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Review Paper

Liposomes as Drug Delivery Vehicles: Insights into Development and Clinical Use

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ABSTRACT

Liposomes are a novel nanostructure used for encapsulating and delivering bioactive agents. They can incorporate a variety of bioactive materials, such as cosmetics, food ingredients, and pharmaceuticals. The first type of nanomedicine to be licensed for clinical use was liposomes. The phospholipid bilayer encircles the empty water center of these spherical vesicles. medications' bio-distribution profiles are altered when liposomes are used for therapeutic administration, improving the medications' therapeutic index. These nano drug delivery systems are the subject of extensive research for a number of uses, including as the delivery of therapeutic genes and anti-inflammatory, anti-fungal, and anti-cancer medications. A comprehensive overview of the history and development of liposomes is given in this bibliographic study, with particular emphasis on preparation techniques such as thin-film hydration, reverse-phase evaporation, ethanol injection, and innovative scalable procedures. This paper reviews the main physicochemical characteristics of liposomes, talks about how they are currently made, and investigates how they might be used as food nanotechnology carriers of nutrients, enzymes, and antimicrobials. We also look at their uses in biomedicine as gene delivery agents and medication carriers. This review also examines the benefits of integrating drugs into the lipid domain of vesicles.

INTRODUCTION

Paul Ehlrich coined the term “the magic bullet concept” in the 1960s to describe the idea of safe and effective drug delivery with the progress in nanotechnology, new drug delivery methods were developed to truly realize this concept. The pharmaceutical sector has seen a radical change because to the liposomal medication delivery system Alec Bangham published the first description of liposomes in 1961 [1]. Since then, a great deal of study has been done in the subject of liposomes, and its uses in drug, biomolecule, and gene delivery are now well- established. The

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multidisciplinary topic of nanotechnology encompasses the invention, processing, and application of materials or devices with dimensions in the nanoscale range. Targeted drug delivery, in contrast to conventional pharmaceuticals, is designed to effectively transport a medication to the desired site of action in order to maximize therapeutic efficacy and reduce side effects. Because of their versatility, liposomes have garnered the most interest among all the carrier systems available. Because they may be easily adjusted to fit a variety of delivery needs, their adaptable physicochemical and biophysical features make them an appealing delivery strategy. Liposomes are spherical vesicles made of bilayers that can be made with ingredients including cholesterol, glycolipids, non-toxic surfactants, and others. Their diameter normally ranges from 0.01 to 5.0 μm [1,2]. Because they may be easily adjusted to fit a variety of delivery needs, their adaptable physicochemical and biophysical features make them an appealing delivery strategy. The application of nanoparticles is particularly beneficial in the diagnosis application of nanoparticles is particularly beneficial in the diagnosis and treatment of cancer, as they exhibit prolonged action, high bioactivity, and enhanced cellular penetration [2].

Furthermore, nanoparticles allow for adjustable release rates and result in fewer adverse effects on healthy tissues. Additional advancements in nanotechnology include the development of nanocarriers. The primary types of nanocarriers consist of micelles, dendrimers, protein-based carriers, and liposomes, all of which can encapsulate drugs within their structure. While these entities can be classified as nanoparticles, certain organic nanocarriers, such as liposomes, may exceed the conventional size limit of 100 nanometers The creation, manipulation, and use of materials or apparatuses with dimensions in the nanoscale range are all included in the multidisciplinary field of nanotechnology. Unlike traditional medications, targeted drug delivery aims to deliver a treatment to the intended site of action with maximum therapeutic efficacy and minimal side effects. Of all the available carrier systems, liposomes have attracted the greatest attention due of their adaptability. They are an attractive delivery technique due to their versatile physicochemical and biophysical characteristics, which may be readily modified to suit a range of delivery requirements. Liposomes are spherical vesicles consisting of bilayers, which can be constituted from various constituents such as glycolipids, cholesterol, and non-toxic surfactants. Typically, their diameter falls between 0.01 to 5.0 μm. It has been demonstrated that encapsulating medications into liposomes increases the therapeutic efficacy of a variety of medicines, mostly by altering their pharmacokinetic and pharmacodynamic characteristics. The multidisciplinary field of nanotechnology encompasses the development, manipulation, and utilization of materials or equipment having dimensions in the nanoscale range. Targeted drug delivery, in contrast to conventional medicine, seeks to maximize therapeutic efficacy and minimize side effects by delivering a treatment to the intended site of action. Because of their versatility, liposomes have garnered the most interest among all the available carrier systems. Their adaptable physicochemical and biophysical properties, which can be easily adjusted to meet a variety of delivery needs, make them a desirable delivery method. Liposomes are spherical vesicles made of bilayers that can be made of a variety of materials, including cholesterol, glycolipids, and safe surfactants. Their diameter usually ranges from 0.01 to 5.0 μm [3,4].

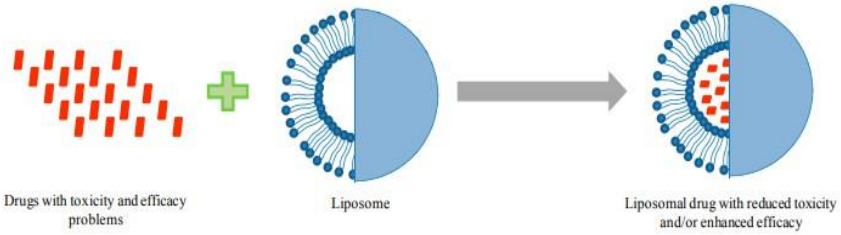

Figure 1. Schematic representation showing the advantages of formulating drugs in liposomes

ADVANTAGES

- Offers liposomal doxorubicin, a selective passive target for tumor tissue.
- Liposomes are immunogenic non-toxic, fully biodegradable, and biocompatible.
- Suitable for the administration of medicines that are hydrophilic, amphiphilic, and hydrophobic.
- Keep the medication enclosed safe from the outside world
- Enhance effectiveness and treatment index
- Enhancement of stability by encapsulation
- Minimize the amount of hazardous drug exposure to delicate tissue
- Boost the stability of proteins.
- Offer a prolonged release.
- Modify the drug's pharmacokinetic and pharmacodynamic properties [5].

DISADVANTAGES

- There is a significant production cost.
- Leakage and fusion of pharmaceutical encapsulation.
- Reactions similar to oxidation and hydrolysis can occasionally occur with phospholipids.
- Limited half-life.
- Insufficient solubility, less stability and rapid absorption by R.E.S. cells
- Reactions with liposomal components may be allergic.
- Difficulty in targeting different tissues because of their size [5,6].

CLASSIFICATION OF LIPOSOMES

➢ **The liposomes classification based on: -**

i. Based on structure
ii. Conventional liposomes
iii. Constituent and its Application
iv. Preparation process
v. Liposome specialty

1) Based on structure

Table 1: diameter side and number of lipids layers of different vesicles

Vesicles types	Abbreviation	Diameter size	No. of lipids layers
Unilamellar vesicles	UV	All size ranges	one
Small Unilamellar vesicles	SUV	20 – 100 nm	one
Medium Unilamellar vesicles	MUV	More than 100 nm	One
Large Unilamellar vesicles	LUV	More than 100 nm	one
Giant Unilamellar vesicles	GUV	More than 1.0 µm	one
Oligolamellar vesicles	OLV	0.1-1.0 µm	Approx 0.5
Multilamellar vesicles	MLV	More than 0.5µm	5-25
Multi vesicular vesicles	MV	More than 1.0µm	Multi compartmental structure

2) Based on method of preparation

Table 2: Different methods of preparation and vesicles developed by those methods

Preparation methods	Vesicle type
Lamellar vesicle of single or oligo formed by reverse phase evaporation	REV
Multi lamellar vesicles formed by the method of reverse phase evaporation	MLV-REV
Stable plurilamellar vesicles	SPLV
Frozen and thawed multi lamellar vesicle	FATMLV
Vesicle prepared by extrusion technique	VET
Dehydration –Rehydration method	DRV

3) Based on composition and application

Table 3: Different Liposomes with their compositions

Types of liposomes	Abbreviations	Compositions
Conventional	CL	Neutral or negatively charged phospholipids and cholesterol
Fusogenic	RSVE	Reconstituted Sendai virus envelops
pH sensitive	-	Phospholipids such as DOPE or PER with either OA or CHEMS
Cationic	-	Cationic lipid with DOPE
Long circulatory	LCL	Neutral high temperature, cholesterol, and 5-10% PEG, DSP
Immuno	IL	CL or LCL with monoclonal antibody linked or sequences of recognition

4) Based upon conventional liposome

i. Glycolipid loaded liposomes
ii. Normalized mixture of Lecithin (PC)
iii. Identical-chain synthetic phospholipids to naturally occurring phospholipids.
i. lipoprotein coated
ii. Bipolar fatty acid
iii. Carbohydrate coated
iv. Methyl/ Methylene x- linked
v. Antibody directed
vi. Multiple encapsulated

5) Based upon specialty liposome

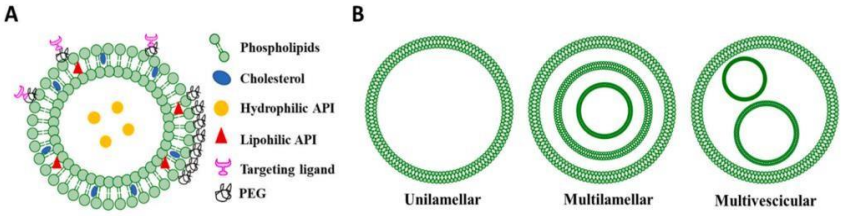

Figure 2. A. Representative liposome structure. A lipid bilayer containing an aqueous core is formed through the self-assembly of cholesterol and phospholipids. Drugs that are hydrophilic are contained in the aqueous core, while drugs that are lipophilic are found in the lipid bilayer. PEGylation of the particle surface is frequently followed by the functionalization of liposomes, i.e., with certain ligands. B. Schematization of liposome subtypes that are most common.

APPLICATION: -

The recent advancements in DNA vaccination and the success of gene therapy represent just a few of the innovative applications of liposomal treatments. Various methods for drug administration utilizing liposomal delivery

systems have been proposed, including the following:

I. Increasing the solubility of drugs (e.g., Paclitaxels, Amphotericin-B, Minoxidil, Cyclosporins)
II. Preserving sensitive drug molecules (such as RNA, DNA, ribozymes, cytosine arabinoside, and anti-sense oligonucleotides).
III. Enhancing intracellular absorption of medications (such as antibiotics, antitumor agents, and antiviral agents)
IV. Modification of pharmacokinetics and biodistribution (e.g., formulations for medicines with short circulatory half-lives that release slowly or continuously) [7,8,9]

❖ Liposomes in Respiratory Drug Delivery Systems

Liposomes are frequently used to treat a range of respiratory ailments. Liposomal aerosols are formulated to minimize side effects, reduce local inflammation, deliver the medication over an extended period of time, and improve stability in the large aqueous core. There are currently a number of injectable liposome-based medications on the market, including Myocet, Fungisome, and Ambisome. A number of variables, including lipid composition, charge, size, drug-to-lipid ratio, and delivery techniques, can affect how well liposomal drug administration works in the lungs [10]. A developing awareness of liposomes' role in macromolecule inhalation is demonstrated by their increasing use in delivering DNA to the lungs. This new information can be used to improve protein compositions based on liposomes. For liposomal inhalation, both liquid and dried forms of the medicinal ingredient are used. Drug powder liposomes have been made using methods like milling and spray drying [11,12].

❖ Liposomes in Eye Disorders

For a long time, liposomes have been used to treat diseases that impact the anterior and posterior portions of the eye. This therapy technique addresses a number of ocular problems, such as endophthalmitis, proliferative vitrreoretinopathy, dryness, keratitis, and corneal graft rejection.1. The primary cause of blindness is abnormalities of the retina [12]. Liposomes are used as monoclonal antibody carriers as well as genetic transfection vectors. Novel approaches have been developed for the treatment of certain malignancies and diseases include neovascular artery blockage, angiography, and stasis of retinal and choroidal blood vessels. Among these is the application of concentrated laser therapy in combination with heat-activated liposomes. Representative liposome structure. Cholesterol and phospholipids self-assemble to form an aqueous core-enclosing lipid bilayer. lipophilic medications 'Verteporfin' is a commercially available liposomal drug specifically designed for ocular applications [13,14,15]

❖ Liposome as Vaccine Adjuvant

Liposomes are a widely recognized immune system adjuvant that greatly improve humoral and cell-mediated immunity. After being injected intramuscularly, these liposomal adjuvants work by progressively and passively releasing encapsulated antigens into the targeted lymph nodes [16]. Phosphatidylserine helps to enhance the targeting of liposomes to lymphoid tissues. By combining liposomes with soluble antigens, microorganisms, and deoxyribonucleic acid (DNA), a liposomal vaccination can be created. This process elicits an immunological response to the expression of antigenic proteins, which subsequently form covalent bonds with the liposomal membrane. Furthermore, liposomal vaccines can be stored in a refrigerated environment for approximately 12 months. The role of liposomes is expected to expand in the

future, encompassing therapeutic, diagnostic, and research dimensions within the field of ophthalmology [17,18].

❖ Liposomes for Targeting the Brain

Recently, due to their biocompatibility and biodegradability, liposomes are a widely used method of delivering drugs to the brain. The Blood-Brain Barrier can be crossed by liposomes (BBB) with ease, regardless of their size they are roughly 100 nm across. Conversely, receptor-mediated or absorptive-mediated transcytosis allows tiny unilamellar vesicles (SUVs), linked to cognitive drug transporters, to pass through the blood-brain barrier. Although cationic liposomes are taken up by cells by absorptive-mediated endocytosis, there is no proof that absorptive-induced transcytosis occurs across the blood-brain barrier. Liposomes coated with mannose have the ability to penetrate the brain and aid in the passage of drugs across the blood-brain barrier. Typically, when administered systemically, substances such as Leu-enkephalin, mefenkephalinykoforphin, and neuropeptides do not effectively penetrate the blood-brain barrier. However, due to the adaptability of this method, the antidepressant amitriptyline is generally able to cross the BBB. Various stabilizers have been utilized to develop nanoparticles (NP). Notably, the concentration of amitriptyline in the brain was significantly enhanced when the drug was adsorbed onto the NP, which remained stable when combined with polyoxyethylene 20 sorbitan trioleate [19,20,21].

❖ Liposomes as Anti-Infective Agents

Protozoa, bacteria, and fungi are examples of intracellular pathogens that dwell in the liver and spleen. Therefore, liposomes can be used as a transport mechanism to effectively transfer therapeutic drugs to these organs in order to eradicate these diseases. Treatments for diseases including tuberculosis, malaria, aspergillosis, giardiasis, candidiasis, erythrococosis, leishmaniasis, and histoplasmosis can be administered by combining and directing the drug with a liposomal carrier [22]. The polyene antibiotic amphotericin B has a reputation for seriously harming the kidneys when used to treat systemic fungal infections. This antibiotic increases the effectiveness of susceptible fungus by binding to sterols in their membranes. The non-specific character of the chemical and its affinity for cholesterol in mammalian cells make it dangerous. Amphotericin B's initial liposomal formulation has recently completed all required clinical trials and is on hand to treat a range of fungal illnesses, such as aspergillosis, blastomycosis, mucormycosis (black fungus), candidiasis, coccidioidomycosis, and cryptococcosis. By passively targeting the liver and spleen, at regular dosing, liposomal amphotericin B reduces overall and renal toxicity. On the other hand, high doses of the medication may cause renal toxicity because they can saturate the macrophages in the liver and spleen [22, 23]. Liposomes can be targeted to the lungs precisely by using coatings like monosialoganglioside, polyoxyl ethylene, or o-stearoyl amylopectin. Antituberculous drugs such as rifampicin and isoniazid that are encapsulated in lung-targeted It has been demonstrated that liposomes increase efficacy and decrease toxicity. Many amphotericin formulations are presently available for purchase in a number of European countries after being approved in a number of clinical trials.

❖ Liposome in Tumor Cell Therapy

Anti-cancer medications often have severe adverse effects if taken for an extended length of time. By reducing these negative effects, liposomal therapy, which targets tumor cells, has greatly revolutionized the treatment of cancer. Although these stable, small liposomes are intended for

passively targeting different types of tumors, they can also extravasate into tissues that have increased vascular density and stay in the bloodstream for long periods of time. The liver and spleen's macrophages' absorption of liposomes has impeded their growth as a method of delivering drugs for more than 20 years. Many natural anticancer drugs have been encapsulated recently. within liposomes to boost bioavailability and optimize targeting [24, 25].

❖ ADVANCEMENT IN LIPOSOME TECHNOLOGY: -

- Ethosomes: These are effective in supplying 30% ethanol and soy phosphatidylcholine to the dermal layers.
- Immuno- liposomes: Antibodies have been added to these liposomes through modification.
- Niosomes: Small unilamellar vesicles formed of non-ionic surfactants make up these.
- Stealth liposomes: This novel class of liposomes is intended to improve stability and lengthen the circulation's half-life. These liposomes are prepared by covering them with polyethylene glycol (PEG) [26, 27].

❖ LIPOSOMES IN BIOMEDICAL RESEARCH APPLICATIONS: AN INVESTIGATION

There is promise for using liposomes in medicine for creating cutting-edge, efficient therapies for a range of diseases. Lipid-based therapeutic carriers have been the subject of a significant rise in research during both in vitro and in vivo experiments. Numerous therapeutic and diagnostic agents, such as bioactive compounds, gene therapy, and therapeutic molecules, are delivered by liposomes [28]. Changes in the charge and content of lipids as well as the addition of ligands and surface coatings are being investigated. Active targeting strategies, which involve early stages of research, binding targeting ligands to the liposome surface, have been thoroughly investigated, especially after parenteral administration [29].

❖ MARKETED EXAMPLES: -

Figure 3. Therapeutic domains where liposome-based medicines are used

1995 marked the release of Doxil® onto the American market, a significant achievement in liposome-based products, specifically for the treatment of patients with AIDS-related Kaposi's sarcoma and ovarian cancer, especially when prior systemic chemotherapy had not worked or was not tolerated [30]. Gabizon and Barenholz started the Doxil® development process in both Israel and the US.The first liposomal formulation in nanosize to be approved by regulators was this product. After that, NeXstar Pharmaceuticals USA introduced DaunoXome®, another liposomal medication that was licensed by the FDA in 1996 for the administration of daunorubicin (DNR) to treat advanced HIV-associated Kaposi's sarcoma [31, 32]. Following the first release of Depocyt® by SkyPharma Inc., Myocet® by Elan

Pharmaceuticals, Mepact® by Takeda Pharmaceutical, and Marqibo® by Talon Therapeutics, a number of other medications were released for the treatment of different malignancies. More recently, Merrimack Pharmaceuticals, Inc. approved a treatment known as OnivydeTM for metastatic pancreatic cancer, based on a combined therapy of fluorouracil and leucovorin. Clinically approved liposomal treatments have primarily targeted cancer, but other medical disorders have also been the subject of development. For example, the use of liposomal versions of Amphotericin B (AmB) greatly improved the management of fungal infections when the U.S. FDA approved Ambisome® and Amphotec® in 1996 and 1997, respectively. Furthermore, liposomes have emerged as crucial delivery systems in vaccine development, with a notable increase in interest surrounding liposomal vaccines, exemplified by the products Epaxal® and Inflexal® V, both developed by Crucell and Berna Biotech for immunization against hepatitis and influenza, respectively [33,34,35].

Table 4: An overview of liposomal products that the FDA (US) and EMA (EU) have approved, omitting lipid medication combinations

Type	Name	API	Approved year / area	Applications
Cancer therapy (drug formulation)	Doxil®/ CaelyxTM	Doxorubicin	1995(US)1996(EU)	Ovaria,breast cancer and Kaposi'sarcoma
	DaunoXome®	Daunorubicin	1996(US,EU)	Kaposi's sarcoma
	Onivyde®	Irinotecan hydrochloride trihydrate	1996(US)2016(EU)	Pancreatic adenocarcinoma Breast
	Myocet®	Doxorubicin Mifamurtide	2000(EU)	cancer Osteosarcoma
	Mepact®	Vineristine	2009(EU)	Leukemia Leukemia
	Marqibo®	Daunorubicin +cytarabine	2012(US)	Breast and ovarian
	Vyxeos®	Doxorubicin	2017(US)2018(EU)	cancer,multiple myeloma,
	Zolsketil®		2022(EU)	kaposi's sarcoma
Other application (drug formulation)	AmBisome®	Amphotericin B Cytarabine	1997(US,EU)	Fungal infection
	DepoCyt®	Verteporphin Morphine sulfate	1999(US)2001(EU)	Lymphomatous meningitis AMD
	visudyne®	Amikacin Bupivacaine	2000(US,EU)	Pain management Lungs
	DepoDur®		2004(US,EU)	infections anaesthesia
	Arikayce®		2018(US,EU)	
	Exparel®		2020(EU)	
Vaccine	Epaxal®	Inactivated hepatitis A virus(RG-SB strain)	1994(EU)	Hepatitis A Influenza
	Inflexal V®	Influenza virus surface antigens(haemagglutin in and neuraminidase),	1997(EU)	Malaria
	MosquirixTM	virosomal 3 different strains	2015(EU)	Shingles and port-herpetic Neuralgia
	shingrix®	proteins found on the surface of the plasmodium falciparum parasites and the hepatitis B virus	2017(US)2018(EU)	COVID-19
	Comirnatytm	recombinant Varicella-zoster virus glycoprotein E	2021(US,EU)	
		mRNA		

DISCUSSION AND CONCLUSION

The current research has shown that liposomes represent a distinctive drug delivery system, capable of facilitating controlled and targeted drug administration. With a variety of extremely potent therapeutics, the liposomal approached liposomal approach can reduce their toxic side effects while also improving pharmacokinetics and therapeutic efficacy. The PEGylated liposomal formulation Doxil® marked the significant market debut of liposomes in 1995. Since then, there has been a surge in interest in these delivery methods, which are now being researched for a variety of ailments, such as the treatment of cancer and pain management. Improved control over pharmacokinetics and pharmacodynamics, greater bioavailability, and decreased toxicity are the main advantages of liposomes. When combined, these characteristics allow liposomes to overcome the drawbacks of conventional treatments. The field of liposome research has yielded a variety of liposome products, including PEGylated liposomes (Lipodox), temperature-sensitive liposomes (ThermoDox), cationic liposomes (EndoTAG-1), and liposomal vaccines (Epaxal and Inflexal V). Numerous liposomal formulations have successfully transitioned into clinical settings, while others are currently undergoing different stages of clinical trials. In conclusion, the liposomes presently undergoing clinical trials hold the potential to offer significant benefits to a diverse patient population across various therapeutic contexts.

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