Current Trends in Biotechnology and Pharmacy
Vol. 17 (1) 735 - 748, Jan 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2023.1.13

A Systematic Review on Recent Advancements and Liposomal Technologies to Develop Stable Liposome

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Abstract

The fundamental problem with liposomal drug delivery systems is stability.The construction of a liposomal drug delivery system allows the precise distribution of medications to various bodily regions. It is advantageous for this delivery technique to transport both hydrophilic and hydrophobic medicinal molecules. Many liposomal drug delivery systems are approved by the regulatory authorities (Foodanddrug administrations and European medicine agency) and developed by various manufacturing industries. Despite of various advantages shown by liposomes for the potent delivery of drugs, they have a major issue of both physical and chemical stability. To avoid stability problems manufacturing scientists have developed some of modified liposomal formulations over the years. Proliposomes is one of the transformed liposomes which is a dried,free-flowing liposomal prodrug when it comes in contact with water forms a liposomal suspension. The other modified liposomes include pH-sensitive liposomes, immunoliposomes, surface-modified liposomes, and elastic liposomes. The pH-sensitive liposomes are uniquelycreated for delivering the drug into the change in pH. They can be delivered inside the cytoplasm via the endocytic pathway. The surface modification of the liposomes is done by using several polymers like polyethylene glycols for enhancing stability. Surface modification can influence blood circulation and eliminate drug interaction risk. The surface-modified liposomes are mainly designed for targeted drug delivery in cancer patients and tumor cells. Another type of stable liposome is the Elastic liposome, which is designed for topical liposomal drug delivery. *In vitro* skin delivery of drugs can be possible by the use of elastic liposomes. The last one is the immunoliposomes. These are mainly prepared by attaching antibodies to the liposomal surfaces for targeting the tumor-specific receptor. Using thioether linkages the immunoliposomes are prepared.The study contains various data on methods of preparations, characterization, and applications of the above-mentioned stability-enhancing liposomes. The objective of this study is to give a summary of the various stability-enhancing liposomes that have been developed over time.

Keywords: Stability, Proliposomes, Ph sensitive liposomes, Surface modification, Elastic liposomes, immunoliposomes

Introduction

Drugs and bioactive substances offer immense potential for treating illnesses, reducing pain, preventing illness, or sustaining health. At the site of action, the drug bioavailability and absorption vary, however, makes the administration of medications by

oral, topical, parenteral, rectal, and nasal routes problematic (1). Currently, a variety of drug delivery methods have been created through encapsulating medications or bioactive substances in a variety of vehicles, such as liposomes (2), nanoemulsions (3), and nano-structured lipid carriers(4).The goals are to improve the quality of the systems themselves, increase drug bioavailability, and control the rate at which the medication is released into the target organ. Due to their capacity to deliver both hydrophilic and hydrophobic membranes to the membranes, long-circulating macromolecular carriers known as liposomes have emerged as the most intriguing of these carriers. The main obstacles to their development are their low physical and biological stability, which can be seen in processes including aggregation, sedimentation, fusion, phospholipid hydrolysis, and oxidation. There have been numerous attempts over time to increase the stability of liposomes. The stability issue of liposomal drug delivery systems was somewhat mitigated by the development of proliposomes, Ph-sensitive liposomes, elastic liposomes, surface-modified liposomes, and immunoliposomes (5–10).

Proliposomes

With various advantages over traditional liposomes, proliposomes are a new form of the carrier-mediateddelivery mechanism. Proliposomes have much more suitability for administrating medications since their stability significantly outweighs that of liposomes (11). When they encounter water inside the system, they transform right away into liposomal dispersion. Liposomes are created when proliposomes (PLs), which are merely soluble particles covered in liposome precursors, are dissolved in water. Liposomes are created when proliposomes (PLs), which are merely soluble particles are dissolved in water (12).

In general, aqueous suspensions of liposomes are susceptible to several negative consequences, which reduce their shelf life. These include aggregation, fusion, and phospholipid hydrolysis. It would be helpful to have a method for manufacturing liposomes fast, on-demand, and with minimal intervention, because the life span of liposomes can be constrained. The “proliposome” approach meets these needs (13). When proliposomes (PLs) are hydrated, they form liposomes because of the liposomal membrane that covers them. *Payne et al*. initially established the idea of proliposomes in 1986 (12). The bioavailability of proliposomes is improved. safeguarding pharmaceuticals against GIT deterioration. By changing the phospholipid content of bi-layers, proliposomes can be employed to regulate release within the vasculature. Targeted drug delivery and controlled drug release are made possible by proliposomes (14).

Various formulation components can be used to produce proliposomes. These may consist of phospholipids, cholesterol, some water-soluble carriers, such as sorbitol, mannitol, and others, as well as solvents, such as ethanol, ether, or chloroform (15). There are numerous ways to make proliposomes including the film deposition carrier method, spray drying method, supercritical anti-solvent method, and fluidized bed method. The most effective technique for producing proliposomes is film deposition on carriers. The proliposomes are made in this manner using a rotating flash evaporator operating under a vacuum(16).

PH-sensitive liposomes

Liposomes, which have been used frequently as medication carriers, serve as both a delivery route for encapsulated substances to enter cells and a controlled release system. Most liposomes that cells internalize do so via a pathwaythat is endocytic and then transported to the lysosome, where lipids and their contentsbreak down enzymatically(17). When given by the majority of liposome compositions described so far, substances that are broken down in or unable to leave the lysosomal compartment would remain inactive. The goal of delivering the liposomes intracellularly is

lost if lysosomotropic transport of the contents after internalization destroys the medications enclosed therein.

There have been several methods used to enhance the cytoplasmic dispersion of medicines delivered by liposomes and prevent lysosomal delivery. Subcellular site-specific delivery would benefit significantly from specially formulated liposomes that would mediate cytoplasmic delivery. When the pH of the surrounding serum changes, pH-sensitive liposomes are precisely engineered to release the medications they are filled with. As a result, they can successfully carry medication or gene fragments into the cytoplasm through the endocytotic pathway. N-palmitoyl-L-homocysteine (PHC), an acylated amino acid, was employed as the acid-sensitive component in the first report of pH-sensitive liposomes, along with di-heptadecanoyl phosphatidylcholine (DHPC) and dipalmitoylphosphatidylcholine to create the liposomes which are pH-sensitive. Thesewere made to become unstable in the low pH environment around tumors, infections, and inflammation, releasing any drugs that were imprisoned there (18).

There are primarily four categories of pH-sensitive liposomes. The first class includes slightly acidic amphiphiles that serve as stabilizers at normalpH with polymorphic lipids like unsaturated phosphatidylethanolamines. Liposomes from the second class have higher permeability to encapsulated solutes because they are made of lipid derivatives. Using pH-sensitive peptides or reconstituted fusion proteins, the third class of liposomes destabilizes thelow-pH membranes. The last and recent class of pH-sensitive liposomes uses pH-titratable polymers for destabilizing membranes once the conformation of the polymer changes at low pH(19).

The water contents of pH-sensitive liposomes are released when the system becomes acidic because they are stable at physiological pH (7.4) but become unstable and develop fusogenic qualities when the pH is low. As a result, these liposomes have the potential to increase medication delivery effectiveness by extending the period that blood circulates (20).Fluorescent markers with different molecular sizes, enzymes, ribozymes, cytotoxic chemicals,DNA,RNA,and proteins can all be delivered to cells much more effectively and efficiently using pH-sensitive liposomes as compared to other delivery methods. The disadvantages of these preparations have prevented any of them from being employed in clinical settings thus far.Thus a therapeutically viable pH-sensitive liposomal formulation needs several crucial components, such as effective pH-triggered release, stability of serum, and sufficient circulation time in vivo. Therapeutic medicines or macromolecules with intracellular targets would have been well-suited to pH-sensitive liposomes as a carrier. Additionally, creating “wise” multifunctional pharmacological nano-carriers by fusing pH-sensitive liposomes with active targeting and other mechanisms may be used in a variety of medical therapies shortly for improved performance(21).

Surface modified liposomes

Drugs can be delivered via nanoparticles and nanocarriers with greater effectiveness and less potential harm. The physical and chemical properties of liposomes, such as their size, surface charge, and lipid organization, can be changed to change how effective they are. The majority of current nanocarrier research focuses on surface modifications thatimprove the efficiency of medicine targeting.Liposomes can be further fine-tuned as nanocarriers by surface alterations and functionalization with moieties that change the range of stimuli perceived. Different surface modifications offer various advantages(22). The advantages of various surface changes vary. The reticuloendothelial system (RES), which typically presents a substantial challenge in intravenous delivery, might be avoided by modifying liposomes with polyethylene glycol (PEG), which may improve blood circulation and eliminate nonspecific

interactions(23). By offering means to get past the present physiological and biological limitations, liposome surface alterations and functionalization can significantly enhance solid tumors and cancer(24).

The shortened half-life of the liposomes is caused by the reticuloendothelial system (RES), which is known to be an important defense mechanism of the body.Opsonin a serum protein detects the liposome as a toxic entity that absorbs the liposome. A liposome that has been opsonized is destroyed by phagocytes in the RES. Liposomes' propensity to leak their contents throughout circulation presents another problem. To lengthen a liposome's circulation time, the surface of the liposome can be changed with the help of hydrophilic polymers, such as polyethyleneglycol (PEG) (25). PEGylated liposomes which are also known as stealth liposomes are the names given to these liposomes.The term "stealth" liposome refers to a liposome that has had its surface PEGylated, which makes it more stable and evasive.

PEGylated liposomes have several benefits. These redesigned nanocarriers have a longer systemic circulation duration and a reduced rate of phagocyte absorption in the RES. Because of the enhanced permeability and retention (EPR) effect and higher concentration in the tumor tissue, stealth liposomes are more accessible than non-PEGylated liposomes and are more effective at eliminating malignancies(26). The development of site-specific liposomes was made possible by the discovery of stealth liposomes. By joining additional moieties, such as antibodies and peptides, to the free ends of PEG polymers, the liposomes can participate in more precise targeting(27). Increased penetration through mucus is another benefit of PEGylation. The human body is shielded by mucus from several outside invaders, such as bacteria and viruses(28). A few polymers can cling to mucosal membrane surfaces, increasing the bioavailability. This is especially important when it comes to drug administration to the airways, as solving the problem of effective delivery of drugsthrough mucus in the airways may pave way for brand-new therapies to treat critical diseases like cystic fibrosis(29).

There are a few limitations to the PEG surface-modified liposomes. The surface affinity and nanoparticle composition must be taken into account when incorporating a specific ligand onto a nanoparticle's surface(30). Even while PEG liposomes have longer circulation durations, their enhanced stealth abilities are limited.The PEGylation effect on in vivo stability is constrained since the stealthiness of these coatings declines and these surface-modified liposomes are finally identified and eliminated by the mononuclear phagocyte system(31).

Elastic liposomes

Pharmaceutical and cosmetic businesses now confront significant obstacles. Cosmetic formulations willnot only support claims that they will change the skin's appearance, but also that they will shield it from the harshness of the environment, slow down the aging process, and significantly improve skin nourishment. Modern cosmetics need to show practicality and physiological benefits to satisfy these requirements. Due to their inherent benefits over other vesicular carriers, elastic liposomes have received the most attention irrespective of the various carriers studied for the delivery of pharmaceuticals topically over the past several years. Elastic liposomes, also called transferosomes, are double-layered vesicular systems that are compatible with biology(32).

According to claims, undamaged elastic liposomes can pass through the skin's layers and into the bloodstream, improving in vitro skin delivery. Gregor Cevc (Idea, Munich) originally identified elastic liposomes and gave them the name Transfersomes®. Amphiphilic phospholipids with a variety of chemical configurations, edge activators (such as sodium cholate, sodium deoxycholate, span,

and dipotassium glycyrrhizinate), and watery parts are the main constituents of elastic liposomesEdge activator decreases the lipid's transition temperature and disrupts the elastic liposomes'double lipid layer, increasing their fluidity and improving how they permeate the skin.The most popular phospholipids are egg phosphatidylcholine (10% w/w) and unsaturated soya phosphatidylcholine (PC)(8,33–35).

For the effective treatment of numerous diseases, researchers have extensively studied elastic liposomes for drug delivery and other therapeutic agents.When results from traditional creams were compared to those from cetrizine-loaded elastic liposomes, a significant reduction in itching has observed(36).Cyclodextrin-colchicine complex elastic liposomes showed superior anti-gout activity compared to other drug solutions(37). When compared to oral delivery, rifampicin-loaded elastic liposomes demonstrated superior pharmacokinetic characteristics(38).Different surfactants were used to load elastic liposomes with 5-fluorouracil (5-FU) to improve medication penetration through the rat skin's stratum corneum (SC) layer(39).isoniazid-loaded ELs employing a surfactant with built-in anti-tubercular action and phosphatidylcholine (PC)(40).Using Taguchi's orthogonal experimental design, the timolol-loaded transferosome formulation was assessed for its effectiveness in treating open-angle glaucoma(41).A local anesthetic-loaded transferosome formulation for the relief of dental and buccal discomfort. The formulation was created to reduce the frequency of administration and improve the safety of the local anesthetic that was provided by delivering a local effect(39).

Immunoliposomes

The fate of liposomes is greatly shaped by their diameter, with nano-sized liposomes gaining in pathological areas as a result of the enhanced permeability and retention effect (EPR), which is based on the fact that the vasculature in pathological areas is "leaky," as opposed to normal tissue(42).The following delivery through the reticuloendothelial system macrophages, phospholipids, and cholesterol compounds are quickly cleared from the circulation (RES)(43).A potential strategy to enhance the therapeutic impact of pharmacological medications in the target tissue has been developed: delivering customized liposomes with ligands attached to their surface that detect cell surface antigens or receptors in target tissues. (44,45).Any biological unit that can bind to a target can be employed; to this end, liposomes have been linked with various biological units such as vitamins, glycoproteins, peptides, oligonucleotides, oligosaccharides, antibodies, or antibody fragments. Liposomes having antibodies bound to its surfaces as targeting ligands are known as immunoliposomes because of their remarkable specificity(46–49).

It is possible to add ligands or antibodies to liposomes both during and after production. To attach antibodies and antibody fragments, the binding must occur either covalently or noncovalently. Recently, numerous chemical techniques for this attachment have been studied(50).A hydrophobic anchor group with a functional group secures the ligand to the liposomes' surface(51).To develop new drugs, it is necessary to consider how the kind and location of attachment may impact pharmacokinetics(52,53).To bind the ligands to the liposomal surfaces thioether linkages are frequently used, such as when thiols and maleimide groups react(54).Many proteins include the sulfhydryl group, but frequently there are few or no -SH groups present, therefore they must be produced from disulfide bonds already present or by adding a heterobifunctional crosslinking agent(50). Alternatively, antibodies may be linked to sterically stabilized long-circulation liposomal membranes at the distal end of the polyethylene glycol chain or in parallel with it(55,56).The utilization of recombinant antibody-binding proteins generated from streptococcal protein G by site-directed mutagenesis is a recent

breakthrough.By simply mixing with lipids, this lipoprotein can be integrated into liposomes and act as an antibody-binding site to produce useful immunoliposomes (57).

Gene delivery is carried out using numerous delivery vehicles, which include liposomes, and is a very promising approach to the treatment of cancer(58,59).When it comes to gene delivery, lipoplexes—complexes made of cationic liposomes and DNAoffer numerous benefits over other systems. Most importantly, lipoplex complexes have insufficient immunogenicity and don't have the potential to develop into new types of infectious viruses because they aren't contagious(59)(60).The creation of liposome-DNA complexes combined with anti-Transferrin receptor antibody fragments is one example of such a method (Single Chain viable fragment)(59).Comparing the single-chain viable fragment-cysteine immunolipoplexes to nonmodified complexes or those connected exclusively with single-chain antibody fragments, they greatly improved the binding to tumor cells (single-chain viable fragment)(61).As a non-invasive, non-viral trans-vascular brain gene delivery technique, Trojan horse immunoliposomes (THL) offer a potential novel belief and have been used for plasmid DNA encapsulation(62). The components of Trojan horse immunoliposomes includenon-viral gene expression plasmids, pegylated immunoliposomes enclosed in carriers, and endogenous peptide or peptidomimetic monoclonal antibodies that function as Molecular Trojan horses (MTHs) and travel through the BBB through receptor-mediated transport(63).

The majority of immunoliposome research is based on various cancer treatments. Most immunoliposome research on breast cancer focuses on the ERBB2-encoded human epidermal growth factor receptor 2. HER2 is the more popular name for this, and it is amplified in 18–20% of breast cancers(64). HER2 overexpression has been observed in various additional cancer types, including those of the brain, lungs, prostate, bladder, and gastrointestinal system (65–67).High levels of HER2 is seen in cancer cells of the breasts (BT-474 and SK-BR-3) and low levels of HER2 (MDA-MB-231), respectively, were treated with a method involving an immunoliposomes coupled withencapsulated paclitaxel and anti-HER2 antibody trastuzumab (Herceptin®) (68,69).The second most common cause of cancer-related deaths, behind lung and breast cancer, is colon cancer. The only form of treatment that effectively removes initial tumors is surgery (70). Recombinant humanized anti-TAG-72 monoclonal antibody (HuCC49) Fab fragments were conjugated to liposomes which are sterically stabilized containing plasmid DNA as one illustration of an immunoliposome in cancer therapy of colon (pDNA) (71).When used to attach to TAG-72 overexpressed LS174T human colon cancer cells, this Immunoliposome outperformed conventional liposomes. Leukemia is a disease for which chemotherapy is the main form of treatment, but immunoliposomes have also been found to be helpful. However, in recent times, the treatment of leukemia has made significant strides. Clinically authorized antibodies, such as alemtuzumab and ritiximab, have shown encouraging results (72,73).

Characterization

The characterization of liposomes depends on a number of factors. A key feature is their particle size. Scanning electron microscopy can be used to analyze the size distribution and surface appearance of particles (74). Zeta potential is a further property that is quite intriguing. It serves as a measure of article charge; the more surface charge there is, the higher the zeta potential absolute value (75). By forming liposome dispersion, separating the drug that hasn't been entrapped, and calculating the amount of drug entrapped, the efficiency of drug entrapment is carried out (76). When a drug is formulated it transforms from crystalline to amorphous, and this can be

detected by differential scanning calorimetry (DSC) and powder X-ray diffractometry (PXRD) (77). A Franz diffusion cell is used to conduct an in vitro drug release assessment test. The substance is deposited on a dialysis membrane that is kept in the diffusion cell's donor and receiver compartments(78).

Liposomal Drugs approved by Regulatory authorities:-

Brand Name	Year Of Authorization	Active Constituents	Therapeutic Indication	Adverse Drug Reaction	References
ARIKAYCE®	2020	Amikacin	pulmonary infections brought on by Mycobacterium avium Complex that are non-tuberculous mycobacterial (NTM).	Severe renal Impairment	(79)
AVASTIN®	2005	Bevacizumab	Epidermal Growth Factor Receptor (EGFR) activating mutations in metastatic carcinoma of the colon or rectum, metastatic breast cancer, or metastatic or recurrent non-squamous non-small cell lung cancer.	Pregnancy, recombinant human or humanized antibodies, or hypersensitivity to Chinese Hamster Ovary (CHO) cell products	(80)
VELCADE®	2004	Bortezomib	Multiple myeloma, Mantle cell lymphoma.	Acute diffuse infiltrative pulmonary and pericardial disease	(81)
EXPAREL®	2020	Bupivacaine	somatic post-operative pain from small- to medium-sized surgical wounds in adults.	Obstetrical paracervical block anesthesia due to risk of foetal bradycardia or death, Renal Impairment, Hepatic Impairment	(82)
MYOCET®	2000	Combination of Doxorubicin and Cyclophosphamide	Metastatic Breast Cancer	Neutropenic fever, neutropenia, thrombocytopenia	(83)
VYXEOS™	2018	Combination of Daunorubicin and cytarabine.	Therapy-related acute myeloid leukemia. (t-AML)	Genotoxicity, carcinogenicity, and reproductive and developmental toxicity	(84)
YONDELIS®	2007	Trabectedin	Soft tissue Sarcoma, Relapsed platinum-sensitive ovarian cancer.	Hepatic Impairment, Renal Impairment, Thrombocytopenia, Neutropenia	(85)
ONIVYDE®	2016	Irinotecan	Metastatic adenocarcinoma of the pancreas.	Hepatic and Renal toxicity, Leukopenia, Neutropenic Fever	(86)
COMIRNATY®	2020	Tozinameran (mRNA)	Injection for active immunization to prevent COVID-19 caused by SARS-CoV-2.	Hypersensitivity and anaphylaxis, Myocarditis and pericarditis	(87)
DOXIL®	1995	Doxorubicin hydrochloride (HCl)	Ovarian Cancer, AIDS-Related Kaposi's Sarcoma, Multiple Myeloma.	Hand-Foot Syndrome (HFS), Stomatitis, Neutropenia or Thrombocytopenia	(88)
ONPATTRO™	2018	Patisiran(siRNA)	Hereditary Transthyretin amyloidosis.	Upper respiratory tract infections and infusion-related reactions.	(89)

liposomal technologies to develop stable liposome

Conclusion

The advantages of employing liposomes as a drug delivery technology include their biocompatibility, capacity to transport large drug payloads, ability to self-assemble, and a variety of physicochemical and physical properties that can be changed to influence their biological aspects. But liposomes have several stability issues. So some modified liposomes are prepared like proliposomes, pH-sensitive liposomes, Elastic liposomes, etc. Proliposomes represent a significant advance in the treatment of the bioavailability,stability and solubility of poorly soluble pharmaceutical problems correlated with liposomes. Additionally, they offer a non-invasive way to distribute drugs through or into the skin. The effectiveness of cytoplasmic transport of different fluorescent markers with different molecular sizes, ribozymes, proteins,enzymes, cytotoxic agents, RNA, and DNA to cells can be greatly increased using pH-sensitive liposomes. Elastic liposomes have been used to enhance not just the physicochemical characteristics of the medications they contain, but also the pharmacokinetic and pharmacodynamic characteristics of such pharmaceuticals in both animal and human studies. Immunoliposome development has led to applications for diagnosis and therapy in a variety of medical fields. More research on these stabilized liposomes can lead us to the treatment of various untreated diseases like Cancer.

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