y" denotes triple bond; "me" denotes methyl isobranching.

Carriers for such cosmetic formulations include liposomes, solid lipid nanoparticles (SLN), and nanostructured lipid carriers (NLC).[265] The use of nanoencapsulation in fragrance products improves their efficiency and allows sustained release of scents over time.[266]

INSIGHTS ON LNP-RELATED PATENTS FROM THE CAS CONTENT COLLECTION

As of June, 2021, there are over 45,000 patents related to LNPs/liposomes in the CAS Content Collection, over 41,000 of which are in the years 2000−2021. The majority of LNP patents come from inventors in the US and China (Figure 18). The largest recipient of LNP patent filings is the World Intellectual Property Organization (WIPO). While the proportion of patents filed with WIPO has stayed nearly constant between 2000 and 2020, the share of patents filed with the China National Intellectual Property Administration (CNIPA) has increased significantly, from less than 1% of patents in 2000 to over 33% of all patents in 2020. Over this period, the fraction of LNP patents filed with the US Patent and Trademark Office (USPTO) decreased significantly, particularly between 2010 and 2018 (Figure 19).

Distribution of LNP-Related Patents by Country and Organization. The top five countries contributing to the growth in LNP patents are the USA, China, Japan, South Korea, and Germany. While the involvement of USA, Japan, and Germany in LNP research has remained stable after the initial growth in the years 2000−2005, the involvement of China and South Korea in LNP research has increased consistently during the same period (Figure 20).

Most Widely Used Lipids in LNP Formulations in Patents. There are many components used in LNPs, with the composition determined by the intended morphology and application. Along with the most common constituents—phospholipids and cholesterol—LNPs frequently include cationic ionizable lipids and PEG−lipid conjugates (PEG-lipids), as well as various other components. A collection of ∼45,000 LNP-related patents were identified in the CAS Content Collection. The most widely used members of various lipid classes were identified in these patents.

Cholesterol (CAS RN 57-88-5) is the lipid component used in the largest number of patents—over 3,200 patents have used LNP formulations including cholesterol.

Phospholipids (Table 10) are the most prevalent class of lipids involving LNPs. Phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), phosphatidylglycerols (PGs), and phosphatidylserines (PSs) are the most common phospholipid constituents. Preferred phospholipid species with respect to their hydrocarbon chains include saturated dimyristoyl (14:0/14:0), dipalmitoyl (16:0/16:0), and distearoyl (18:0/18:0) chains, as well as unsaturated dioleoyl (18:1c9/18:1c9) chains (Table 10). Phospholipids from natural sources, such as soya total phospholipids, soya phosphatidylcholines, hydrogenated soya phosphatidylcholines, and egg phosphatidylcholines, have also been commonly used in LNP formulations.

PEG−Lipid Conjugates. Since the discovery that PEG−lipid conjugates can significantly increase the circulatory half-lives in the sterically stabilized "stealth" liposomes, PEG-lipids have been widely used in pharmaceutical LNP formulations. The major classes PEG-lipids found in patents are listed in Table 11, with their structures depicted in Figure 21.

Cationic Lipids. The most often used cationic lipids in LNP formulations were identified and listed in Table 12. They typically comprise various amine derivatives, *e.g.*, DOGS and DC-Chol, quaternary ammonium compounds, *e.g.*, DOTMA,

Table 11. Number of Patents for the Most Widely Used PEG-lipids in LNPs

R1/R2	Common/ Commercial Name	CAS RN	Number of patents
		PEG-PE[a]	
18:0/18:0	DSPE-PEG	145035-96-7; 170931-04-1	483
16:0/16:0	DPPE-PEG	145035-97-8; 170931-03-0	94
18:1c9/18:1c9	DOPE-PEG	145035-95-6; 262601-19-4	43
14:0/14:0	DMPE-PEG	211567-66-7; 211733-74-3	38
18:1c9/16:0		170127-34-1	5
12:0/12:0		2055341-27-8	4
18:2c9,12/18:2c9,12		736998-47-3	4
		mPEG-glycerides	
14:0/14:0	DMG-PEG	160743-62-4	245
		1397695-86-1	
18:0/18:0	DSG-PEG; Sunbright DSG 2H; Sunbright DSG 20H	308805-39-2	36
		850628-36-3	
16:0/16:0	DPG-PEG	162409-28-1	17
18:1c9/18:1c9		160743-61-3	5
		mPEG-PE	
18:0/18:0	DSPE-mPEG; Sunbright DSPE 020CN	156543-00-9; 247925-28-6; 474922-77-5; 459428-35-4	329
16:0/16:0	DPPE-mPEG	205494-72-0	29
14:0/14:0	DMPE-mPEG	474922-82-2; 261764-82-3	33
18:1c9/18:1c9		226940-29-0	20
		amino-mPEG	
14:0/14:0	ALC-0159	1849616-42-7	6
12:0/12:0		1849616-44-9	1
12:0/14:0		1849616-45-0	1
16:0/16:0		1849616-43-8	1
18:0/18:0		741737-56-4	1
		Chol-PEG	
PEG-cholesterol	PEG-cholesterol	27321-96-6	54
mPEG-cholesterol	mPEG-cholesterol	99559-58-7	11

[a]For the structures of the various PEG-lipid subclasses, see Figure 21, lower panel.

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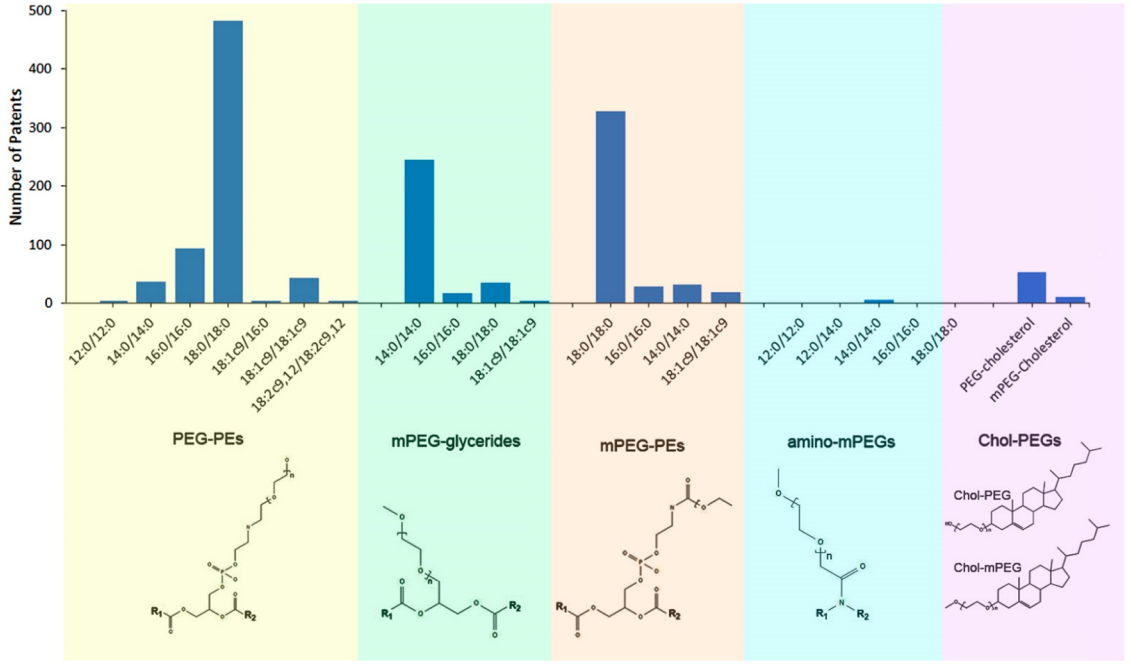

Figure 21. Number of patents for the most widely used PEG-lipids in patents on LNPs.

DOTAP, DORIE, and DMRIE, cationic phosphatidylcholines such as EDOPC and EDMPC, combinations of amines, *e.g.*, DOSPA and GAP-DLRIE, and amidinium salts, *e.g.*, Vectamidine.[18,19,267−275] Cationic multicharged head groups such as DOSPA and DOGS have been reported to be more effective than single-charged cationic lipids such as DOTMA, DOTAP, DC-Chol, and DMRIE.[276,277] The increased effectiveness may be related to the greater ability of highly charged cationic lipids to condense and protect nucleic acids, but the increased binding of multicharged ligands to nucleic acids may also obstruct or inhibit nucleic acid release inside the cell. In addition, the combination of quaternary ammonium salts and polyamines enhances delivery efficiency. Indeed, the earliest cationic lipid incorporating both quaternary ammonium and polyamine moieties, Lipofectamine (CAS RN 158571-62-1), comprising a 3:1 mixture of DOSPA and dioleoylphosphatidylethanolamine (DOPE), is a highly effective transfection agent.

SUMMARY AND OUTLOOK

Insights on LNP Compositions Inferred from the Research Landscape. Based on the landscape analysis of the LNP-related documents in the CAS Content Collection, the following aspects may be worth considering when selecting lipid compositions for LNP formulations.

- Biocompatibility. Naturally occurring lipids are preferable, because they are likely to be metabolizable in the target species. The most widely used class of lipids in LNP formulations are phospholipids, which are also the major class of biomembrane lipids.
- Fluidity. Cholesterol is well-known as a powerful modulator of lipid bilayer fluidity; it is able to enhance the fluidity of solid bilayers and to reduce the fluidity of liquid bilayers. It is also one of the major components of biomembranes and is highly biocompatible.
- Phase state and phase transition temperature. Phase state is an important characteristic of LNPs—it contributes to their stabilities and encapsulation efficiencies and controls their interactions with biomembranes and cargo release. The phase transition temperatures of the individual lipids in the LNP as well as their miscibilities should be considered. Generally, lipids with longer alkyl chains and higher degrees of saturation have higher transition temperatures.
- Electric charge (zeta potential). The electric charges of LNPs affect their stability, their rate of cargo release, their circulating half-lives in the bloodstream, and their fusion with biomembranes. Naturally occurring membrane lipids are either zwitterionic (PCs, PEs) or negatively charged (PGs, PSs). In many uses, such as in nucleic acid delivery, the presence of positive-charged lipids is beneficial, leading to the development and use of synthetic cationic lipids. Since cationic lipids are not natural constituents of cells, their biocompatibilities and the toxicities of their degradation products should be considered.
- Toxicity is especially relevant to formulations including cationic lipids, which are synthetic and whose toxicities may not be known or have been observed in biological systems. In many cases, the effectiveness of a cationic lipid in LNP formulations correlates to increased toxicity. For example, multivalent cationic lipids have been more effective than monovalent cationic lipids in LNP formulations but are also much more toxic to cells. Identification of cationic lipids with similar structures to natural lipids known to be effective in LNPs such as cationic ethylphosphatidylcholines or cationic cholesterols may yield LNPs with reduced side effects.
- Size is a critical parameter in determining LNP circulation half-life and drug encapsulation. The size of LNPs strongly depends on how they are prepared. Ultrasonication, extrusion, and microfluidic methods have been most often used to control LNP size.
- Circulation time and phagocytic uptake. Coating LNPs with an inert polymer such as PEG considerably extends their residence in blood circulation by preventing phagocytes from reaching the surface of LNPs and

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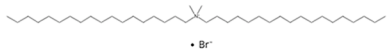
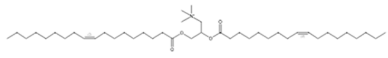
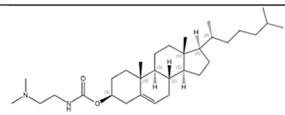
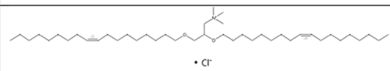
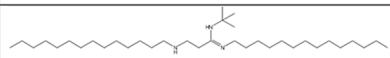
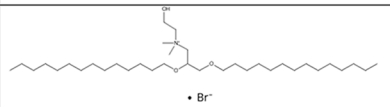
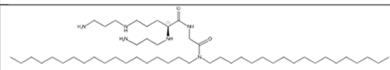
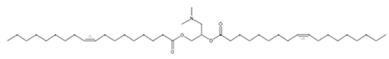
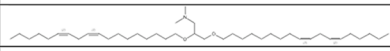
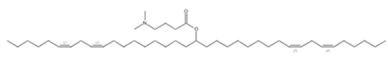
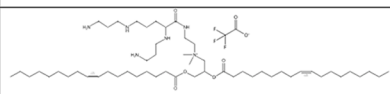
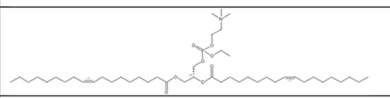
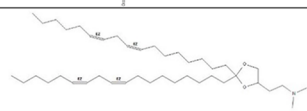
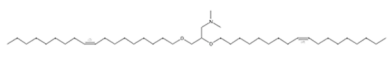
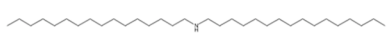
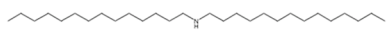
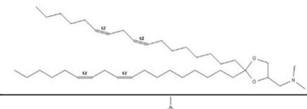
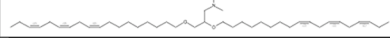
Table 12. List of the Most Widely Used Cationic Lipids in LNPs in Patents

CAS Registry Number	Chemical Structure	Common/Commercial Name	Number of References
3700-67-2		DDAB	2532
113669-21-9		DOTAP	2422
137056-72-5		Cholesterol (2-dimethylaminoethyl) carbamate; DC-Chol	904
112-99-2		Dioctadecylamine ; Armeen 2-18; Distearylamine; Genamin SH 200	821
104162-48-3		DOTMA	806
105488-80-0		CLONfectin; Vectamidine	585
153312-64-2		DMRIE	357
124050-77-7		DOGS Transfectam	323
127512-29-2		1,2-Di(oleoyloxy)-3-(dimethylamino) propane; DODAP	303
871258-12-7		DLinDMA	245
1224606-06-7		DLin-MC3-DMA; MC 3; RV 28	238
168479-03-6		DOSPA	229
183283-20-7		EDOPC	204
1190197-97-7		DLin-K-XTC2-DMA; Dlin-KC2-DMA; XTC	200
104162-47-2		DODMA; MBN 305A; N-[2,3-Di(oleyloxy)propyl]-N,N-dimethylamine	181
16724-63-3		Armeen 2-16 Dipalmitylamine	174
17361-44-3		Alamine 205 Dimyristylamine NSC 91530	122
1169768-05-1		DLin-K-DM4	91
874291-25-5		DLenDMA	90

https://doi.org/10.1021/acsnano.1c04996
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Table 12. continued

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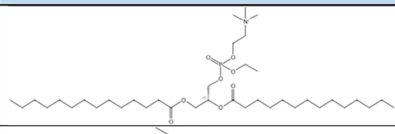
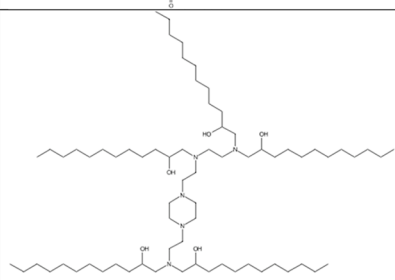
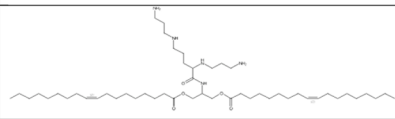
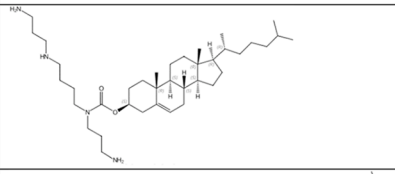
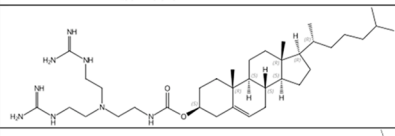
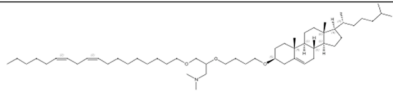
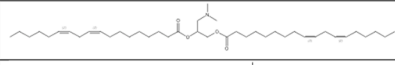
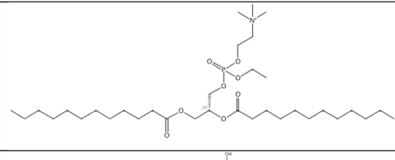
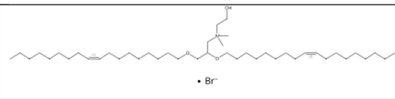
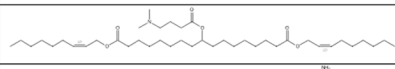
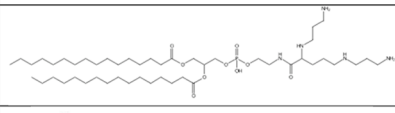
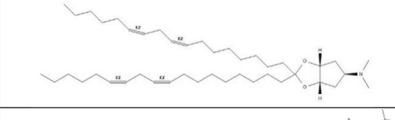
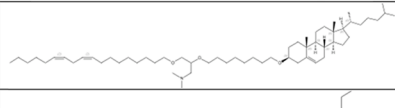
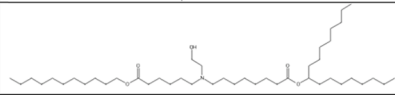
183283-19-4		EDMPC	88
1220890-25-4		C12-200 Tech G 1	87
178532-92-8		DOSPER	87
179075-30-0		GL 67 N4-Spermine cholesteryl carbamate	83
182056-06-0		BGTC; Bisguanidinium tren-cholesterol	73
908860-82-2		CLinDMA	52
1019000-51-1		DLinDAP	49
230949-32-3		EDLPC	48
153312-60-8		DORIE	44
1351586-50-9		L 319 RV 92	41
124076-29-5			39
1226778-72-8		ALN 100 ALNY 100	36
1208381-69-4		Octyl CLinDMA	36
2089251-47-6		SM-102	36

https://doi.org/10.1021/acsnano.1c04996
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Table 12. continued

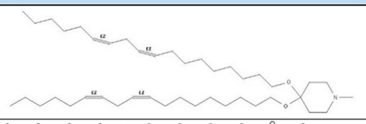
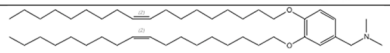
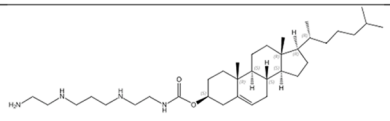
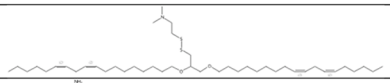
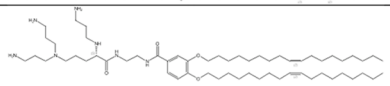
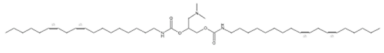
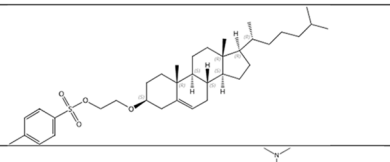
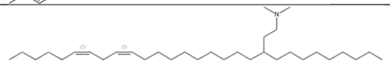
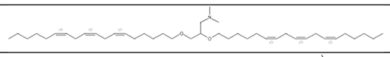
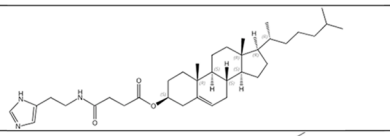
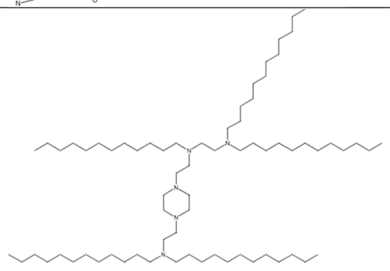
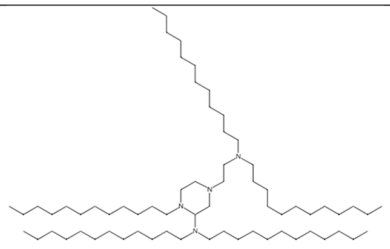
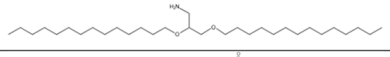
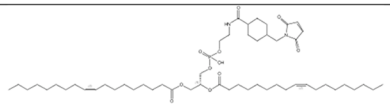
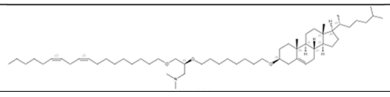
CAS Registry Number	Chemical Structure	Common/Commercial Name	Number of References
1318793-78-0		YSK 05	35
908860-85-5		DMOBA	33
200337-52-6		CDAN GL 138	31
1415795-37-7		HGT 4003	29
760939-62-6		MVL 5	29
1169768-13-1		DLin-C-DAP	28
30656-75-8		Cholest-5-en-3β-oxyethane tosylate	28
1361106-13-9		Compound 32	28
1260141-95-4		γ-DLenDMA	28
449791-79-1		HisChol	28
1413010-97-5		KL22	27
1413010-89-5		KL10	27
396727-98-3		Dimyristyloxypropylamine	26
789482-14-0			26
1208381-72-9			25

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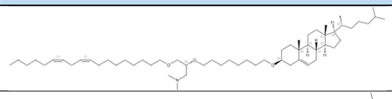
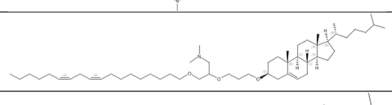
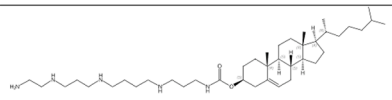
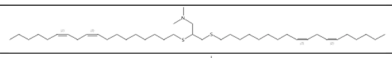
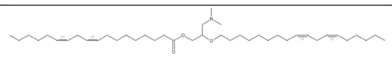
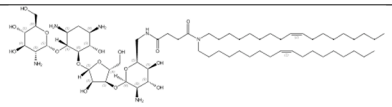
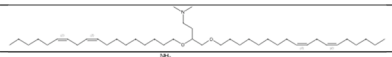
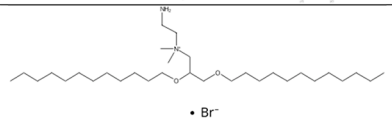
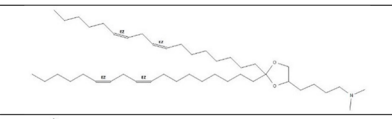
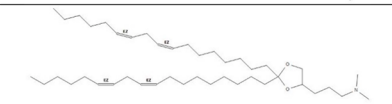
Table 12. continued

CAS Registry Number	Chemical Structure	Common/Commercial Name	Number of References
1208381-70-7			25
908860-83-3		CpLinDMA PCLinDMA	24
200337-57-1		CTAP	21
1169768-15-3		DLin-S-DMA	13
1169768-10-8		DLin-2-DMAP	13
959664-11-0		Dioleylamine-A-succinyl paromomycin DOSP	13
1192257-55-8		C2-DLinDMA	11
208040-06-6		GAP-DLRIE	10
1217306-47-2		DLin-K-C4-DMA	9
1217306-46-1		DLin-K-C3-DMA	9

inhibiting their ability to uptake LNPs through steric hindrance.

- Cargo release. Effective LNPs rely on a delicate balance between stability and cargo release. LNPs need to be stable enough to safely transport their cargo to their targets yet capable of falling apart to release their cargo at the desired location. In many cases, external stimuli can be used to facilitate drug release. For example, when the pH at the desired location differs from the pH at other sites, ionizable lipids can facilitate drug release at that desired location. Cargo release from LNPs containing cationic lipids can also be triggered by lipid exchange with biomembranes, inducing the formation of nonlamellar phases.
- Encapsulation efficiency and stability. Replacing traditional liposome formulations with solid lipid nanoparticles (SLN) or especially with nanostructured lipid carriers (NLC) may enhance the stability and encapsulation efficiency of lipid nanocarriers significantly.

Perspectives. Nanotechnology has significantly widened the horizons in science and particularly in medicine. Due to their small size and high surface area, drug nanoformulations such as the LNPs have different properties than the corresponding bulk materials, and the changes in the biochemical, electronic, magnetic, and optical properties of nanoparticle drug formulations have been used to therapeutic benefit. As a result, nanomedicine has brought impressive progress in modern drug therapy against many diseases. Application of nanotechnological strategies to drug delivery has improved the effectiveness, selectivity, residence time, and biodistribution of conventional drug carrier systems while reducing their limitations. Furthermore, nanoparticle drug formulations have reduced the toxicities and improved the solubilities and bioavailabilities of conventional medicines. The continuous efforts in synthesis and screening of functionalized lipid nanomaterials by chemically optimizing their molecular structures to enable tunable biodegradability *in vivo* would promote the development of more versatile, highly efficient, and biocompatible delivery vehicles.

As ongoing research attempts to address the needs of personalized medicine, more sophisticated and multifunctional nanocarrier designs are being developed. LNPs with complex structures are being designed to overcome biological barriers specific to individual patient or disease status as demanded by precision, or personalized, medicine. The objective of precision medicine is to utilize patient information such as genetic profile, age, lifestyle, environmental conditions, or comorbidities in order to develop an individualized treatment approach. Tailored nanocarrier designs, adapted by patient data and engineered to permeate particular barriers, can markedly improve the delivery of and response to precision medicine therapies.[278]

The use of LNPs in medicine is likely to expand significantly. The development of LNP types and varieties with enhanced drug delivery properties such as the nanostructured lipid carriers and the ionizable cationic nanoparticles brings further advantages to the LNP formulations and enlarges the prospects of their applications. LNPs hold great promise in genetic medicine where gene editing, vaccine development, immuno-

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oncology, and other genetic therapies rely on the ability to efficiently deliver nucleic acids into cells. LNPs have advantages over other gene and vaccine delivery systems because they are easier to manufacture, are less immunogenic, can carry larger payloads, and can be designed for multiple dosages. Nucleic acid therapeutics are an emerging class of drugs showing potential for treating disease. LNPs have come out as successful and efficient carriers for such drugs. The successful use of LNPs as a delivery vector for the COVID-19 mRNA vaccines will likely broaden the horizons for research in mRNA vaccines.

From a materials science perspective, the success of LNPs in medicine is important, as it motivates further fundamental and applied nanoparticle research. The use of LNPs in the controlled synthesis of metal nanoparticles may also be important in expanding their use in display technologies and other uses. Expansion of the LNP technologies in other areas is also noticeable. Numerous cosmetic products are already in the market, with much more in development. Additional areas such as nutrition, nutraceuticals, agrochemistry, and nanoreactors are already exploring the benefits of lipid-based nanoencapsulation. Further, LNPs may have environmental applications, such as in metal detoxification. Based on the current progress and success, LNPs can certainly be recognized as one of the most advantageous and promising areas in modern nanotechnology.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsnano.1c04996.

Table S1. Clinically approved LNP formulations. Table S2. Marketed LNP cosmetic preparations. Figure S1. Classification of liposomes according to their size and lamellarity. Figure S2. Mechanism of action of mRNA mediated vaccination. (PDF)

AUTHOR INFORMATION

Corresponding Author

Qiongqiong Zhou − *CAS, a division of the American Chemical Society, Columbus, Ohio 43210, United States;* orcid.org/0000-0001-6711-369X; Email: qzhou@cas.org

Authors

Rumiana Tenchov − *CAS, a division of the American Chemical Society, Columbus, Ohio 43210, United States;* orcid.org/0000-0003-4698-6832

Robert Bird − *CAS, a division of the American Chemical Society, Columbus, Ohio 43210, United States*

Allison E. Curtze − *CAS, a division of the American Chemical Society, Columbus, Ohio 43210, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsnano.1c04996

Notes

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VOCABULARY

Lipid nanoparticle (LNP), nanosized particle composed mainly of lipids, used predominantly in drug delivery but also in cosmetics, nutrition, *etc.*; liposome, a vesicle comprising at least one lipid bilayer; major types of liposomes are small unilamellar liposomes (SUV) having a single bilayer and multilamellar liposomes (MLV) having several lipid bilayers; solid lipid nanoparticles (SLN), LNPs comprising solid lipids; nanostructured lipid carriers (NLC), LNPs comprising a mixture of solid and liquid-crystalline lipids. Both SLN and NLC are widely used in drug delivery because of their enhanced physical stabilities, high loading capacities, high bioavailabilities of their cargoes, and facile production on a large scale; cationic lipid, synthetic lipid compound similar to the natural lipids, except for the presence of an ionizable (cationic) head group instead of the zwitterionic or anionic head group of the natural lipids; invented and applied mainly for delivery of nucleic acids; "stealth" liposome, sterically stabilized liposome coated with biocompatible inert polymers (mostly PEG), making them invisible to phagocytes, thus exhibiting long circulation half-life; immunoliposome, targeted liposome generated by coupling a ligand, typically an antibody, to the liposomal surface, allowing for active tissue targeting through binding to cell-specific receptors.

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