

Review

Innovative and Patented Liposome-Based Drug Carriers

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Abstract: Liposome-based drug carriers are multipurpose colloidal drug delivery systems developed mainly for targeted therapy. Researchers have expanded their research on liposomes due to their unique characteristics (e.g., non-toxicity, biodegradability, biocompatibility, and non-immunogenicity). This review summarizes historical advances, from the first scientific papers and patents to the latest inventive solutions, in the field of liposome-based drug carriers and their production processes. Various bibliometric studies on the use of liposomes as drug carriers have been published; nevertheless, they focus on published scientific works rather than patent documents. Patent information is important for the pharmaceutical, nutraceutical, and cosmetic industries because technical knowledge in patent documentation is often not published in any other document. The research in this review was conducted using the Espacenet—European Patent Office database, with keywords and classification codes defined by the International Patent Classification. Innovative formulations, including the usage and administration route, are broadly researched to produce effective and safe drug delivery systems with negligible side effects. Global patenting trends in liposome drug carriers' production process were also discussed, and this evaluation unifies up-to-date development in this field. Patent database reviews and analyses could help as inspiration for future investigations as well as for problem-solving resources.

Keywords: drug delivery systems; liposomes; nanoparticles



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1. Introduction

The goal of pharmaceutical and technological progress is to discover novel processes for converting an active ingredient (e.g., drug) into a dosage form acceptable for application, which enables the concentration of the active ingredient in the site of action to quickly reach a therapeutic level and maintain an approximately constant level over time, according to the prescribed therapeutic needs [1]. The processes applied to reach this aim are very complex and challenging and demand sophisticated and functional drug delivery systems (DDS). Progress from the existing drug molecule, through the conventional drug dosage form to a new and innovative drug delivery system, could significantly improve features of active ingredients, considering efficiency, safety, and compliance (acceptability for patients). The drug targeting or targeted therapy concept implies an adaptable carrier (polymeric or colloidal) at which the active ingredient is bound (by sorption or chemical bonding). Colloidal drug delivery systems are mainly developed as target systems, i.e., carriers for achieving the target result [2,3]. Drug carriers should transport and/or direct the active ingredient precisely at a controlled rate to a specific group of cells or organs in which it should be released and act [4,5]. The key benefit of carriers is their capability of incorporating drugs, enhancing their bioavailability and selectivity; thus, reducing side effects to humans. The main task in formulating a drug delivery system is that the selected carrier should protect the incorporated/adsorbed drug from the negative effects of the organism on the way from the application site to the target site (diseased organ or

cells) and release the active substance to the intended target site [6]. Nanoparticles used as DDS exhibit slow kinetic transportation in diseased cells and tissues due to vascular barriers [7]. In order to increase the possibility of crossing the vascular wall, a relatively long circulation time of nanomaterials is needed. The prototypical nanoparticles are blocked in the bloodstream due to rapid clearance by the reticuloendothelial system (RES).

Liposomes are among the most studied advanced pharmaceutical drug delivery systems. They represent microparticulate lipid vesicles, i.e., spherical self-enclosed structures, composed of curved lipid bilayers in which a part of the solvent (water) is enclosed, in which these structures freely float [3,4]. Liposomes were discovered in 1965 by the scientist Bangham, as a system similar to cell membranes [8]. Some years later, scientists Gregoriadis, Leathwood, and Ryman found novel pharmaceutical applications for the enzymes fructofuranosidase and amyloglucosidase, as well as ^{131}I -labeled albumin and human serum albumin delivery, using liposomes in genetic disorder treatments [9–11]. In the following decades, numerous studies were developed in the area of novel and improved liposome production processes and their innovative possibility of applications in humans [12]. Conventional liposomes are usually made only of phospholipids (neutral or negatively charged) and/or cholesterol and are characterized by a relatively short blood circulation time [13,14]. Drug delivery systems found solutions to improve colloidal stability in aqueous media, e.g., charge-repulsion-based stabilization and steric stabilization using hydrophilic, neutral, and flexible polymers, e.g., polyethylene glycol (PEG), as well as many others [7]. The applications of liposomes as carriers to protect insulin against proteolysis in the gastrointestinal tract, from the first study in 1976 to today, is at the preclinical level because of its limited stability in gastrointestinal conditions [15,16]. In contrast, in this period, many liposomal technologies were significantly improved and reached large-scale production thanks to plentiful investigations about possibilities in drug delivery, their stability in physiological fluids, many clinical trials, and approved products, and many are in various stages of testing, which confirms the remarkable progress [17–20]. In numerous cases, lipid complexes and liposomal drug forms provide less toxicity and better efficacy than the drug itself or the conventional pharmaceutical form of the same drug [4,6]. Due to their relatively high biocompatibility, liposomes are acceptable for therapeutic purposes and in vivo diagnostics. Liposomes are used to improve the “transfer/delivery” as carriers of various drugs, such as immunomodulators, chemotherapeutic agents, diagnostic agents, antigenic, genetic material, etc. (e.g., doxorubicin, epirubicin, amphotericin B, calcitonin, interferon, or technetium-99 as imaging agents) [6]. Encapsulation of drugs in liposomes has become a notable strategy for the invention of pharmaceutical preparations applied through different administration routes, such as ocular, oral, vaginal, urinary, or rectal routes [5,21]. Nowadays, there are many pharmaceutical preparations available on the market, developed on liposomes as carriers, mainly for parenteral administration (for intravenous, intramuscular, subcutaneous, epidural, intraperitoneal, and intrathecal applications) [22–24], and dermatological products for topical usage [25–27]. Encapsulation of active therapeutic ingredients in liposomes could enhance their penetration into the skin’s deeper layers with the accumulation and drug “depot” formation, which provides a sustained release and reduces side effects and systemic absorption [1,28]. The stability of the active substance also increases with encapsulation in liposomes, which delays their shelf life [29].

Liposomes are generally formed of phospholipids (neutral or negatively charged) and/or cholesterol and characterized by a relatively short circulation time in the blood [8,21]. Phospholipids, as basic ingredients of plant, animal, and bacterial membranes, make liposomes non-toxic, biodegradable, and non-immunogenic carriers for bioactive ingredients. The phosphate part, called the polar “head”, is the hydrophilic part, while the long chain of saturated and/or unsaturated fatty acids is the hydrophobic/non-polar part of the phospholipid molecule [1]. Phospholipids are solvable in organic solvents and their mixtures. As a result of their amphiphilic nature, they form aggregates in an aqueous medium, directly above the critical micellar concentration (CMC, about 10^{-8} M), the structure of which

depends on the chemical structure of the dimension and saturation degree of the fatty acids chain and the polar “head”, and the pH and ionic strength of the aqueous medium. Stealth (long-circulating)/sterically stabilized liposomes were developed to overcome the short retention time in the circulation of conventional liposomes. They have a coating/polymer protective layer, formed from polyethylene glycol (polyoxyethylene derivatives) on the surfaces of liposomes. It avoids rapid clearance by the immune system and prevents liposomes from leaving the circulation [5,6]. The prolonged presence in the blood allows them to pass to places with permeable vascularization, often in tumors. They have a potential application in the therapy of malignant lung cancer cells.

Target liposomes (immuno-liposomes) were additionally improved by incorporating monoclonal antibodies or antibody fragments into the membranes of conventional or sterically stabilized liposomes (by covalent or other bonds) for precise and specific targeting of tumors cells and to deliver drugs [6]. Cationic liposomes were developed to improve the transfer of genetic material, as the cationic lipid components react with deoxyribonucleic acid (DNA) and neutralize their negative charge. The lipid–DNA complex provides protection and helps direct the condensed plasmid to the target cell’s surface and release the genetic material into the cytoplasm or nucleus. The new generation of stimulus-responsive liposomes was invented to overcome the disadvantages of conventional liposomes. Smart liposomes could be sensitive to the change in thermo, light, pH, ion, enzyme, and magnet stimulus, or the dual- or multi-sensitive liposomes. They can encapsulate different active ingredients, with low toxicity, high levels of viability for healthy cells, and increased bioavailability in damaged tissues. Strategies of surface modification of liposome-based carriers, their classification, and the possibility of active ingredients’ encapsulation (drugs, proteins, small molecules, and targeting moieties, e.g., antibodies, peptides, aptamers, etc.), conjugated on the carrier’s surfaces by various linkers, covalent or non-covalent bonds, and electrostatic interactions, are schematically presented in Figure 1 [19]. Many products based on liposomes as carriers have been approved and are on the drug market, and numerous others are in various testing stages [21].

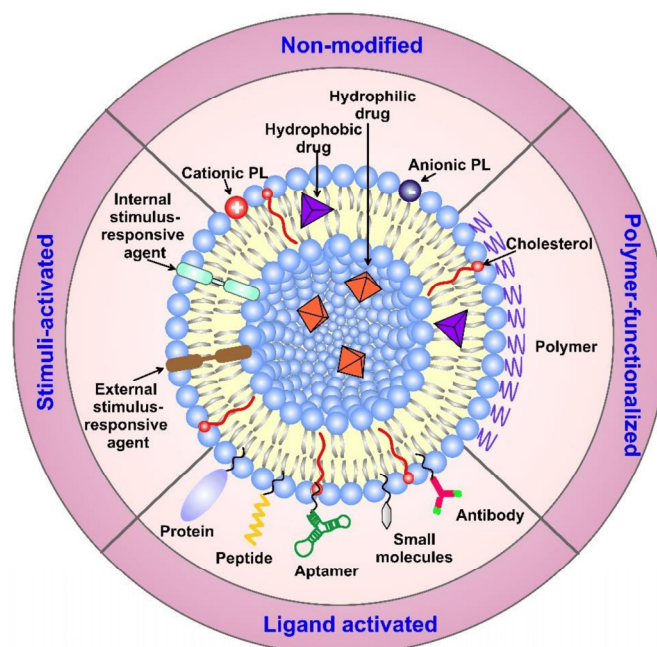


Figure 1. Surface modification strategies of liposomes, together with their classification. The modified carriers can contain active components, such as drugs, small molecules, proteins, and/or targeting moieties, such as antibodies, peptides, aptamers, etc., conjugated on the surface of the vehicles through different linkers, non-covalent or covalent bonds, and electrostatic interactions. Abbreviation: PL—phospholipid. Reprinted from [19] under an open-access creative common CC-BY license.

A few scientific review papers provide short reports on patent documents for liposome-based carriers, without a whole, up-to-date patent analysis [30,31]. This overview aims to provide a patent analysis of liposome-based carriers, from the first patent to the newest inventive patented solutions, focused on the aspects of procedures for obtaining them and pharmaceutical applications. Patent activity and filing trends of liposome-based drug carriers can be used as inspiration for future research and data sources for problem-solving in pharmaceutical or cosmetic applications.

2. Patent Searching for Liposome-Based Drug Carriers

The search methodology for relevant patent information was conducted on the reference database Espacenet, with free access to over 150 million patent documents from over 100 countries, provided by the European Patent Office (EPO) [32]. This search was carried out by including keywords and classification symbols (using both the International Patent Classification (IPC) and Cooperative Patent Classification (CPC)). The IPC was provided by the World Intellectual Property Organization (WIPO) [33], used by above 100 National Patent Offices and frequently used for searching. It is a hierarchical system for the classification of patents and utility models according to the different areas of technology to which they pertain, independent from language symbols [34]. The CPC is a classification system developed by the EPO and the United States Patent and Trademark Office (USPTO), based on the previous European classification system (ECLA), a more detailed version of the ICP [35]. Both classifications, the IPC and CPC, are hierarchical classification systems that separate all technical knowledge using the descending order of hierarchical levels: section, class, subclass, group, and subgroup [36]. The hierarchy among subgroups is determined solely by the number of dots (•) before their titles, not by the numbering of the subgroups. To avoid repetition, dots before a group title are used instead of the titles of its hierarchically upper-level groups. Liposomes and their derivatives are classified in the A61K9/00 group and A61K9/127 subgroup, while the classification symbol for processes for preparing is A61K9/1277. Medicinal preparations containing active ingredients are classified into group A61K31/00, while those characterized by the non-active ingredients used, e.g., carriers or inert additives, targeting or modifying agents chemically bound to the active ingredient, are classified into group A61K47/00. The main groups and particularly relevant subgroups of IPC/CPC codes, selected and applied for this search, are listed in Table 1.

Table 1. List of IPC/CPC codes used for the liposome drug delivery systems patent search [34,35].

IPC Code	IPC Description, as Indicated by the Relevant CPC Symbol
A61K9/00	Medicinal preparations characterized by special physical form (...)
A61K9/10	• Dispersions; Emulsions (...)
A61K9/127	•• Liposomes
A61K9/1271	••• Non-conventional liposomes, e.g., PEGylated liposomes, liposomes coated with polymers (...)
A61K9/1272	•••• With substantial amounts of non-phosphatidyl, i.e., non-acylglycerophosphate, surfactants as bilayer-forming substances, e.g., cationic lipids (...)
A61K9/1273	•••• Polymersomes; Liposomes with polymerizable or polymerized bilayer-forming substances (...)
A61K9/1274	••• Non-vesicle bilayer structures, e.g., liquid crystals, tubules, cubic phases, cochleates; Sponge phases
A61K9/1275	•••• Lipoproteins; Chylomicrons; Artificial HDL, LDL, VLDL, protein-free species thereof; Precursors thereof
A61K9/1276	•••• Globules of milk or constituents thereof
A61K9/1277	••• Processes for preparing; Proliposomes
A61K9/1278	•••• Post-loading, e.g., by ion or pH gradient
A61K31/00	Medicinal preparations containing organic active ingredients
A61P35/00	Antineoplastic agents

Table 1. Cont.

IPC Code	IPC Description, as Indicated by the Relevant CPC Symbol
A61K38/00	Medicinal preparations containing peptides (enzymes, hormones, porphyrin hemoglobin)
A61K43/00	Drugs for specific purposes, not provided for in groups A61P1/00–A61P41/00
A61K45/00	Medicinal preparations containing active ingredients not provided for in groups A61K31/00–A61K41/00
A61K45/06	• Mixtures of active ingredients without chemical characterization, e.g., antiphlogistic and cardiac
A61K47/00	Medicinal preparations characterized by the non-active ingredients used, e.g., carriers or inert additives; Targeting or modifying agents chemically bound to the active ingredient
A61K47/06	• Organic compounds, e.g., natural or synthetic hydrocarbons, polyolefins, mineral oil, petrolatum, or ozokerite
A61K47/08	•• Containing oxygen, e.g., ethers, acetals, ketones, quinones, aldehydes, peroxides
A61K47/10	••• Alcohols; Phenols; Salts thereof, e.g., glycerol; Polyethylene glycols (PEG); Poloxamers; PEG/POE alkyl ethers
A61K47/16	•• Containing nitrogen, e.g., nitro-, nitroso-, azo-compounds, nitriles, cyanates
A61K47/18	••• Amines; Amides; Urea; Quaternary ammonium compounds; Amino acids; Oligopeptides having up to five amino acids
A61K47/24	•• Containing atoms other than carbon, hydrogen, oxygen, halogen, nitrogen, or sulfur, e.g., cyclomethicone or phospholipids
A61K47/28	•• Steroids, e.g., cholesterol, bile acids, or glycyrrhetic acid
A61K47/34	•• Macromolecular compounds obtained other than by reactions only involving carbon-to-carbon unsaturated bonds, e.g., polyesters, polyamino acids, polysiloxanes, polyphosphazines, copolymers of polyalkylene glycol, or poloxamers (. . .)
A61K47/48	• The non-active ingredient being chemically bound to the active ingredient, e.g., polymer drug conjugates (5)
A61K48/00	Medicinal preparations containing genetic material, which is inserted into cells of the living body to treat genetic diseases; Gene therapy

The final patent search results were obtained using the keywords (“liposome” and “drug delivery system” or “drug carrier” and “process” or “procedure” or “obtaining” and “method”) in the search fields: full text, title, abstract, and claims.

Using a combination of the keywords with the classification codes, according to the abovementioned search criteria, 6296 patent documents were found from 1971 to July 2024, graphically presented in Figure 2 (with data for the first 10 countries, applicants, and IPC subgroups).

The first patent application, DE 2249552, named: “Encapsulation of chemical substances—in liposomes for medicinal use”, claimed by a German company, Bayer AG (DE) (after the transmission of property from the Swiss company Inchema S.A., Wädenswil), was filed on 12 October 1971, published on 30 May 1973, and granted on 9 September 1976.

The patent application distribution shows a peak in the period 2016–2021, with more than 300 yearly, and 2021 is the year with the highest number of published patent applications, with 447 documents. Today, it seems to be decreasing, with “only” 19 records in the first 7 months of 2024. The United States is the country with the greatest number of priority patent applications filed in this technical field (3298), followed by WO patent applications (3150) using the international patent application system (PCT) administered by the WIPO [37], the Republic of China (2907), European Patent (EP; 2259), Japan (1636), Canada (1477), Australia (1343), the Republic of Korea (1008), Spain (570), and Brazil (436), as the first 10 countries. The largest number of patent applications come from universities (The University of Texas System (US), The Regents of the University of California (US), Shenyang Pharmaceutical University (CN), and Sichuan University (CN)) and companies (The Liposome Company, Inc. (US), Translate Bio Inc. (US), Alza Corp. (US), Yissum Research Development Company of the Hebrew University of Jerusalem Ltd. (IL), and L.E.A.F. Holdings Group LLC (US)), as the first ten applicants.

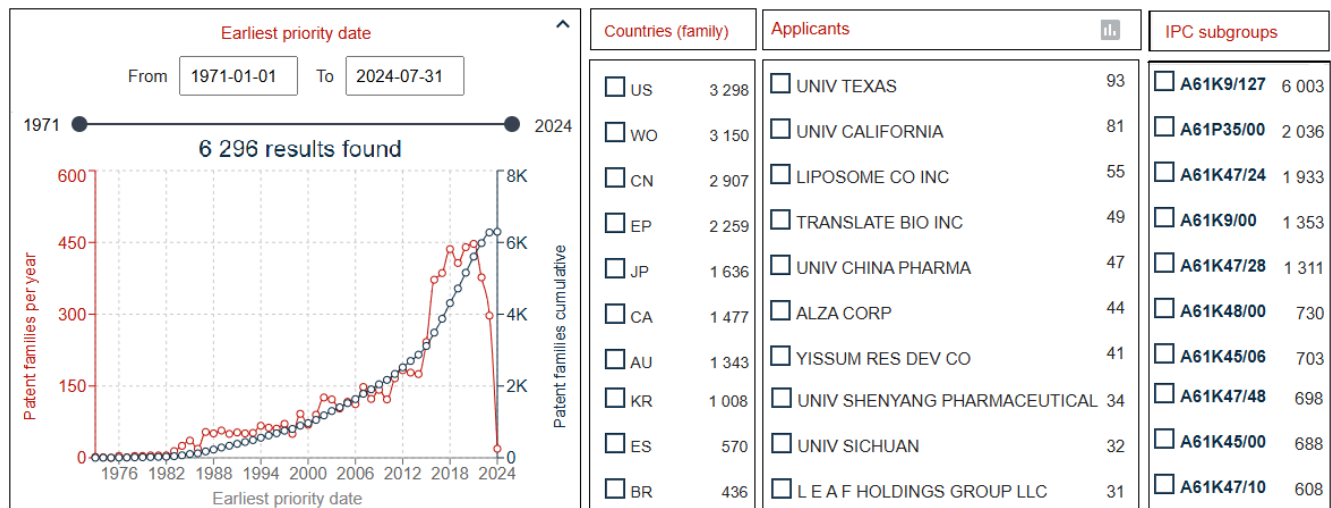


Figure 2. Liposome-based drug delivery systems: cumulative and yearly number of patent applications depending on earliest priority date, for the first ten countries, applicants, and IPC subgroups. Two-letter codes: US, WO, CN, EP, JP, CA, AU, KR, ES, and BR are abbreviations for the following countries: United States of America, the international publication of patent application using the Patent Cooperation Treaty (PCT) of the World Intellectual Property Organization (WIPO), Republic of China, European Patent, Japan, Canada, Australia, Republic of Korea, Spain, and Brazil, respectively. Data were obtained using the Espacenet database [32].

Searching criteria were narrowed to include only granted patents. During the time from the first application, a total of 2057 patents were granted in the period 1976 to July 2024, focusing on liposome drug delivery systems (Figure 2). Regarding countries, the Republic of China has the largest number of granted patents in this search criteria (2231), followed by Australia (1184), Japan, (1092), the Republic of Korea (793), and Taiwan Province of China (145), as the first five. The greatest number of granted patents also comes from universities (The University of Texas System (US), Shenyang Pharmaceutical University (CN), China Pharmaceutical University (CN), Zhejiang University (CN), The Regents of the University of California (US), and Shanghai Jiao Tong University (CN)) and companies (Translate Bio Inc. (US), The Liposome Company, Inc. (US), Shire Human Genetic Therapies, Inc. (US), Alza Corp. (US), and Insmed Inc. (US)), as the first 11 patent owners. The distribution shows a peak in the period 2016–2022 with more than 100 granted patents yearly, and the highest number of patents (186) was granted in 2018 (Figures 3 and 4), with a decreasing trend this year.

During the last ten years (from 2014 to 2024), 1306 patents were granted. The most numerous results and activities come from companies and universities in the Republic of China (Figure 4). This result suggests that the liposome-based drug delivery systems and correlated technologies are near the peak of research. By analyzing the obtained data, it was observed that many of the applications were based on liposomes and their application for medicinal preparations containing active ingredients. The most common were anti-cancer drugs, with a large and diverse class of medications. Data analysis indicated that the medicinal preparations containing genetic material (inserted into the cells of the living body) for genetic disease treatment, gene therapy, and mixtures of active ingredients were objects of patent protection. Also, objects of protection were drugs for specific purposes. Additional narrowed search criteria were included only for granted patents in specific fields, e.g., for different obtaining methods (classical or supercritical) or their pharmaceutical applications.

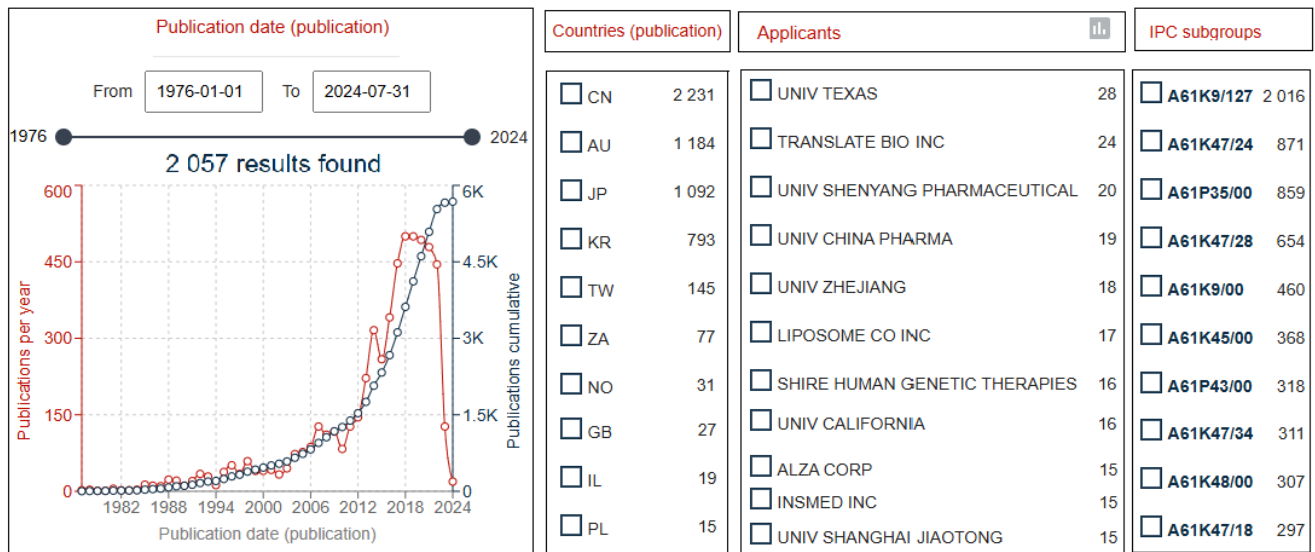


Figure 3. Liposome drug delivery systems: cumulative and yearly numbers of granted patents, depending on publication date, showing the first ten applicant origin countries, applicants, and IPC subgroups. Two-letter codes: CN, AU, JP, KR, TW, ZA, NO, GB, IL, and PL are abbreviations for the following countries: Republic of China, Australia, Japan, Republic of Korea, Taiwan Province of China, South Africa, Norway, United Kingdom, Israel, and Poland, respectively. Data were obtained using the Espacenet database [32].

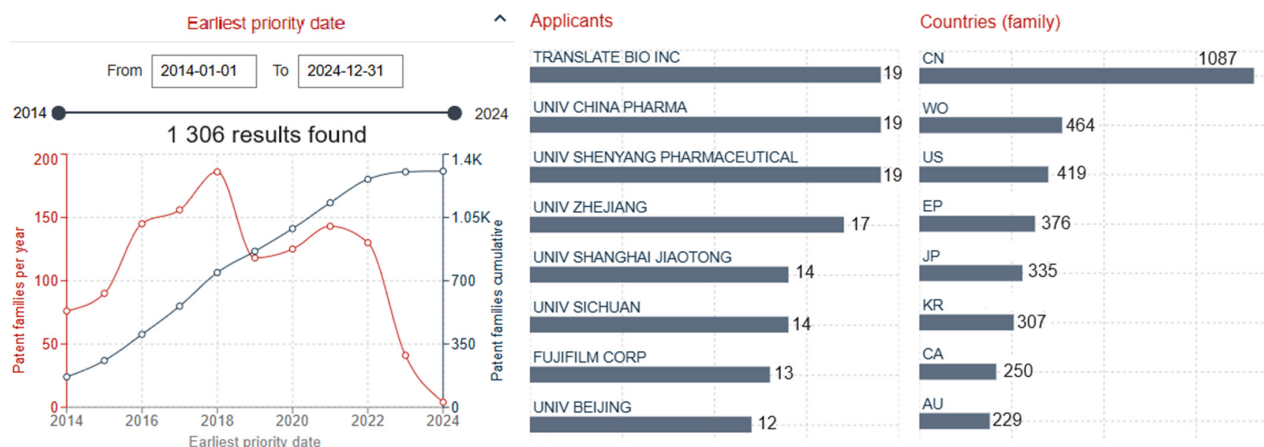


Figure 4. Liposome drug delivery systems: cumulative and yearly numbers of granted patents during the last ten years (from 2014 to 2024), depending on the earliest priority date, showing the first eight applicant origin countries and applicants. Two-letter codes: CN, WO, US, EP, JP, KR, CA, and AU are abbreviations for the countries: Republic of China, the international publication of patent application using the Patent Cooperation Treaty (PCT), United States of America, European Patent, Japan, Republic of Korea, Canada, and Australia, respectively. Data were obtained using the Espacenet database [32].

3. Processes for the Liposomes' Production

Engineering approaches for liposome formation were developed during the past five decades and include both “bottom-up” and “top-down” techniques (Figure 5 [19]).

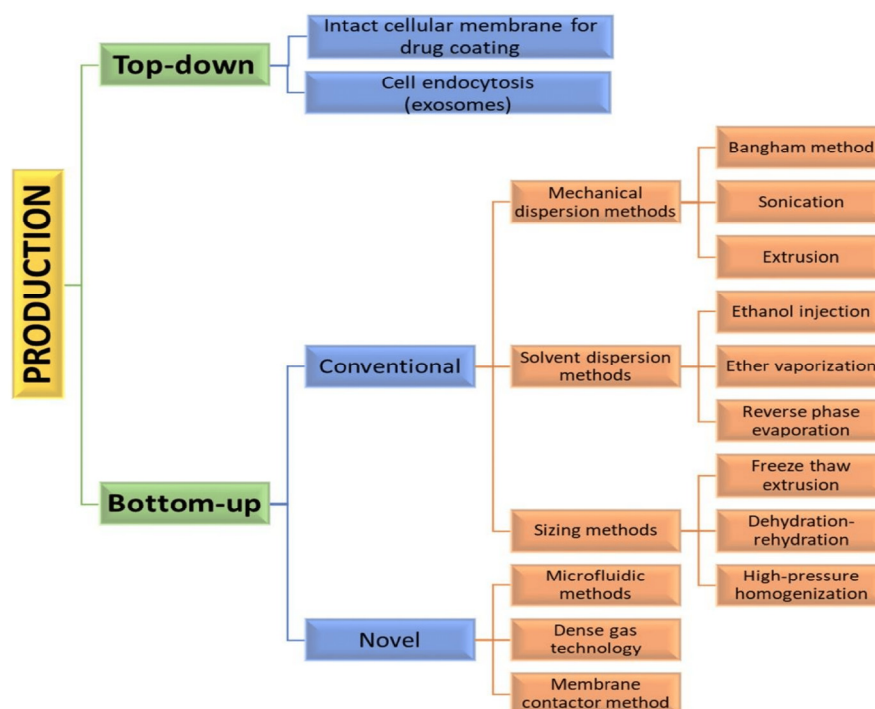


Figure 5. Diagram of various manufacturing engineering approaches and processes used for the synthesis of liposome vesicles. Reprinted from [19] under an open-access creative common CC-BY license.

The “bottom-up” strategy for liposome fabrication processes includes conventional methods (mechanical dispersion, solvent dispersion, or size-adjusting) or novel methods (microfluidic systems [38], dense gas technology, or membrane contactor methods). The conventional methods of mechanical dispersion include the well-known Bangham method (thin-layer dehydration rehydration), sonication, as well as the extrusion technique. With solvent dispersion methods, it is possible to apply ethanol injection, ether vaporization, or reverse-phase evaporation [39]. The size-adjusting conventional methods comprise improvement by applying freeze–thaw extrusion, dehydration–rehydration, and high-pressure homogenization techniques to obtain a narrower particle size distribution of liposomes. In general, all traditional procedures, which work at ambient temperature and pressure, lead to improvements with expensive post-processing stages, to obtain the nanometric dimensions of liposomes. However, these techniques have various disadvantages, e.g., low average encapsulation efficiencies of active ingredients (about 20–40%), loss of drugs, high residue of the solvent, problematic control of particle size distribution, large micrometric dimensions, expensive operating costs, and difficult repeatability in batch mode [40].

To overcome the problems related to conventional liposome production techniques, novel processes were proposed (microfluidic systems, dense gas technology, or membrane contactor methods). High-pressure systems, e.g., supercritical anti-solvent [41], reverse-phase supercritical evaporation [42], or depressurization of the expanded solution in an aqueous medium [43], were developed in semi-continuous formations to solve the mentioned problems. The crucial difference is the inversion of the traditional production phases so that the droplets of water were obtained first, and then they were rapidly encircled by phospholipids in supercritical conditions using high-pressure carbon (IV) oxide (in the range of 100–200 bar at 35–45 °C). By applying supercritical conditions, the encapsulation efficiency was increased by about 50–60%, with better particle size distribution control. A novel supercritical-assisted process was invented as a one-shot production method, with improved liposome stability over six months, excellent encapsulation efficacy of hydrophilic

or lipophilic compounds (up to 99%), and lessened residual solvent [44]. Inverted, solvent-free liposomes were formed, with a second layer of phospholipids around the first one.

The “top-down” strategy comprises a cellular membrane/biomembrane for the drug-coating technique or cell endocytosis (exosome membranes) [19].

Even though novel, efficient production processes of liposomes were developed, batch-mode techniques with mostly low repeatability and expensive raw materials were industrialized [45].

3.1. Overview of Patented Liposome Production Processes

The patent search in the Espacenet database was further narrowed by adding field IPC or CPC A61K9/127/low and A61K9/1277 keywords: “liposom”, “process”, “procedure”, “obtaining” or “method”, and “technique”, in the claim search field, according to the abovementioned production techniques, “bottom-up” or “top-down”. Selected granted patents for the liposome production processes, both conventional and novel, summarized in Tables 2–4, respectively, are briefly analyzed.

3.1.1. Conventional Liposome Production Processes

The advanced search in the Espacenet database was narrowed by adding keywords that describe conventional liposome production processes: “mechanical dispersion”, “solvent dispersion”, “size-adjusting”, “thin-layer hydration”, “sonication”, “extrusion”, “freeze–thaw extrusion”, “dehydration–rehydration”, and “high-pressure homogenization”. Also, keywords for novel processes: “microfluidic systems”, “dense gas technology”, “membrane contactor”, cellular membrane”, “biomembrane”, “cell endocytosis”, and “exosome membrane”, were excluded from the search criteria. As a search result, conventional liposome production processes were the subject of 518 patent documents, which included patent applications (“A” documents) and granted patents (“B” documents) from 1974 to July 2024 in the Espacenet database according to title, abstract, and claims, from which a total of 186 patents were granted (including filter “B” for publication number). After narrowing this analysis by excluding abstracts and titles in the query, 170 patents were found. During the last decade, 22 granted patents presented expansion with narrowing obtained results (publication date from 2014 to 2024). Finally, the search was narrowed to involve only granted patents with publication dates after 2020, and the documents were sorted by relevance. Several granted patents for conventional liposome production processes, selected by relevance and patenting levels, are summarized in Table 2 and briefly analyzed.

Table 2. Selected relevant patents for conventional liposome production processes.

Title	Publication No. [Reference]	Applicants	Earliest Priority
Encapsulation of chemical substances—in liposomes for medicinal use	FR2221122B1 [46]	Bayer AG	12 October 1971
Fusogenic liposomes and methods of making and using same	EP0758883B1 [47]	The Liposome Company, Inc.	12 April 1994
Liposome sensitive to pH or reductive condition and processes for the preparation thereof	KR100853172B1 [48]	Postech Acad Ind Found	4 April 2007
Improved lipid formulation	US8158601B2 [49]	Chen J.; Ansell S.; Akinc A; Dorkin J.R.; Qin X.; Cantley W.; Manoharan M.; Rajeev K.G.; Narayanannair J.K.; Jayaraman M.; Alnylam Pharmaceuticals, Inc.	10 June 2009

Table 2. Cont.

Title	Publication No. [Reference]	Applicants	Earliest Priority
Improved lipid formulation	US8802644B2 [50]	Chen J.; Ansell S.; Akinc A; Dorkin J.R.; Qin X.; Cantley W.; Manoharan M.; Rajeev K.G.; Narayanannair J.K.; Jayaraman M.; Tekmira Pharmaceuticals Corp	10 June 2009
Liposome formulation and manufacture	EP3135274B1 [51]	Biorest Ltd.	14 March 2013
Preparing method of proliposomes	KR101682821B1 [52]	Seoul National University R&DB Foundation	21 July 2015
Platinum-modified liposome as well as preparation method and application thereof	CN107550865B [53]	Guangzhou Heji Biological Tech Co., Ltd.	21 June 2017
Compositions and systems comprising transfection-competent vesicles free of organic solvents and detergents and methods related thereto	EP3864163B1 [54]	The University of British Columbia	9 October 2018
Alkannin active drug-loading liposome as well as preparation and application thereof	CN115105473B [55]	Suzhou Yutai Pharmaceutical Tech Co., Ltd.	19 Marc 2021
Hydrogel–liposome combined drug delivery system as well as preparation method and application thereof	CN115192581B [56]	The First Affiliated Hospital of Shandong First Medical Univ. Shandong Qianfoshan Hospital	21 July 2022
Preparation method and application of acid-response-type drug delivery platform based on liposome nano-vesicles	CN115337267B [57]	University of Science and Technology China	15 August 2022
Probucol liposome for hypercholesterolemia, preparation and preparation method of probucol liposome	CN115364056B [58]	Pingdingshan No. 2 People’s Hospital, Shangdong New Time Pharmaceutical Co., Ltd.	26 August 2022
Thermo-sensitive nano-liposome capable of realizing stepped release of active matters and application of thermo-sensitive nano-liposome	CN115590818B [59]	University of Jiangnan	9 September 2022
Alendronate sodium liposome and preparation thereof	CN116650418B [60]	Zhengzhou Central Hospital	5 June 2023
A stabilized nano-liposome delivery carrier comprising nucleic acid and a method for preparing the same	KR102678046B1 [61]	Binotec Co., Ltd.	22 October 2023
Anti-alopecia hair-strengthening nano-liposome and preparation method thereof	CN118001207B [62]	Wangshuhe Biomedical Wuhan Co., Ltd.	2 February 2024

The first patent, FR2221122B1, relates to the encapsulation of chemical substances in liposomes for medicinal use and liposomes’ preparation with a maximum diameter of 1000 Å, preferably from 200 to 500 Å [46]. The liposome walls consist of mono-, bi-, or multi-molecular layers, preferably bimolecular with a thickness of 30–100 Å. Two simple methods were used for the liposome preparation. In the first method, a lipid was added to the aqueous phase, the mixture was slightly heated, then energetically shaken, followed by sonication until liposomes were formed, manifested by partial illumination and a slight

bluish opalescence appearance. In the second method, the dispersion system, as a linear colloid, was obtained most simply by transforming the dispersed phase into thin films, evaporating the solvent, and bringing these films into contact with a continuous aqueous or non-aqueous phase, with vigorous mixing and treating with ultrasound for a long period.

Fusogenic liposomes' composition, production methods, and applications were disclosed in patent EP0758883B1 [47]. They were obtained according to known methods by drying chloroform solutions of lipids under nitrogen, then removal of the residual solvent under a high vacuum. Formed lipid films were hydrated by vortex-mixing with buffers to produce multilamellar liposomes (MLVs), and five freeze–thaw cycles were used to achieve homogeneous mixtures. The MLVs were extruded ten times to make large unilamellar liposomes. Composition was comprised of a liposome with (i) an outermost lipid bilayer comprising a fusion-promoting ionizable lipid with a cationic protonatable headgroup and unsaturated acyl chain, and (ii) a section contiguous to the terminal lipid bilayer, which comprised an aqueous solute with the first pH. The first pH was lower than the pKa of the ionizable lipid in an outermost lipid bilayer. Outside of the liposome, in the structure there was an aqueous solution with the second pH (bigger than the pKa of the ionizable lipid in the outermost lipid bilayer), such that there was a pH gradient across the outermost lipid bilayer. An ionizable lipid was accumulated in the inner monolayer of the outermost lipid bilayer in response to the gradient.

The proliposome preparation method by patent KR101682821B1 comprised of the following steps: (1) the first solution was prepared by dissolving a sugar-based carrier in water, (2) the second solution was prepared by dissolving a bioactive ingredient, lipids, and a stabilizer in an organic solvent, (3) the first and the second solutions were mixed and condensation-stirred to a uniform consistency, and (4) the condensation-stirred mixed solution was freeze-dried [52]. The condensation-stirring method could significantly reduce the excipients amount, compared to conventional proliposomes. The obtained proliposome can enhance the bioavailability of the encapsulated insoluble drug by promoting intestinal elution and absorption.

Korean patent KR100853172B1 disclosed pH-sensitive liposomes, used as a desirable drug delivery system based on a self-assembling cucurbituril derivative, stable in blood, and processes for their preparation, wherein the dispersing step is performed by ultrasonic wave using a sonication method [48]. The obtained pH-sensitive liposomes disintegrated after absorption in the cell, allowing the drug to act on a tissue of a desired target portion, while preventing side effects of a systemic drug by encapsulating.

The granted US patents US8158601B2 and US8802644B2 relate to the cationic lipids, and lipid particles comprising these lipid formulations are prepared by an extrusion method [49,50]. Moreover, these patents provide production methods of these compositions, and methods of introducing nucleic acids into cells through obtained compositions, e.g., for treatments of various disease conditions. The invention presented in the EP3135274B1 patent relates to a novel process for obtaining liposomal formulations of uniform size with desirable, independently controllable features [51]. This formulation for use in the prevention of restenosis was manufactured via a method of extruding the vesicles, consisting essentially of repeatedly extruding through a single filter 10–18 times before ultrafiltration of the vesicles. Compositions and systems comprising transfection-competent vesicles free of organic solvents and detergents, and production methods via extrusion, were disclosed in the granted patent EP3864163B1 [54]. An alkannin/shikonin active drug-loading liposome (CN115105473B) was prepared by dissolving phospholipids, cholesterol, and PEGylated phospholipids in a volatile organic solvent, evaporating the organic solvent under reduced pressure to form a dry lipid film, adding copper ion salt solution, and performing hydration treatment and extrusion to reduce the particle size of the liposomes to form nano-sized liposomes [55]. After that, agarose gel chromatography columns were used to exchange the extracellular aqueous phase of the liposomes, the blank liposomes were incubated with the dimethyl sulfoxide solution of shikonin at 55–65 °C for 30 min, and then they were cooled for 5 min. The shikonin active drug-carrying liposome can be

applied in the preparation of antitumor drugs. CN115337267B protects the pH-responsive lipid nanovesicles that are prepared by using acidic phospholipids, acid-sensitive silyl ether prodrug Chol-R848, and 1,2-distearoyl-rac-glycero-3-PE-N-polyethyleneglycol-2000 (DSPE-mPEG2000) as raw materials through film hydration and extrusion [57].

The specific preparation method to obtain a hydrogel-liposome combined drug delivery system includes dissolving hydrogenated soybean phosphatidylcholine, cholesterol, cyclic arginine-glycine-aspartic acid-modified DSPE-PEG, and elastane in an organic solvent, obtaining crude liposomes after sonication, and mixing the crude liposomes with an aqueous solution of temozolomide (TMZ) to form an emulsion. After removing the organic solvent, the liposome was processed by ultrasonic wave and extrusion (CN115192581B) [56]. Probucol liposome for hypercholesterolemia (CN115364056B) [58] was prepared via several steps, which included (1) preparation of the initial mixture of phospholipids, cholesterol, polysorbate, and probucol, (2) the sonication step and formation of liposomes of 30–80 nm by the French extrusion method, and (3) freeze-drying to obtain the final product.

The CN107550865B patent describes a high-pressure homogenization method at a frequency of 500–1500 bar and 20–60 Hz for preparing a liposome, wherein the phospholipid and the platinum-containing aqueous solvent are used in a weight ratio of 1:30 to 80 [53]. The liposome obtained by the invention has excellent stability and can be stored for a long time, while still maintaining a small particle size. Granted patent KR102678046B1 [61] described a stabilized nano-liposome as a carrier for encapsulated nucleic acid delivery obtained by the high-pressure homogenization method. A thermo-sensitive nano-liposome capable of realizing stepped release of active matters (CN115590818B) [59], anti-alopecia hair-strengthening nano-liposome (CN118001207B) [62], and alendronate sodium liposome (CN116650418B) [60] were also prepared using a high-pressure homogenization method.

Analysis of the granted patents showed that sonication, extrusion, dehydration–rehydration, and high-pressure homogenization methods were most usually applied for novel pharmaceutical applications.

3.1.2. Novel Liposome Production Processes

New technologies have been developed to support less scalable liposome production technologies, including self-assembling liposome systems and microfluidic production.

Microfluidic methods allow precise control of many factors during preparation, e.g., liposome size distribution and fluids in a constrained volume (by laminar flow and lipid concentrations in microfluidic channels), pH, temperature, vesicle size, salinity, and osmolarity [63]. This is an effective drug encapsulation method to achieve self-assembled liposomes. Besides the advantages of this simple and low-cost method, the main disadvantages are unsuitability for bulk production and difficulty in removal of organic solvents. In the membrane contactor method, a dissolved lipid phase in alcohol was pushed throughout a porous membrane in an aqueous phase flow, from where lipid molecules were self-assembled in homogenous-sized liposomes [64]. Dense gas technology is one more novel method for liposome preparation, which utilizes supercritical fluids (e.g., supercritical carbon dioxide) as excellent solvents for many lipids. After mixing with the water phase, liposomes with a narrow size distribution are synthesized [65].

Search criteria in the Espacenet database were carried out using keywords that describe novel processes: “microfluidic systems”, “dense gas technology”, “membrane contactor”, “cellular membrane”, “biomembrane”, “cell endocytosis”, and “exosome membrane”. All previously mentioned keywords for conventional liposome production processes (Section 3.1.1) were excluded from the search criteria. Novel liposome production processes were the subject of 661 patent documents (patent applications and granted patents) from 1973 to July 2024 according to the title, abstract, and claims, and 195 patents were granted therefrom. Throughout the last 10 years, 431 patents were found, and after narrowing this result by claims only in the query, 82 granted patents were found. A narrowed search included only granted patents chosen by relevance. Selected granted patents for

novel liposome production processes are summarized in Tables 3 and 4, respectively, and briefly described.

Table 3. Selected relevant patents for novel liposome production processes.

Title	Publication No. [Reference]	Applicants	Earliest Priority
Novel functionalized liposomes and a process for production thereof	EP0247497B1 [66]	Wako Pure Chemical Industries, Ltd.	20 May 1986
Synergistic liposomal formulation for the treatment of cancer	EP3046542B1 [67]	Council of Scientific and Industrial Research	18 September 2014
Liposome compositions and methods of use thereof	EP2603201B1 [68]	Rhode Island Board of Governors for Higher Education; Yale University, Inc.	12 August 2011
Targeted hybrid exosome loaded with curcumin and miR140 as well as preparation method and application of targeted hybrid exosome	CN117965429B [69]	Sichuan University	29 March 2024
Hybridosomes, compositions comprising the same, processes for their production, and uses thereof	US10561610B2 [70]	Anjarium Biosciences AG	20 January 2015
Hybridosomes, compositions comprising the same, processes for their production, and uses thereof	US11484500B2 [71]	Anjarium Biosciences AG	2 January 2020
Process for the production of hybridosomes	EP3096741B1 [72]	Anjarium Biosciences AG	21 January 2014
Preparation method of exosome bionic preparation for synergistically promoting wound healing and preparation thereof	CN113577272B [73]	China Pharmaceutical University	28 July 2021
Co-loaded liposome and preparation method thereof	CN116270473B [74]	Chengdu Jinrui Found Biotechnology Co., Ltd.	25 May 2023
A method for preparing a functional synthetic cell in form of a giant unilamellar vesicle	EP3630068B1 [75]	Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V.	16 June 2017
PEG2, N-lipid derivative-modified nano-carrier, preparation method and application	CN113350512B [76]	Shenyang Pharmaceutical University	7 June 2021
Compound responding to endocytosis and release and application thereof	CN113683769B [77]	Shanghai Jiao Tong University	19 May 2020
All-trans retinoic acid liposome preparation and preparation and application thereof	EP3501500B1 [78]	Shanghai Jiao Tong University	18 August 2016
Granular-type adjuvant as well as preparation method and application thereof	CN108324938B [79]	Institute of Process Engineering, Chinese Academy of Sciences	1 March 2018
mRNA–liposome complex and application thereof	CN112107680B [80]	Zhejiang Zhida Pharmaceutical Co., Ltd.	21 June 2019
Drug delivery vector and pharmaceutical formulation	CN114007653B [81]	Fudan University; JSR Corporation	5 May 2019

Novel functionalized liposomes containing a high-molecular-weight amphiphilic compound, as one of the matrix materials, were obtained by the reverse-phase evaporation method, described in patent EP0247497B1 [66]. They have a very high encapsulation

efficiency and readily undergo lysis. An immunological substance (antigens, antibodies, etc.) or a physiologically active substance could be immobilized on the liposomes efficiently, with a sufficient binding rate, using the amphiphilic compound as a spacer.

The synergistic liposomal formulation for use in the treatment of cancer is prepared by reverse-phase evaporation vesicles or multilamellar vesicles (EP3046542B1) [67]. The reverse-phase evaporation technique encapsulates up to 50% of solute (the first to use ‘water-in-oil’ emulsions). The preparation method involves a rapid injection of aqueous solution into an organic solvent containing dissolved lipids. After water droplet formation (‘water-in-oil’ emulsion) by bath sonication of the two-phase mixture, the emulsion is dried down to a semi-solid gel in a rotary evaporator. The following step is vigorous mechanical shaking of the gel to induce a phase change from a “water-in-oil” emulsion to a vesicle suspension. Some water droplets collapse, attach to adjacent, intact vesicles, and form the outer leaflet of the bilayer of a large unilamellar liposome (0.1–1 μm diameter).

The invention is based on the discovery that pH (Low) Insertion Peptide (pHLIP) liposomes target acidic tissue, and release liposome content, i.e., cargo, into a cell (EP2603201B1) [68]. In the method of delivering cargo into a target cell (tumor cell, ischemic cell, inflamed cell, bacterially infected cell, fungus-infected cell, or virally infected cell), pHLIP+ liposome both fuses with a cell membrane of an endosomal compartment of a target cell and is taken up by cell endocytosis.

The granted Korean patent CN117965429B disclosed a targeted hybrid exosome loaded with microRNA 140 (miR140) and curcumin [69]. The obtaining method comprises the following phases: (1) obtaining a cell strain capable of stably expressing chondrocyte-targeted peptide, (2) addition of miR-140, carrying out co-culture, separation, and extraction to obtain an exosome loaded with miR-140-5p, (3) addition of the curcumin into the cholesterol and soybean lecithin solutions, addition of buffer solution, and ultrasonication after rotary evaporation to form a curcumin-loaded liposome buffer solution, adding sucrose, and (4) the exosome obtained in phase 2 and the solution obtained in phase 3 were subjected to a freezing circulation method, and the targeted hybrid exosome was obtained.

Granted patents US10561610B2, US11484500B2, and EP3096741B1 describe hybrid biocompatible carriers (hybridosomes). The process for manufacturing hybridosomes, hybrid biocompatible carriers, is comprised of contact and uniting of a first vesicle and a second vesicle [70–72]. The first vesicle, produced in vitro, comprises a membrane, a therapeutic agent, and a fusogenic, ionizable, cationic lipid at a molar concentration of at least 30% of the total lipid of the first vesicle. The second vesicle, produced in vivo, comprises a lipid bilayer and is released into the extracellular environment. These inventions further provided pharmaceutical compositions comprising pharmaceutical applications and appropriated pharmaceutical methods.

The other example is an exosome bionic preparation, which synergistically promotes wound healing, prepared by the method described in Chinese patent CN113577272B [73]. This procedure comprises several steps: preparing a catalase-photosensitizer micelle, preparing an reactive oxygen species (ROS) response liposome carrying the catalase-photosensitizer micelle, extracting, separating, and purifying the exosome, mixing the exosome with the liposome, mediating membrane fusion between the liposome and exosome using the extrusion method, mixing the membrane fusion carrier and the gel, and homogenously stirring the mixture to prepare the final product.

The invention CN116270473B relates to adopting a film dispersion method of a co-loaded liposome preparation, which comprises 5–35% magnolol and the sum of honokiol (as active pharmaceutical ingredients), 50–80% phospholipid, 8–15% cholesterol, 3–10% polyethylene glycol or PEGylated phospholipid, 1–5% cholesterol, and 1–5% polyethylene glycol [74]. The raw materials and the auxiliary materials are mixed and then dissolved with an organic solvent, rotary evaporation is performed to form a film, and after that, hydration and homogenization are performed to obtain the co-loaded liposome. The encapsulation efficacy reaches 90% or above, the particle size distribution is uniform, and the stability of the preparation is remarkably improved.

European patent EP3630068B1 outlines a preparation method for a protocell in the form of a giant unilamellar vesicle by a microfluidic device or bulk technique by adding destabilizing molecules. It comprises the following stages: (1) forming a water-based droplet (0.5–1000 µm) encapsulated by a surface polymer shell, which borders the inner space of the droplet, containing at least one lipid, (2) transforming the droplet lipid content into a lipid bilayer, which covers the inner surface of the polymer shell and oil phase to form a polymer-shell-stabilized giant unilamellar vesicle, (3) alternatively, incorporating one or more nuclei and/or proteins into the polymer-shell-stabilized giant unilamellar vesicle, and (4) alternatively, removing the polymer shell and oil phase from the polymer-shell-stabilized giant unilamellar vesicle and transferring it from the oil to the water phase [75].

The PEG2, N-lipid derivative-modified nano-carrier, was prepared using a method described in patent CN113350512B, capable of eliminating an ABC phenomenon caused by a PEGylation nano-carrier [76]. PEG2 forms a compact hydration layer on the carrier surface, with improved physical and biological stabilities. In addition, this novel nano-carrier overcame insufficient circulation time defects of many PEG substitute materials, and a firmer foundation was laid for its clinical transformation.

A compound having a response to the endocytic release of cells, and its usage, were described in the patent CN113683769B [77]. This compound was composed of a lipophilic head, a hydrophilic chain, and a group having a response to the pH. The modified complex is stable in body fluid circulation and has a high transfection efficacy.

The invention EP3501500B1 relates to an all-trans retinoic acid liposome and a liposome vector, its preparation, and its application [78]. The all-trans retinoic acid liposome preparation obtained through this active drug-loading method significantly improves the plasma drug concentration in all-trans retinoic acid liposome and prolongs the half-life. The granular-type adjuvant was prepared from a biocompatible wall material, biocompatible grease, and all-trans retinoic acid according to patent CN108324938B [79]. The biocompatible wall material includes a biocompatible polymer poly(lactic-co-glycolic acid (PLGA) and liposomes, and the mass percentage of the cationic liposome in the biocompatible wall material is less than or equal to 80%. An inner core formed by the biocompatible grease was covered in a shell, formed by the biocompatible wall material. All-trans retinoic acid was coated in the inner core. Systemic and mucosal immunizations were activated, and a dual-activation effect was realized, according to this invention.

According to patent CN112107680B [80], the method of obtaining the messenger ribonucleic acid (mRNA) and liposome complex comprised diluting the mRNA, adding the liposome, or preparing it in a continuous online mixing mode, or using a microfluidic mixing system.

The Chinese granted patent CN114007653B described a drug delivery vector containing a membrane-penetrating peptide-modified cationic liposome with 1,2-dioleoyl-3-trimethylammonium-propane (cationic lipid), 1,2-di-(9Z-octadecanoyl)-sn-glycerol-3-phosphoethanolamine (non-cationic lipid), cholesterol, and polyethylene glycol phospholipids conjugated to membrane-penetrating peptides (PEGylated phospholipids) [81].

Table 4 highlights a few granted patents for novel supercritical-assisted processes.

Table 4. Selected patents for supercritical-assisted liposome production processes.

Patent Title	Publication No. [Reference]	Applicants	Earliest Priority
A delivery device	EP3334413B1 [82]	PTT Holding Aps	11 August 2015
Pharmaceutical composition, preparation, and uses thereof	EP3236934B1 [83]	Curadigm SAS	25 November 2014
Method for preparing medicinal extract containing intermediate peashrub seeds	CN104382984B [84]	Northwest Institute of Plateau Biology Chinese Academy of Sciences	1 December 2014

Table 4. Cont.

Patent Title	Publication No. [Reference]	Applicants	Earliest Priority
Preparation method of temperature and fluorescence probe of liposome loaded with gold nanocluster and anti-cancer drug	CN103599070B [85]	Shanghai Jiao Tong University	26 November 2013
Antitumor liposome formulation and method for producing the same	JP4715133B2 [86]	Konica Minolta Med and Graphic	26 August 2004
Method for producing liposome-containing formulation and liposome-containing formulation	JP4599849B2 [87]	Konica Minolta Med and Graphic	18 February 2004
Method for producing liposome-containing formulation and liposome-containing formulation	JP4649841B2 [88]	Konica Minolta Med and Graphic	18 February 2004

The invention EP3334413B1 relates to a delivery device suitable for delivering a chemical substance, e.g., a medical device, such as a catheter, a microcapsule, an implantable capsule, or a P-ring [84]. This device is formed by loading a drug into the matrix using a solvent in its supercritical, near supercritical, or dense gas states. A preferred drug carrier includes CO₂ in a liquid and/or supercritical state. The delivery device for the chemical substance comprises a drug (for treating symptoms of Alzheimer's, e.g., dual agonist of the peroxisome proliferator activated nuclear receptor delta/gamma, aka PPAR δ / γ (T3D-959), donepezil, galantamine, memantine, rivastigmine, or any combinations thereof) trapped in liposomes. Together with the metal particles, the liposomes are dispersed in a polar liquid, e.g., an aqueous liquid.

The invention EP3236934 (B1) relates to a pharmaceutical composition comprising the combination of at least one (i) biocompatible nanoparticle and (ii) carrier comprising at least one pharmaceutical compound, a polymer selected from dextran, polysialic acid, hyaluronic acid, chitosan, heparin, polyvinyl pyrrolidone, polyvinyl alcohol, polyacrylamide, or poly(ethylene glycol), and a PEG-based copolymer [85]. The carrier can respond to an intracellular or extracellular stimulus when it is exposed to electromagnetic radiations, ultrasounds, and a magnetic field.

The drug-containing liposome nanoparticles and medicinal extract of *Caragana korshinskii* seeds were prepared by the patent CN104382984B [84]. The *Caragana korshinskii* seed extract, with membrane material, cholesterol, lecithin, and deionized water, at a temperature of 35–40 °C and a pressure of 25–30 MPa, was dispersed and mixed in supercritical CO₂ medium for 35–45 min. The mass ratio of lecithin and cholesterol was 2:1. The mass ratio of the film material and the seed medicinal extract was 3:1.

The object of patent CN103599070B is the preparation method of fluorescence and temperature probes of liposome membrane, loaded with the water-soluble anti-cancer drug and gold nanocluster dispersion [85]. They are incubated by a supercritical CO₂ method at a defined temperature and pressure.

A highly safe antitumor liposome formulation with a stabilized structure, affording retention stability of an anti-cancerous compound and achieving efficient delivery of an anti-cancer agent, was described in Japanese patent JP4715133B2 [88]. The liposome with an average particle size diameter of 0.05 to 0.7 μ m was produced by mixing the lipid membrane components, constituting the phospholipid membrane, with carbon dioxide in a supercritical or subcritical state, in the presence of at least one compound having a polyethylene glycol (PEG) group.

Patent JP4599849B2 provides a production method for liposome-containing formulations, with improved content of an enclosed substance in liposomes under supercritical conditions [89]. A mixed solution of supercritical carbon dioxide, an aqueous solution con-

taining an encapsulating substance and liposome suspension, was subjected to a heat and pressure treatment (115–135 °C and 0.17–0.31 MPa). The method includes the encapsulated anti-cancer substance or a contrast-enhancing compound.

The JP4649841B2 patent relates to a method for producing a liposome-containing preparation consisting of a micronized liposome with a high encapsulation rate of an encapsulated substance [90]. In the described effective method for their production, even though the main solvent is supercritical carbon dioxide, organic solvent (as a solvent aid) addition is unavoidable, and the organic solvent tends to lower the film strength. The obtained preparation can be applied to internal medicines, external preparations for skin, cosmetics, and contrast agents, and similar applications.

Inventive designs of novel liposomal production processes contribute to drug activity optimization *in vitro* and, consequently, *in vivo*. Published patents showed new possibilities to develop stable liposomes, which exhibited excellent abilities as drug carriers. Novel liposome preparation processes developed in the laboratory are not easily translated to the semi-industrial and industrial levels. This may slow down the further development and application of new liposome systems.

4. Liposome-Based Drug Delivery Systems

Any drug delivery system aims to beneficially modulate the pharmacokinetics parameters of the drug and/or distribution on the tissue. Nanoparticles as drug carriers show kinetically slow transportation in diseased tissues due to vascular barriers. A relatively long circulation time is needed to extend the time of crossing the vascular wall [7]. Liposome-based drug carriers have become increasingly important due to their ability to protect drugs and improve delivery across biological barriers. A key aspect is their unique structure that allows them to encapsulate both hydrophilic and hydrophobic drugs. Liposomes pass through biological barriers, of which the blood–brain barrier is one of the most challenging because it protects the brain from potentially harmful substances, but also limits the entry of many drugs. Mechanisms for overcoming biological barriers include surface functionalization with ligands or antibodies that bind to specific receptors on target cells. Stimuli-responsive liposomes overcame biological barriers by releasing the drug in response to specific stimuli in the body (temperature, pH, light, concentration, and pressure). Stealth liposomes have a central role in improving pharmacokinetics (biodistribution, blood circulation, and tissue targeting) [7]. The stealth effect shows the ability of nanoparticles to be invisible to the immune system, especially the reticuloendothelial system (RES), as a result leading to lessening clearance and increased retention time in the bloodstream. The most important effect of liposome PEGylation is shifting blood pharmacokinetics from non-linear saturable to linear non-saturable kinetics [7,89,90]. For non-linear kinetics, the saturation can happen during any kinetic clearance processes; however, it is generally in correlation with saturating RES clearance, thus bringing a net effect of the RES-blockade [91]. Due to the RES-blockade, non-PEGylated liposomes exhibit biphasic clearance (short α -phase, followed by a relatively long β -phase) until the PEGylated liposomes tend to exhibit a long half-life monophasic clearance. Furthermore, monophasic clearance of PEGylated liposomes exhibits a constant clearance rate, dose-independent, with above-wide dosage ranges. The analysis demonstrated that 85% of the reported stealth nanomaterials/drug delivery systems encounter a sharp α -phase clearance in blood circulation (a rapid drop in blood concentration to half of the administered dose within 1 h post-administration), while a relatively long β -phase could occur. The term pseudo-stealth effect describes this common pharmacokinetics behavior. The stealth effect and pseudo-stealth effect improve pharmacokinetics [7]. It is important to keep a good balance between the stealth effect and the interaction with diseased tissue. Dynamic modulation of the stealth effect throughout a stimuli-responsive strategy can further advance the functionality and increase the therapeutic efficacy [91].

Analysis of the obtained results from the Espacenet database search showed numerous applications based on liposomes with encapsulated medicinal active ingredients. The patent search for liposome-based carriers was further narrowed by adding keywords for specific fields, e.g., “anti-cancer”, “pulmonary”, “ocular”, “RNA”, “neural”, “orthopedic”, “internal”, “anti-inflammation”, and “dermal”. Selected patents for liposome-based carriers are summarized in Tables 5–10 and subsequently analyzed.

4.1. Liposome-Based Drug Carriers for Cancer Treatments

Keywords “anti-cancer”, “cancer”, “tumor”, and “tumour” were added to the search criteria. A total of 1822 patent documents (applications and granted patents) from 1983 to July 2024 according to the title, abstract, and claims, and 637 granted patents, were found. Obtained data are graphically presented in Figure 6 as cumulative and yearly numbers of patent applications and granted patents, the first seven applicant origins and countries, and the IPC/CPC subgroups for granted patents. During the last 10 years, 476 patents were granted. Table 5 highlights the selected granted patents for liposome-based drug carriers used in drug delivery for cancer treatments, chosen by relevance.

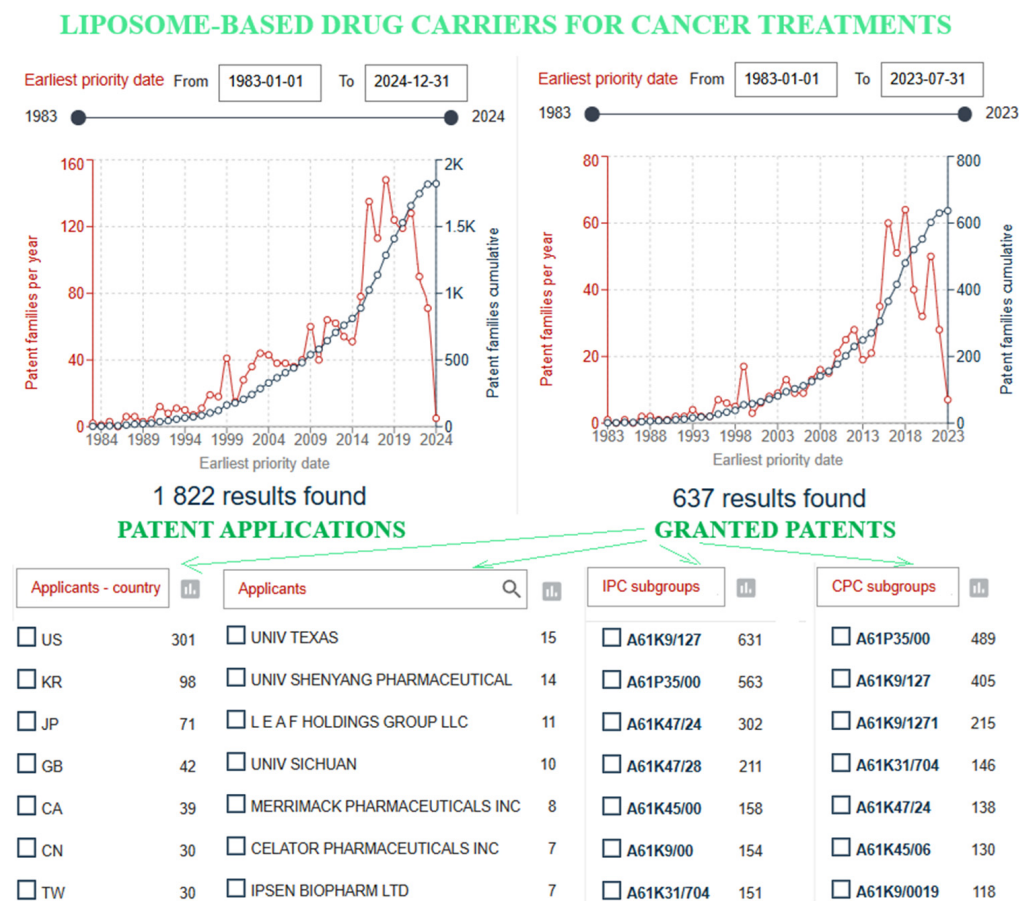


Figure 6. Liposome-based drug carriers for cancer treatments: cumulative and yearly numbers of patent applications and granted patents depending on the earliest priority date (from 1983 to 2024), the first seven applicant origins and countries, and the IPC and CPC subgroups for granted patents. Two-letter codes: US, KR, JP, GB, CA, CN, and TW, are abbreviations for the following: United States of America, Republic of Korea, Japan, Canada, United Kingdom, Republic of China, and Taiwan Province of China, respectively. Data were obtained using the Espacenet database [32].

Table 5. Selected granted patents for liposome-based drug carriers for cancer treatments.

Patent Title	Publication No. [Reference]	Applicants	Earliest Priority
Controlled drug release liposome composition	EP2968146B1 [92]	Taiwan Liposome Company Ltd.; TLC	15 March 2013
Method of reconstituting liposomal annamycin	US11980634B2 [93]	Board of Regents, The University of Texas System	28 June 2019
Protamine short-peptide-modified paclitaxel liposome and preparation method thereof	NL2033676B1 [94]	Institute of Biological Resources, Jiangxi Academy of Sciences; Anhui Zishanyuan Ecological Agricultural Technology Co., Ltd.	6 December 2022
Paclitaxel liposome composition for treatment of cancer and preparation thereof	EP1332755B1 [95]	Nanjing Zhenzhong Bioengineering Comp. Ltd.	19 October 2000
Liposome of irinotecan or its hydrochloride and preparation method thereof	EP2508170B1 [96]	Jiangsu Hengrui Medicine Co., Ltd.; Shanghai Hengrui Pharmaceuticals Co., Ltd.	3 December 2009
Irinotecan liposome preparation and preparation and application thereof	CN109260155B [97]	HighField BioPharmaceutical Corporation	5 November 2018
Pegylated lipid, liposome modified by pegylated lipid, pharmaceutical composition containing liposome, and preparation and application of pharmaceutical composition	CN115197078B [98]	Xiamen Sinopeg Biotech Co., Ltd.	8 April 2021
Liposomes with ginsenoside as membrane material and preparations and use thereof	EP3337456B1 [99]	Shanghai Ginposome Pharmatech Co., Ltd.	19 August 2015
Dianhydrogalactitol, diacetyldianhydrogalactitol or dibromodulcitol to treat non-small-cell carcinoma of the lung and ovarian cancer	EP3125920B1 [100]	Del Mar Pharmaceuticals Inc.	4 April 2014
Multicellular targeting liposome	JP7470418B2 [101]	Shanghai Jiao Tong University	14 September 2017
Tumor-targeted therapeutic drug carrier as well as preparation method and application thereof	CN102133404B [102]	Chengdu Nuoen Biological Technology Co., Ltd.	23 March 2011
Application of redox-responsive chitosan-liposome	CN107982547B [103]	University Dalian Minzu	27 July 2017
Pharmaceutical composition comprising jasmonates	KR101751918B1 [104]	Fehr Pereira Lopes, Jose E.	15 July 2008
Liposomal delivery of vitamin-E-based compounds	AU2002361812B2 [105]	Research Development Foundation	19 December 2001
Liposomes useful for drug delivery	US8147867B2 [106]	Hermes Biosciences Inc.	3 May 2004
Liposomes useful for drug delivery	US8703181B2 [107]	Merrimack Pharmaceuticals	3 May 2004
Liposomes useful for drug delivery	US9782349B2 [108]	Ipsen Biopharma Ltd.	11 December 2015
Preparation method of temperature and fluorescence probe of liposome loaded with gold nanocluster and anti-cancer drug	CN103599070B [85]	Shanghai Jiao Tong University	26 November 2013

Table 5. Cont.

Patent Title	Publication No. [Reference]	Applicants	Earliest Priority
Granular-type adjuvant as well as preparation method and application thereof	CN108324938B [79]	Institute of Process Engineering, Chinese Academy of Sciences	1 March 2018
Interleukin-2 variant proteins fused to human IgG4 Fc and uses thereof	US11896648B2 [109]	Gilead Sciences, Inc.	22 October 2020

The pharmaceutical composition for inhibiting the cancer cell growth, according to patent EP2968146B1, comprised: (1) at least one liposome having a particle-forming component, selected from (i) phospholipid and (ii) a mixture of at least one phospholipid and cholesterol, (2) dextran sulfate or a pharmaceutically acceptable salt, (3) ammonium sulfate, and (4) a vinca alkaloid [92]. The method of reconstituting pre-liposomal annamycin, a cancer chemotherapeutic agent, lyophilized to form a liposomal annamycin suspension, was presented in patent US11980634B2 [93].

The disclosure in patent NL2033676B1 provides protamine, short-peptide-modified paclitaxel liposome, pharmaceutical formulations, and their preparation method [94]. The method includes mixing paclitaxel, lecithin, and cholesterol, dissolving the mixture in a solvent, and forming a film by removing the solvent after sterilization, then washing the film with an aqueous glucose solution. Paclitaxel liposome suspension was obtained by homogenization and lyophilized as a powder by freeze-drying. Protamine peptide combined with liposome increases the water solubility of paclitaxel, increases paclitaxel content in cells, and significantly reduces the vascular density in tumors, which has antitumor effects.

The other patent, EP1332755B1 [95], disclosed a paclitaxel-based liposome composition for the treatment of cancer, which consists of paclitaxel (2–5 parts), phosphatide (20–200 parts), cholesterol (2–30 parts), amino acids (0.3–4 parts), and lyophilized excipient (10–75 parts). According to this invention, the products do not contain polyoxyethylated castor oil, the toxic and expensive adjuvant, which was substituted with nontoxic media and an easily obtained adjuvant. This invention has low toxicity, better stability, good water solubility, and good patient tolerance, and can be realized in the industry.

The object of patent EP2508170B1 was the liposome comprising irinotecan or its hydrochloride (widely used in the treatment of malignant tumor), neutral phospholipid, and cholesterol, wherein the weight ratio of cholesterol to neutral phospholipid was 1:3–5. Said liposome was prepared using the ion gradient method [96].

The irinotecan liposome preparation provided by the invention CN109260155B includes monovalent sulfonate and disulfonate as internal water phases, and stably encapsulates irinotecan in the form of insoluble sulfonate and disulfonate in the aqueous phase of the lipid body and obtains a good sustained-release effect [97]. The irinotecan liposome preparation has the advantages of a high drug loading amount, easy preparation, good biocompatibility and targeting modification, and is favorable for improving drug efficacy. The irinotecan liposome of the invention has a long half-life in vivo and high bioavailability.

Registered patent CN115197078B [98] protects cationic-modified liposomes, which contain cationic lipids, used as a drug delivery system to cells, specifically, antitumor drugs and nucleic acids.

A blank liposome with ginsenoside as a membrane material as a carrier for active substances (e.g., drugs, cosmetically active substances, and substances with healthcare function), their preparation, and usage, especially against human gastric cancer, are outlined by patent EP3337456B1 [99]. Their preparations can comprise a thermo-sensitive or pH-sensitive excipient, a surfactant, or an ion additive.

Granted patent EP3125920B1 [100] provides a novel therapeutic modality for non-small-cell lung carcinoma and ovarian cancer treatment, based on dianhydrogalactitol, an alkylating agent on DNA that produces N7 methylation. Dianhydrogalactitol is active

against tumors that are refractory to temozolomide, cisplatin, and thymidine kinase inhibitors, is also effective in suppressing cancer stem cell growth, and acts independently of the repair mechanism. Liposomes and other delivery vehicles or carriers for hydrophobic drugs were used to improve the therapeutic potential. A neutral liposome is incorporated into a pegylated substituted hexitol derivative, wherein the polyethylene glycol strand is conjugated to at least one mobile peptide or targeting agent.

JP7470418B2 [101] describes the multicellular-targeting liposomes used in antitumor drug preparation. One liposome with multiple antibodies against different cells, with a stable structure and long circulation characteristics in vivo, was formed by molecular self-assembly. It can simultaneously or sequentially bind to multiple different cells using the action of antibodies to promote the cytotoxicity, communication between, the identification, and the clearance or pro-apoptosis of diverse cells.

A tumor-targeted therapeutic drug carrier consisting of liposome, lecithin, 1,2-bis(oleoyloxy)-3-(trimethylammonium)propane (DOTAP), or methylsulfated DOTAP, its preparation method, and its application were described in the granted Chinese patent CN102133404B [102].

The chitosan-liposome, redox-responsive to a disulfide bond and encapsulation of superparamagnetic ferro-ferric oxide nanoparticles, used as a drug carrier for anti-human non-small lung cancer cell A549, was described in patent CN107982547B [103]. Dithiobissuccinimidyl-substituted ester was used for the synthesis of the double fatty chain substituent phosphatidylethanolamine-s-s-chitosan. The chitosan-liposomes have strong cell adhesion characteristics, high drug delivery efficiency, and antiserum abilities, and they are biocompatible and suitable for intravenous injections.

A pharmaceutical formulation that contains methyl jasmonate or jasmonic acid as an active ingredient is described in patent KR101751918B1 [104]. Jasmonates are contained within, associated with, or linked to nano-carriers or microcarriers, such as cyclodextrins, pegylated liposomes, or ceramide liposomes, liposomes selected from nano-emulsions resembling low-density lipoprotein (LDE), poly(ethylene glycol)-600-hydroxystearate, which minimize side effects. The pharmaceutical formulation could be used in chemotherapeutic treatment for cancer in humans to decrease the side effects, as a single treatment or in association with mixed therapies.

A composition based on vitamin E and an anti-cancer compound contained within a delivery vesicle was presented in Australian patent AU2002361812B2 and provides a method for treating a cell proliferative disease [105]. The vitamin-E-based compounds are 2,5,7,8-tetramethyl-(2R-(4R,8R,12-trimethyltridecyl) chroman-6-yloxy) acetic acid, α -tocotrienol, β -tocotrienols, γ -tocotrienol, and δ -tocotrienol, and the lipid is 1,2-dilauroyl-sn-glycero-3-phosphocholine. Anti-cancer drugs are 9-nitrocamptothecin, cisplatin, paclitaxel, doxorubicin, or celecoxib. This composition comprises a liposome and is to be administered by aerosol delivery.

The objects of patents US8147867B2, US8703181B2, and US9782349B2 are compositions comprising liposomes with an interior space separated from the medium by membranes, containing one or more lipids, in which the inner space of the liposome contains substituted ammonium [106–108]. Liposomal compositions comprise one or more lipids and encapsulated anti-cancer therapeutic agents (e.g., camptothecin, topoisomerase inhibitor, and irinotecan) and sucrose hexasulfate. The toxicity of the liposomal composition administered to the mammal is at least 2–4-fold less than the substance administered in free non-liposomal form. There is a half-release time from the liposome of at least 10–24 h.

Patent CN103599070B described the method of fluorescence and temperature probes of the liposome membrane, loaded with the water-soluble anti-cancer drug (paclitaxel, doxorubicin, berberine, or cisplatin) and gold nanocluster dispersion [85].

Hydrophobic immunoregulation substances, all-trans retinoic acid and antigens, are loaded, delivered, and released at the same time and act as a therapeutic vaccine against mucosa-associated tumors, according to patent CN108324938B [79].

Interleukin-2 variant proteins fused to human immunoglobulin heavy constant gamma 4 (IgG4 Fc) (US11896648B2) [109] are used for preventing, reducing, and/or inhibiting the recurrence, growth, proliferation, migration, and/or metastasis of a cancer cell or population of cancer cells in a subject in need. An effective amount of the heterodimer is administered depending on the cancer cell type or population of cancer cells, selected from melanoma and colon cancer.

4.2. Liposome-Based Drug Carriers for Nucleic Acid Delivery

Search criteria were conducted using the keywords “nucleic acid”, “RNA”, and “DNA”. From 1975 to July 2024, were found 1580 patent documents (applications and granted patents) according to the title, abstract, and claims, and 637 granted patents. Cumulative and yearly patent applications and granted patents according to these results, the first seven applicant origins and countries, and the IPC and CPC subgroups only for granted patents are graphically presented in Figure 7. During the last 10 years, 476 patents were granted. Selected patents for liposome-based drug carriers for RNA delivery are presented in Table 6 and analyzed afterward.

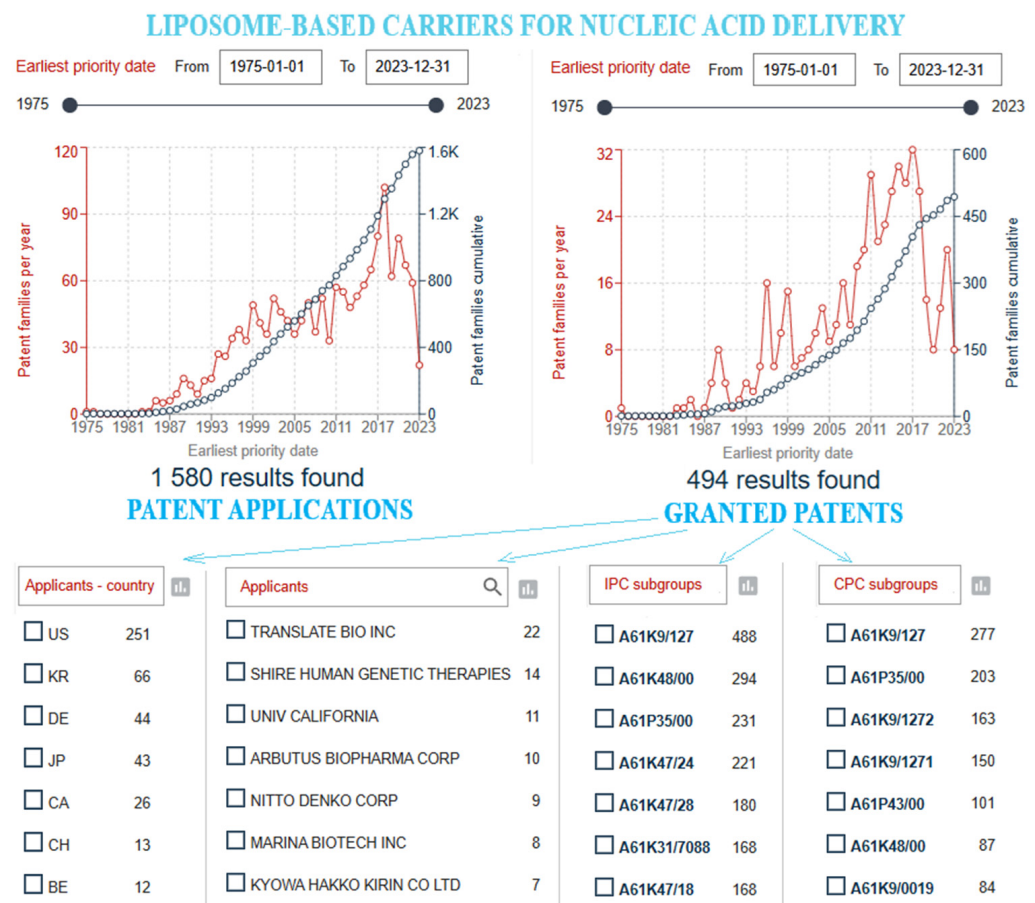


Figure 7. Liposome-based drug carriers for nucleic acid delivery: cumulative and yearly numbers of patent applications and granted patents depending on the earliest priority date (from 1975 to 2024), the first seven applicant origins and countries, and the IPC and CPC subgroups for granted patents. Two-letter codes: US, KR, DE, JP, CA, CH, and BE, are abbreviations for the following: United States of America, Republic of Korea, Germany, Japan, Canada, Switzerland, and Belgium, respectively. Data were obtained using the Espacenet database [32].

Table 6. Selected patents of liposome-based carriers for nucleic acid delivery.

Patent Title	Publication No. [Reference]	Applicants	Earliest Priority
Messenger RNA vaccines and uses thereof	CN112384205B [110]	Translate Bio, Inc.	30 May 2018
Methods and compositions for delivering mRNA-coded antibodies	EP2970456B1 [111]	Translate Bio, Inc.	14 March 2013
Preparation and storage of liposomal RNA formulations suitable for therapy	US11395799B2 [112]	BioNTech SE	20 October 2017
Compositions and systems comprising transfection-competent vesicles free of organic solvents and detergents and methods related thereto	EP3864163B1 [54]	The University of British Columbia	9 October 2018
Cationic lipid, liposome containing cationic lipid, and nucleic acid, pharmaceutical composition containing liposome, and preparation and application thereof	CN115197078B [100]	Xiamen Sinopeg Biotech Co., Ltd.	8 April 2021
Reduced and oxidized polysaccharides and methods of use thereof	CN108430458B [113]	Harvard College	26 October 2015

The patent CN112384205B outlined compositions comprising a messenger RNA (mRNA) encoding an antigen encapsulated in a PEG lipid nanoparticle and the method of preparation of a vaccine for inducing an immune response in vivo, wherein the lipid nanoparticle comprises one or more cationic lipids [110]. Obtained vaccines could be administered subcutaneously, intradermally, intramuscularly, or intravenously.

The other patent, EP2970456B [111], demonstrates compositions for delivering mRNA-coded antibodies comprised of the first mRNA encoding the antibody heavy chain (of approximately 50–70 kD), and the second mRNA encoding the antibody light chain (of approximately 25 kD), for use in therapy in a patient administered intravenously. The first and the second mRNA (i) each comprised a 5' cap structure, a 3' poly-A tail, a 5' untranslated region, and a 3' untranslated region, and (ii) encapsulated within liposomes comprising a cationic lipid, a neutral lipid, a cholesterol-based lipid, and a PEG-modified lipid (up to 150 nm). The first mRNA encoding the heavy chain and the second mRNA encoding the light chain are encapsulated in the same liposome, and the antibody is a tetramer composed of two identical pairs of polypeptide chains.

Preparation and storage of liposomal RNA formulations for delivery of RNA to target tissues after parenteral or intravenous administration were described in the granted patent US11395799B2 [112].

Methods were outlined for preparing RNA lipoplex particles in an industrial GMP-compliant manner. Additionally, they protect methods and compositions for storing RNA lipoplex particles without significant loss of product quality and RNA activity. Systems comprising transfection-competent vesicles free of organic solvents and detergents configured to efficiently and safely deliver nucleic acid, e.g., DNA, RNA, ribonucleoprotein (RNP), and protein cargoes in target cells, were disclosed in the granted patent EP3864163B1 [54].

Pharmaceutical compositions based on cationic liposomes and nucleic acid drugs (i.e., DNA, plasmid, aptamer, interfering nucleic acid, antisense nucleic acid, mRNA, small interfering RNA (siRNA), microRNA (miRNA), antagomir, and ribozyme) have good biocompatibility and higher gene transfection efficiency (patent CN115197078B) [98].

According to Chinese patent CN108430458B, the therapeutic or diagnostic agents were encapsulated in lipid-based nanoparticles (liposomes or virosomes), highly oxidized polysaccharides—alginate, human mesenchymal stem cells, small molecules, and biolog-

ical agents (peptides, antibodies, or fragments thereof, vaccines, DNA, RNA, or peptide nucleic acid (PNA) molecules) [113]—and formulated as the implantable or injectable device, or the drug delivery composition.

4.3. Liposome-Based Drug Carriers for Pulmonary and Ocular Treatment

Keywords “pulmonary”, “lung”, “inhal”, and “respiratory” were added to the search criteria. A total of 3187 patent documents (applications and granted patents) from 1975 to July 2024 according to the title, abstract, and claims, and 928 granted patents, were found (Figure 8). Using the keywords “ocular”, “eye”, “sclera”, and “retina”, a total of 1752 patent documents (applications and granted patents) were found from 1975 to July 2024 according to the title, abstract, and claims, and 542 granted patents (Figure 9). During the last 10 years, 476 patents were granted. Table 7 presents certain patents for liposome-based drug carriers for pulmonary and ocular treatment that are subsequently analyzed.

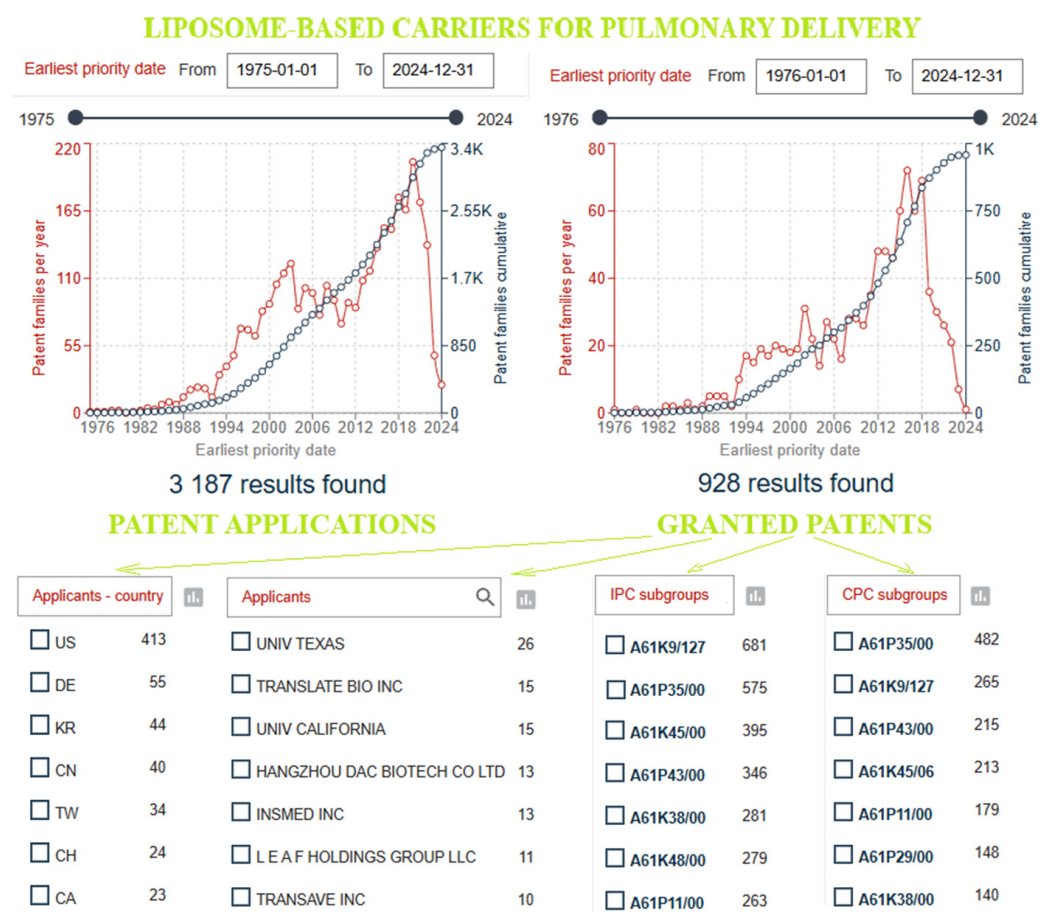


Figure 8. Liposome-based drug carriers for pulmonary treatments: cumulative and yearly numbers of patent applications and granted patents depending on the earliest priority date (from 1983 to 2024), the first seven applicant origins and countries, and the IPC and CPC subgroups for granted patents. Two-letter codes: US, DE, KR, CN, TW, CH, and CA, are abbreviations for the following: United States of America, Germany, Republic of Korea, Republic of China, Taiwan Province of China, Switzerland, and Canada, respectively. Data were obtained using the Espacenet database [32].

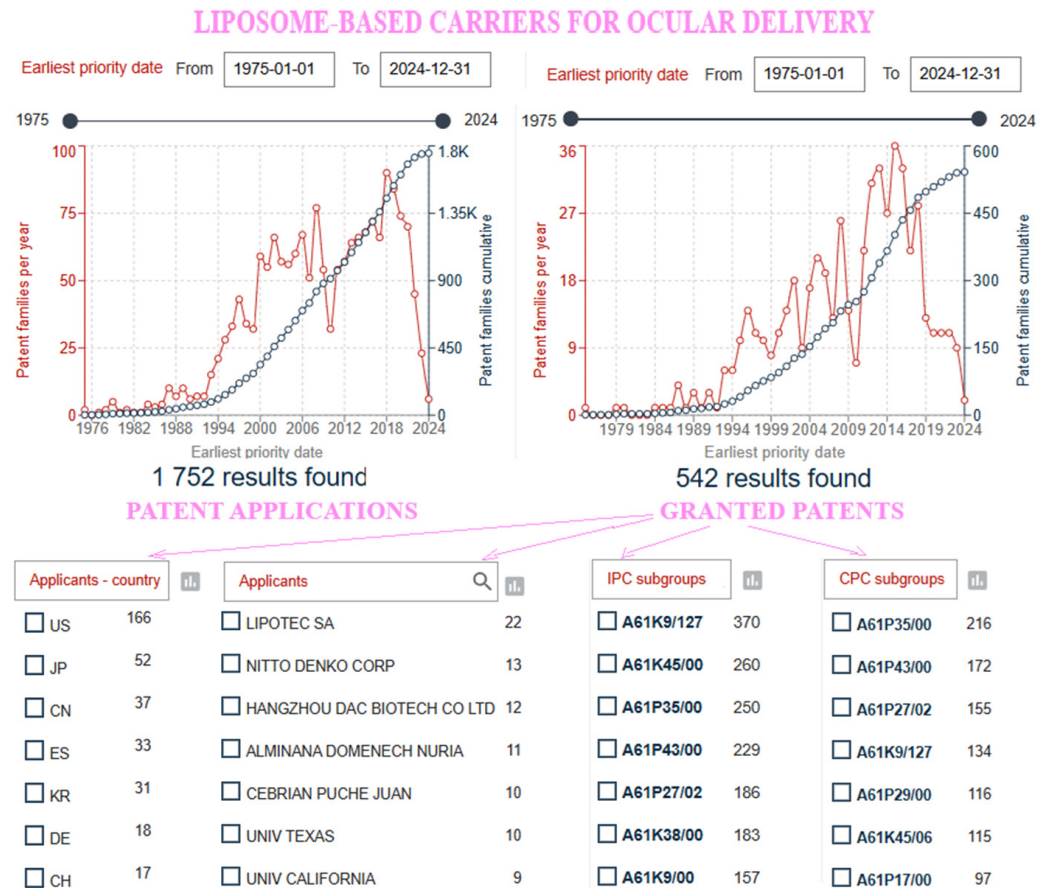


Figure 9. Liposome-based drug carriers for ocular treatments: cumulative and yearly numbers of patent applications and granted patents depending on the earliest priority date (from 1983 to 2024), the first seven applicant origins and countries, and the IPC and CPC subgroups for granted patents. Two-letter codes: US, JP, CN, ES, KR, DE, and CH, are abbreviations for the following: United States of America, Japan, Republic of China, Spain, Republic of Korea, Germany, and Switzerland, respectively. Data were obtained using the Espacenet database [32].

Table 7. Selected patents of liposome-based drug carriers for pulmonary and ocular treatment.

Patent Title	Publication No. [Reference]	Applicants	Earliest Priority
Inhalable compositions for use in the treatment of pulmonary diseases	EP3890767B1 [114]	Breath Therapeutics GmbH	4 December 2018
Slow-release salbutamol sulfate inhalation preparation and production process thereof	CN111700883B [115]	Shenzhen Daphne Medicine Co., Ltd.	23 July 2020
Tobramycin liposome used for aerosol inhalation and production method thereof	CN111228243B [116]	Zhuhai Essex Bio-Pharmaceutical Co., Ltd.	24 December 2019
Liposome atomization treatment preparation containing acetaldehyde dehydrogenase as well as preparation method and application thereof	CN108434446B [117]	Hangzhou Hibio Technology Co., Ltd.	21 March 2018
Liposomes containing steroid esters	EP0170642B1 [118]	Draco Ab	30 July 1984

Table 7. Cont.

Patent Title	Publication No. [Reference]	Applicants	Earliest Priority
Liposomal eye drops solution and uses thereof for the treatment of dry eye syndrome	EP3673896B1 [119]	Dr. Rolf Lambert Pharma-Consulting GmbH	28 December 2018
Sustained-release ophthalmic pharmaceutical compositions and uses thereof	TWI786328B [120]	Taiwan Liposome Company, Ltd.; TLC Biopharmaceuticals, Inc.	10 September 2018

The aerosol inhalation technology has the advantages that a drug can be directly delivered to a respiratory tract, quickly absorbed, takes effect quickly, and the bioavailability and the stability are high. A pharmaceutical composition comprising cyclosporine A, as an inhalable immunosuppressive macrocyclic active ingredient for the prevention or treatment of pulmonary disease or condition, administered by inhalation in the form of an aerosol, generated by nebulization of the pharmaceutical composition, were disclosed in patent EP3890767B1 [114].

A slow-release salbutamol sulfate inhalation preparation (capable of relieving acute attack of asthma) and its production process are objects of patent CN111700883B [115]. The invention disclosed in patent CN111228243B is a tobramycin liposome used for aerosol inhalation, composed of 0.1–15.0% tobramycin, 0.5–36.0% of a phospholipid, 0.05–20.0% of a stabilizer, 0.01–10.0% of a charge modifier, 0.01–5.0% of an antioxidant, 5.0–50.0% of an organic-phase medium, and the balance of an aqueous-phase medium [116]. The production process is simple, easy, and rational for industrial production.

The invention CN108434446B provides a therapeutic preparation process for a liposomal aerosol [117]. By using the pulmonary method of administration, it is possible to avoid enzyme destruction in the digestive tract of oral preparations, as well as the insufficient activity and safety problems of injectable preparations. The proportion of inhalable particles after administration via the aerosol inhalation route satisfies the pharmacopoeia. This preparation has good application forecasts for treating drunkenness, alcoholism, and other acetate dehydrogenase deficiency.

A pharmaceutical composition for the local administration primarily to the respiratory tract when treating and controlling anti-inflammatory and anti-allergic conditions comprising lyophilized liposomes in the presence of lactose (range of 0 to 95% of the final composition), in combination with active steroid esters and budesonide-21-palmitate, was described in patent EP0170642B1 [118]. The lecithin (derived from egg, soybean, or synthetic) has different lengths of fatty acid chains and, therefore, has different phase-transition temperatures. The administration routes involve powder aerosols, nebulization, instillation, and aerosols.

Ophthalmic formulations for the treatment of dry eye syndrome, based on an eye drop solution composed of liposomes, were protected by patent EP3673896B1 [119]. Eye drop solutions are comprised of liposomes built with non-hydrogenated phospholipids (containing linseed oil, Vitamin E tocopherol polyethylene glycol succinate (TPGS), and Vitamin A Palmitate) and water phase (with Vitamin B12 and Pycnogenol). Vitamin E TPGS inside the liposomes and Vitamin B12 and Pycnogenol outside the liposomes have a protective effect against UVA/UVB rays. A liposomal eye drop solution containing a specific, peculiar system composed of 2-amino-2(hydroxymethyl) propane-1,3-diol, which makes an agent for Pycnogenol and borate buffer to improve the filterability, has a satisfying filtration procedure to sterilize liposomal eyes drops (up to 0.2 µm), avoiding the steam sterilization (which can destroy components and liposome's structure).

Sustained-release ophthalmic pharmaceutical compositions based on liposomes (comprising a bilayer membrane) and a therapeutic agent for treating an eye disease with a high drug-to-lipid ratio and encapsulation efficiency were described in the granted patent

TWI786328B [120]. It provides a method for treating age-related macular degeneration or diabetic eye disease using this ophthalmic composition, which can be administered by injection (intravitreal or suprachoroidal) or topical (by eye drop or ointment).

4.4. Liposome-Based Drug Carriers for Some Neural, Orthopedic, and Internal Diseases

Using the keywords “neural”, “brain”, “cerebral”, “orthopedic”, “bone”, “cartilage”, “osteoarthritis”, “arthritis”, “internal diseases”, “gastric”, “intestinal”, and “liver”, a total of 1809 patent documents (applications and granted patents) from 1975 to July 2024 according to the title, abstract, and claims, and 549 granted patents, were found (Figure 10). In the last 10 years, 476 patents were granted. A few examples of patents for drug carriers based on liposomes for neural, orthopedic, and some internal diseases are analyzed and summarized in Table 8.

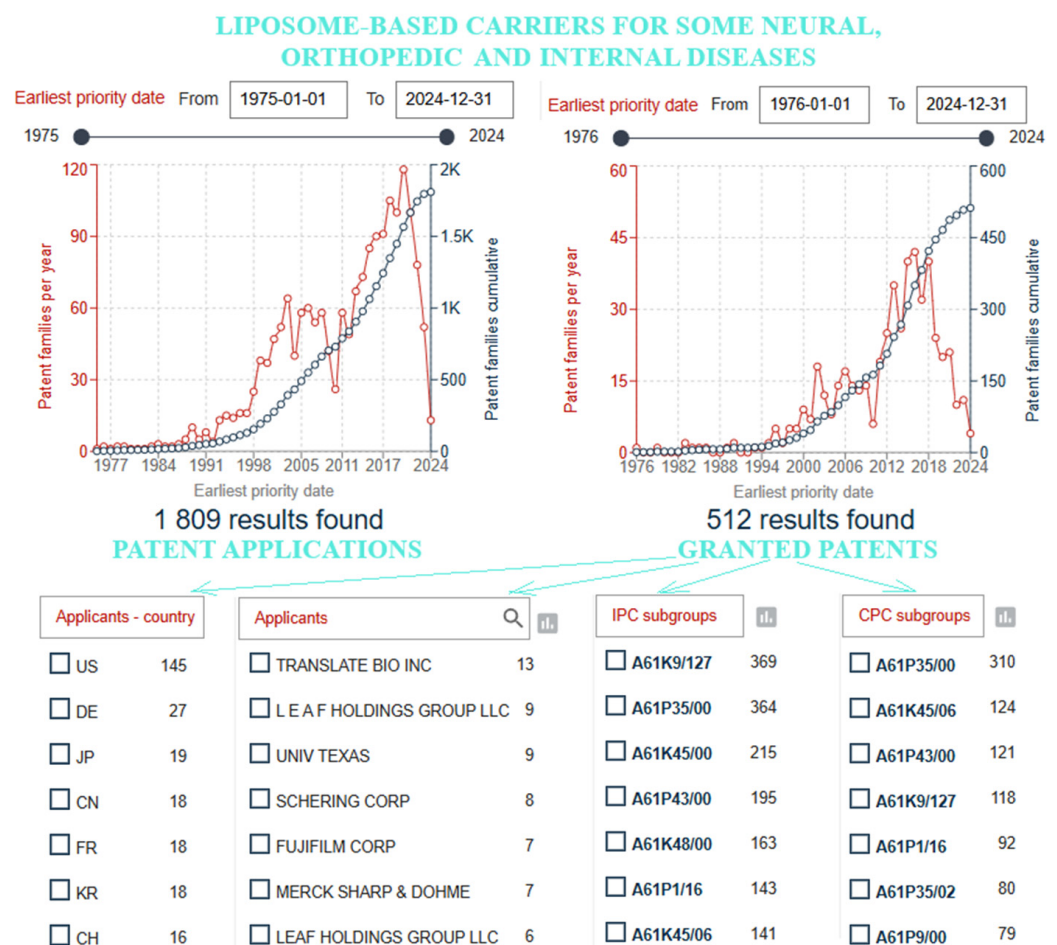


Figure 10. Liposome-based drug carriers for some neural, orthopedic, and internal diseases: cumulative and yearly numbers of patent applications and granted patents depending on the earliest priority date (from 1975 to 2024), the first seven applicant origins and countries, and the IPC and CPC subgroups for granted patents. Two-letter codes: US, DE, JP, CN, FR, KR, and CH, are abbreviations for the following: United States of America, Germany, Japan, Republic of China, France, Republic of Korea, and Switzerland, respectively. Data were obtained using the Espacenet database [32].

Table 8. Selected patents of liposome-based drug carriers for neural, orthopedic, and internal diseases.

Patent Title	Publication No. [Reference]	Applicants	Earliest Priority
A formulation useful for delivery of neuro-protecting agent	EP3200770B1 [121]	Council of Scientific and Industrial Research	29 September 2014
Sustained-release pharmaceutical compositions comprising a therapeutic agent for treating diseases due to reduced bone density or cartilage loss and uses thereof	JP7431419B2 [122]	Taiwan Liposome Company Ltd.; TLC Biopharmaceuticals, Inc.	14 November 2018
Methods of treating arthritis	EP2854769B1 [123]	Taiwan Liposome Company Ltd.; TLC Biopharmaceuticals, Inc.	5 July 2012
Manufacturing of bupivacaine multivesicular liposomes	GB2603047B [124]	Pacira Pharmaceuticals Inc.	22 November 2021
Formulations of volatile anesthetics and methods of use for reducing inflammation	US9744142B2 [125]	Spakevicius, D.; Ozsoy H.; Board of Regents, The University of Texas Systems	5 May 2009
Method for selecting cationic or anionic liposomes for treatment of a mucosa membrane, and kit comprising the same	EP1694298B1 [126]	Yissum Research Development Company	3 November 2003
Pharmaceutical composition for treating alcoholic fatty liver and preparation method thereof	CN117860678B [127]	China Agricultural University	13 March 2024
Cilostazol liposome solid agent	CN112006992B [128]	Pu-Pharma. Co., Ltd.	16 September 2020
Targeted hybrid exosome loaded with curcumin and miR140 as well as preparation method and application of targeted hybrid exosome	CN117965429B [69]	Sichuan University	29 March 2024

The EP3200770B1 patent [121] discloses a novel delivery system based on liposomes, with the potent and prudent therapeutic potential of a new formulation using the standardized extract fraction of a new NMITLI118RT+ chemotype of *Withania somnifera* roots. It is useful for brain function restoration and neuroprotection against cerebral stroke. A new chemotype of *Withania somnifera* roots, a variety of Ashwagandha, is phytochemically characterized by a particular abundance of two withanolides, i.e., withanolide and/or withanone, for neuroprotection against cerebral stroke, prescribed for administering the formulation in a predetermined dose and period.

Pharmaceutical compositions with sustained release of a therapeutic agent for treating diseases due to reduced bone density or cartilage loss, and uses thereof, were described in patent JP7431419B2 [122]. Sustained-release compositions comprised (a) at least one liposome with a lipid bilayer membrane, (b) a scavenger (triethylammonium sucrose octasulphate and/or ammonium sulphate), and (c) a cathepsin K inhibitor. The injection with a therapeutic agent for treating reduced bone density or cartilage loss could be administered via an intraarticular, subcutaneous, subdermal, intradermal, or intramuscular route. The other sustained-release composition for use in the treatment of arthritis, comprising liposomes, cholesterol, and one or more therapeutic agents, e.g., the water-soluble steroid dexamethasone sodium phosphate, and the nonsteroidal anti-inflammatory drug indomethacin, a disease-modifying anti-rheumatic drug, were objects of the granted patent EP2854769B1 [123].

Composition of multivesicular liposomes (MVLs) with encapsulated bupivacaine, residing within a plurality of internal aqueous chambers of the MVLs, separated by lipid membranes, was disclosed in patent GB2603047B [124]. The internal aqueous chambers

may comprise lysine and/or dextrose, or/and cholesterol and tricaprilyn. These compositions could be useful in treating or ameliorating pain, particularly via the interscalene brachial plexus nerve block, via local infiltration to a surgical site, or via femoral nerve block to provide regional analgesia. Another volatile anesthetic for treating or reducing inflammation or a wound in need of wound treatment or inflammation treatment by delivering a volatile anesthetic to the wound or inflammation site was formulated for topical, mucosal, rectal, vaginal, or buccal administration.

The invention EP1694298B1 [126] concerns a method for selecting cationic or anionic liposomes, a medicament for the treatment/prevention of a gastrointestinal mucosa disorder, and a kit for its use. The application of charged lipid assemblies is based on the principles of the differential adhesion of negatively vs. positively charged lipid assemblies to diseased and healthy mucosa, respectively.

The invention CN117860678B [127] discloses a pharmaceutical composition for treating alcoholic fatty liver and an appropriate obtaining method. Ethyl *p*-methoxycinnamate and nano-enzymes are enclosed in liposomes to form a composite material. According to this invention and using a new strategy, liposomes are delivered, in a targeted manner, to the alcoholic fatty liver for precise treatment with improved bioavailability.

The invention CN112006992B discloses a cilostazol liposome solid agent, prepared by a subsequent method: cilostazol (improves blood flow) was dissolved in acetic acid, and mixed with dioleoyl phosphatidylcholine, dimyristoyl phosphatidylcholine, poloxamer P188, and cholesterol succinic acid monoester, to form a lipid membrane solution [128]. After that, an acetic acid sodium acetate buffer solution was added to disperse and emulsify to obtain a uniform liposome solution. Then, lactose, sodium carboxymethyl starch, microcrystalline cellulose, and magnesium stearate were added, mixed, refined and dispersed. Obtained a uniform mixed suspoeulsion was freeze-dried. The cilostazol loading capacity and liposome encapsulation efficiency are improved, the technological process is shortened, and the raw materials are safe and non-toxic.

The hybridized exosome, described in Korean patent CN117965429B, showed a synergistic effect of loaded curcumin and miR140, inhibited activation of an NF-kappa B pathway, enhanced the anti-inflammatory effect, and reduced the cytotoxicity of the curcumin. It is used in the preparation of a drug for treating osteoarthritis [69].

4.5. Liposome-Based Drug Carriers with Anti-Inflammation and Antibiofilm Agents

Search criteria were narrowed using the keywords “anti-inflammation”, “inflammation”, “antibiofilm”, “microbial”, and “biofilm”. From 1975 to July 2024, 1231 patent documents (applications and granted patents) were found according to the title, abstract, and claims, as well as 368 granted patents. The cumulative and yearly numbers of patent applications and granted patents, according to these search results, with the first seven applicant origins and countries and the IPC and CPC subgroups (only for granted patents), are graphically presented in Figure 11. A few examples of granted patents for drug carriers based on liposomes with anti-inflammation and antibiofilm agents (presented in Table 9) are briefly described.

Table 9. Selected granted patents for liposome-based drug carriers for anti-inflammation agents.

Patent Title	Publication No. [Reference]	Applicants	Earliest Priority
Sterile pharmaceutical composition and process for a solution of propofol emulsion having microbial growth retardation	US7468394B1 [129]	Amphastar Pharmaceuticals Inc.	11 March 2003
Liposomes for inhibiting biofilm formation	EP3589278A1 [130]	Combioxin SA	2 March 2017

Table 9. Cont.

Patent Title	Publication No. [Reference]	Applicants	Earliest Priority
Methods for the manufacture of liposomal drug formulations	AU2019262117B2 [131]	Insmad Inc. Worsham Robert	2 May 2018
Interdigitation-fusion liposomes containing arachidonic acid metabolites	EP0729352B1 [132]	Liposome Co., Inc.	16 November 1993
Pharmaceutical composition comprising jasmonates	KR101751918B1 [104]	Fehr Pereira Lopes, Jose.E.	15 July 2008

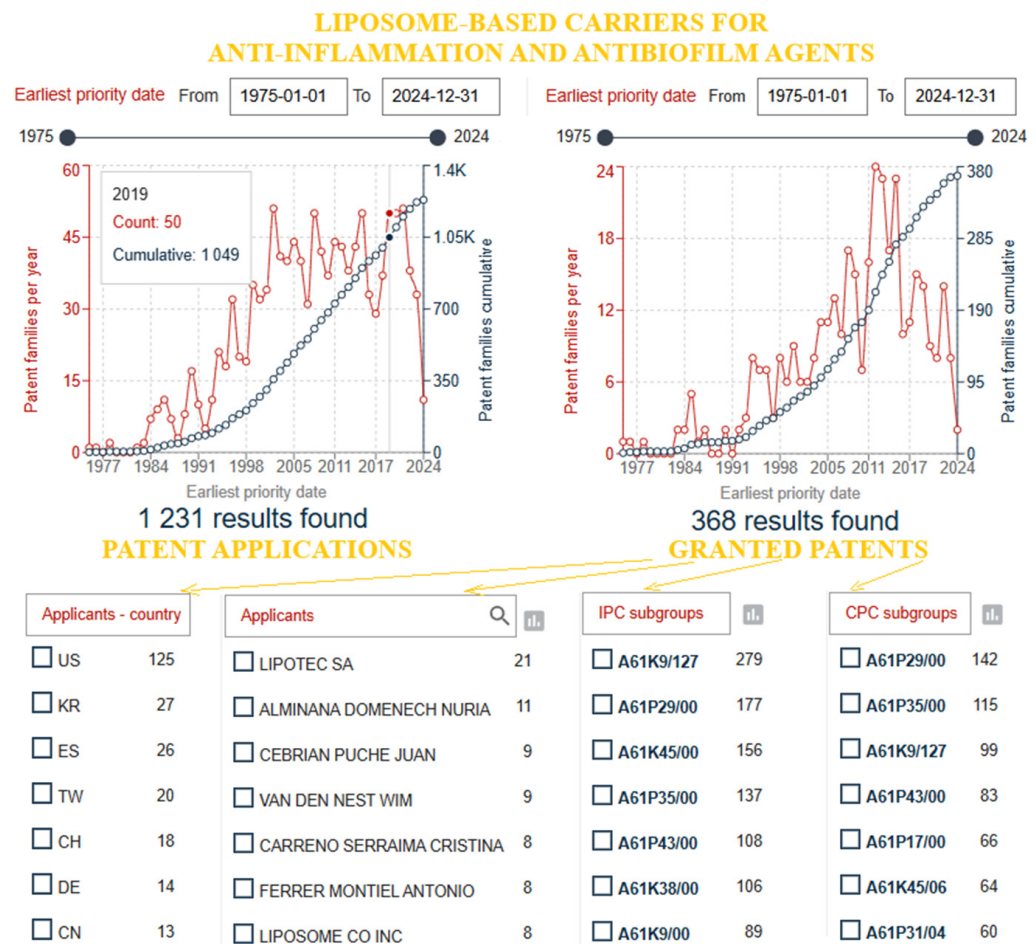


Figure 11. Liposome-based drug carriers for nucleic acid delivery: cumulative and yearly numbers of patent applications and granted patents depending on the earliest priority date (from 1975 to 2024), the first seven applicant origins and countries, and the IPC and CPC subgroups for granted patents. Two-letter codes: US, KR, ES, TW, CH, DE, and CN, are abbreviations for the following: United States of America, Republic of Korea, Spain, Taiwan Province of China, Switzerland, Germany, and Republic of China, respectively. Data were obtained using the Espacenet database [32].

A sterile pharmaceutical composition and process for an oil-in-water propofol emulsion having microbial growth retardation, without the side effects associated with other growth retardation additives, are disclosed in the US7468394B1 patent [129]. Several methods for the optimization of the innate microbial retardation capability of propofol are described. This composition contains liposome particles, emulsion particles, additives to act as a microbial growth retardation agent, and supporting aqueous phase. The oil-in-water propofol emulsion contains soybean oil and egg lecithin. The described process

improves the propofol emulsion solution's safety by controlling microbial growth, without the side effects associated with growth retardation additives.

Liposomes for preventing or reducing biofilm formation, or for eradicating or reducing existing biofilm, are described in patent EP3589278A1 [130]. A method for a large-scale production of liposomal drug formulations, containing amikacin sulfate formulation as an aminoglycoside (antibiotics for serious bacterial infections, especially Gram-negative), with valuable lipid/drug characteristics, was presented in patent AU2019262117B2 [131]. The method employs a particular relative flow rate ratio of lipid to drug streams to obtain liposomes with a high aminoglycoside encapsulation efficiency. The invention EP0729352B1 offers an interdigitation-fusion liposome, including an arachidonic acid metabolite, a lipid bilayer containing a lipid, and an aqueous compartment comprising a release-inhibiting buffer [132]. The preferred arachidonic acid metabolites are the prostaglandins, particularly PGE1. The liposomal formulations can be used to treat animals, particularly humans, for diseases, disorders, or conditions that can be ameliorated by prostaglandins, e.g., cell activation/adhesion disorders and inflammatory disorders.

A pharmaceutical formulation for use in the treatment of bacterial or fungal diseases, containing methyl jasmonate or jasmonic acid as an active ingredient, is described in patent KR101751918B1 [104].

4.6. Liposome-Based Drug Carriers for Dermal Applications

Using the keywords “dermatological”, “derma”, “skin”, “wound”, “cosmetic”, and “stratum corneum”, a total of 1809 patent documents (applications and granted patents) from 1975 to July 2024 according to the title, abstract, and claims, and 549 granted patents, were found (Figure 12). In the last 10 years, 476 patents were granted. Several examples of granted patents for drug carriers based on liposomes for dermal applications, shown in Table 10, are briefly described.

Table 10. Selected relevant patents of liposome-based carriers for dermal applications.

Patent Title	Publication No. [Reference]	Applicants	Earliest Priority
Method for preparing medicinal extract containing intermediate peashrub seeds	CN104382984B [84]	Northwest Institute of Plateau Biology of Chinese Academy of Sciences	1 December 2014
Composition for increasing expression of PGC-1 alpha	EP3403655B1 [133]	Benebiosis Co., Ltd.	13 January 2016
Peptides which inhibit activated receptors and their use in cosmetic or pharmaceutical compositions	EP2773366B1 [134]	Lipotec SA	4 November 2011
Cosmetological and pharmaceutical formulae for the rejuvenation and restoration of skin, including after surgical procedures	US8846064B2 [135]	Martynov, A.; Farber, B.S.; Farber, S.S.; Sitenko, A.	22 November 2010
Cosmetic or pharmaceutical compositions comprising metalloproteinase inhibitors	ES2330291B1 [136]	Lipotec SA	29 February 2008
Pharmaceutical composition, in particular dermatological or cosmetic, comprising hydrous lipidic lamellar phases or liposomes containing a retinoid or a structural analogue thereof, such as a carotenoid	FR2591105B1 [137]	Moet Hennessy Recherche	11 December 1985

Table 10. Cont.

Patent Title	Publication No. [Reference]	Applicants	Earliest Priority
Hyaluronic drug delivery system	EP0963196B1 [138]	Hyal Pharmaceutical Corporation; JagotecAg	29 September 1996
Preparation method of exosome bionic preparation for synergistically promoting wound healing and preparation thereof	CN113577272B [73]	China Pharmaceutical University	28 July 2021

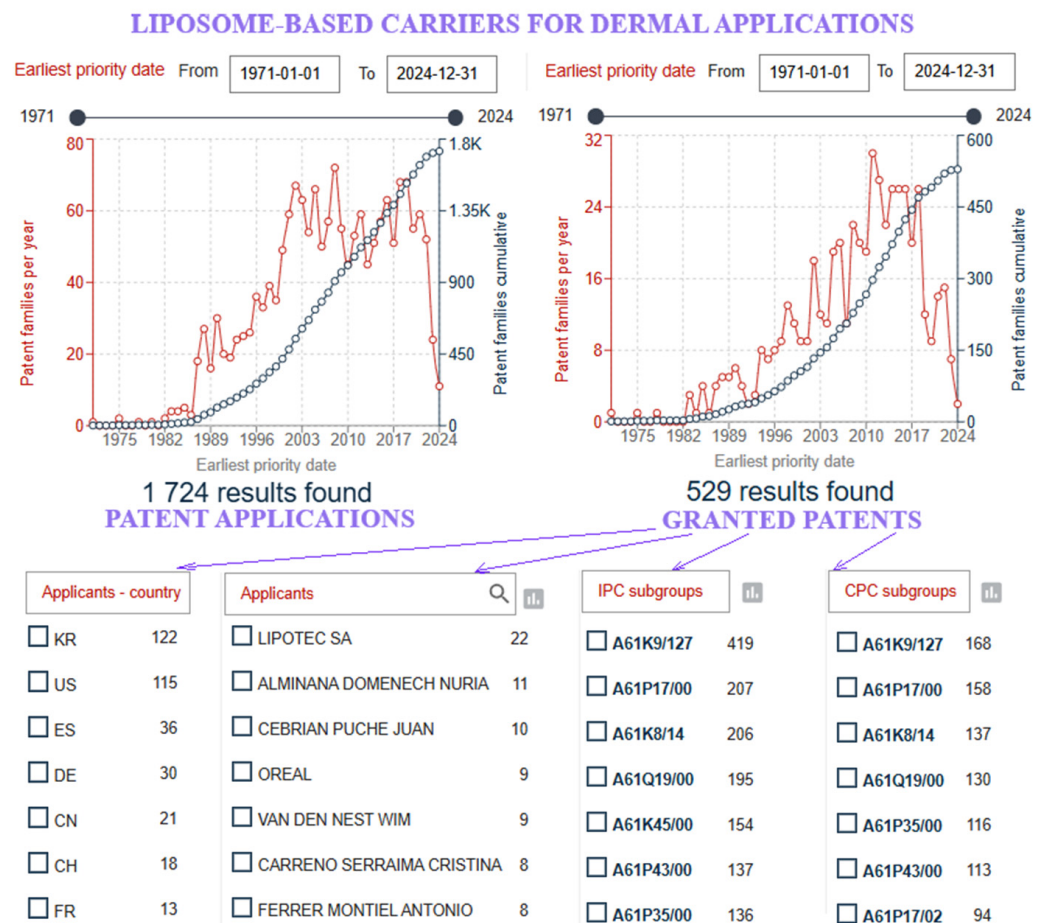


Figure 12. Liposome-based drug carriers for dermal applications: cumulative and yearly numbers of patent applications and granted patents depending on the earliest priority date (from 1971 to 2024), for granted patents the first 7 applicant names and countries, and the IPC and CPC subgroups. Two-letter codes: KR, US, ES, DE, CH, CN, and FR, are abbreviations for the following countries: Republic of Korea, United States of America, Spain, Germany, Republic of China, Switzerland, and France, respectively. Data were obtained using the Espacenet database [32].

Medicinal extract of *Caragana korshinskii* seeds for the drug-containing liposome nanoparticles prepared by the patent CN104382984B have the characteristics of targeted drug delivery and long-term release [84]. Due to their cell-like structure, they are cell-compatible and can release drugs through the cell membrane. Drug-containing liposome nanoparticles with *Caragana korshinskii* seed extract improve therapeutic index and are more effective in skin disease treatments, e.g., psoriasis, dermatitis, impetigo, and skin itching.

Registered patent EP3403655B1 relates to a composition for preventing or treating diseases or symptoms associated with a reduction in the expression of peroxisome proliferator-

activated receptor coactivator 1- α (PGC-1 α), consisting of an active ingredient, and its salt or solvate, encapsulated in liposomes [133]. The composition is used for reducing the subcutaneous adipose tissue volume (of the femoral region, a lower part of the neck, neckline, chest, buttocks, lips, face, cheeks, eyelids, and/or hands), or for the triglyceride content reduction in adipose tissue.

The object of patent EP2773366B1 [134] is a pharmaceutical or cosmetic composition with a sustained-release system, formed by liposomes, mixed liposomes, or some other carriers, and the peptide, which inhibits activated receptors.

Cosmetic and pharmaceutical compositions with liposomes and proteins were described in US patent US8846064B2 [135]. Used proteins were partially acylated (0.1–10% of their mass) and present in the supramolecular assembly. These partially acylated proteins were derived from collagenase, hyaluronidase, insulin, or their mixtures.

Spanish patent ES2330291B1 protects a pharmaceutical or cosmetic composition consisting of the peptide (its stereoisomers, mixtures, and cosmetically or pharmaceutically acceptable salts), incorporated in a vehicle or a system for sustained delivery, as well as an obtaining method [136]. Liposomes and mixed liposomes were chosen, among other cosmetic or pharmaceutically acceptable carriers. Obtained compositions were applied for the treatment and/or care of disorders, conditions, and/or skin pathologies, mucosae, and scalp that are a result of the matrix metalloproteinases (MMP) activity increase or an MMP overexpression.

Dermatological, pharmaceutical, or cosmetic compositions based on liposomes, or hydrated lipid lamellar phases, comprising retinoids (or analogues, e.g., carotenoids), are described in patent FR2591105B1 [137]. Defined compositions have additional effects against acne and are less irritating to the skin, and they have pharmaceutical and certain dermatological or cosmetic applications.

Pharmaceutical compositions based on multilamellar, negatively charged liposomes with encapsulated cyclosporin A and hyaluronic acid, described in patent EP0963196B1, were used for topical administrations for psoriasis treatment [138].

The obtained exosome bionic preparation synergistically helps wound healing according to Chinese patent CN113577272B [73].

The patent analysis of liposome-based drug carriers for cancer treatments, nucleic acid delivery, pulmonary and ocular treatment, neural, orthopedic, and internal diseases treatment, anti-inflammation and antibiofilm treatments, as well as dermal applications highlighted that published patents protect novel and alternative formulations, which are not found in scientific journals before their priority date because novelty in patent applications is the first demand for patentability and granted patent rights. Patent publications on liposomal formulations expanded new methods of drug delivery. Liposome formulations have been developed to establish novel approaches in drug delivery systems, e.g., to optimize the content of drug loading, stability, and release profiles, including long-circulating structures and further functionalization of liposomes for the co-delivery of drugs to the targeted site. New liposome-based compositions and strategies to optimize the stabilities and capabilities of drug vehiculation found more acceptable fabrication methods. Continued utilization of liposomes in disease treatments is based on defining stable, simpler, and cost-effective formulations, which could be applied in the pharmaceutical industry in the future to provide more beneficial therapeutic solutions for many diseases.

5. Conclusions

Many inventors have significantly contributed to improvements in liposome-based carriers by synthesizing more complex vesicles and developing new targeted and selective release mechanisms, without causing side effects. This has resulted in enhanced therapy efficiency and reduced treatment costs. New drug release methods have been identified to activate administration only at specific sites when needed. Drugs can be administered into the body via different methods, some of which are oral, topical, intranasal, sublingual, and intravenous. Future trends in liposome technology as drug carriers may include

new patent applications aimed at improving the protection of encapsulated drugs and delivery efficiency by overcoming biological barriers. New inventive solutions may be directed toward development of stealth liposome technology, incorporating PEG or other polymers, so that liposomes avoid the immune system more effectively, achieving long circulation (sterically stabilized) in the bloodstream with optimized and increased stability. Also, further progress in the design of liposomes to overcome biological barriers, such as the blood–brain barrier, is important, as it represents a major challenge for the treatment of neurological disorders. For the treatment of pulmonary and gastrointestinal diseases, it is important to improve liposome-based carriers so that they improve and optimize the passages through the mucosal barriers for more efficient delivery of inhaled or orally administered drugs. “Smart” liposomes can be designed as bioreactive carriers that release contents in the body in a controlled manner in response to biological signals (such as pH, temperature, light, or enzymes) exclusively in targeted areas (such as infected tissues or tumors). Liposomal formulations are intensively developed for precision gene therapy to improve encapsulation of genetic materials (such as RNA and DNA), protection during delivery, and enhancement of cellular uptake. The trend of future developments will probably be directed toward new methods of obtaining nano-liposomes, at a nanoscale below 100 nm, which can penetrate deeper into tissues (e.g., solid tumors) to improve drug delivery and treatment outcomes. Significant progress is expected in the technology of lipid nanoparticles, closely related to liposomes, especially in the delivery of nucleotides (e.g., mRNA in vaccines) and their optimization. One of the key trends for future technologies may be targeted drug delivery by modifying and functionalizing the surface of liposomes with ligands, antibodies, or aptamers that can selectively bind to specific cell receptors. This is particularly important for the more precise delivery of drugs for the treatment of cancer and genetic disorders, improving therapeutic outcomes and reducing side effects. The future of liposome technology will focus on reproducible, scalable, and environmentally friendly manufacturing processes to meet the growing demand for liposomal drugs.

Patent data are authoritative for recognizing trends in technical development, assessing industrial technological attractiveness, monitoring the process of protecting competition’s patent rights, and defining technological advantages and new strategies. The review of the patents could help in future investigations as a rich source of inspiration in several ways. Patent searching can help researchers avoid duplicating efforts and save time by analyzing and recognizing what has already been patented and, thus, focusing on novel aspects, strategies, or procedures. By informing with patents in force and existing patent rights, researchers can guarantee that their innovations do not infringe on the owners’ patent rights (other companies). Patent applications often reveal trends (e.g., new processes, methods, or materials) and could motivate researchers to modify or combine current methods and investigate similar technologies. Through the patent analysis of liposome-based drug carriers in the period 1976–2024, it is interesting to point out that the activities were aimed at finding new, improved production processes, as well as formulations for the targeted treatment and prevention of diseases (mostly for pulmonary, dermal, ocular, neural, and gastric). Numerous scientific papers on various drug delivery systems have been published in scientific journals, but a smaller number requested patent applications and fewer have registered patents. It is important that companies and academic researchers first protect their inventions by patenting new products, production processes, and/or formulations, and then publish their research in peer-reviewed journals. Currently, the largest number of patents are from universities, and additional cooperation with companies is needed to improve the technological procedures in industry by applying efficient and modern technologies.

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