

Advancement and Patents on Liposomal Drug Delivery

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Abstract: Liposomes derived from Greek: 'Lipos' means fat and 'Soma' means body. Liposomes increase efficacy and therapeutic index of drug, stability via encapsulation. They are biocompatible and completely biodegradable in nature. On structure parameters, liposomes are classified into large unilamellar, medium lamellar, giant unilamellar, oligolamellar etc. On preparation parameters, liposomes are classified into Single or oligolamellar vesicle made by reverse phase evaporation method, Multilamellar vesicle made by reverse phase evaporation method, Stable plurilamellar vesicle. On Composition and application, liposomes are classified into conventional liposome, fusogenic liposome, cationic liposome, long circulatory liposome etc. Liposomes are prepared by passive loading technique and active loading techniques. Various marketed formulations are available in market of liposomes for different diseases.

Key words: Liposomes • Method Of Preparation • Therapeutic Application • Marketed Formulation • Patents

INTRODUCTION

Liposome is derived from Greek: 'Lipos' means fat and 'Soma' means body [1]. They consist of microscopic spherical one or more lipid bilayer surrounding aqueous volume enclosed by lipid volume, where the polar head groups are oriented in the pathway of the interior and exterior aqueous phases. Particle sizes of liposomes are ranging from 30 nm to several micrometers [2]. Liposomes consist by natural or synthetic phospholipids. Liposomes have been known for their considerable potential such as biocompatibility, non-immunogenicity and biodegradable properties as drug carriers. Due to their inherent structural specificities and properties, Liposomes are capable of encapsulating hydrophilic drugs inside their aqueous phase and hydrophobic drugs inside their phospholipids bilayers [3, 4].

Advantages of Liposome: There are various advantages of liposomes;

- Liposomes have efficacy and therapeutic index of drug (Actinomycin-D).
- Liposomes increase stability via encapsulation.
- Liposomes are biocompatible, completely

biodegradable, non-toxic, flexible and non immunogenic for systemic and non-systemic administrations.

- Liposomes reduce toxicity because of its capsulation character (Amphotericin B, Taxol).
- Liposomes help to reduce exposure of sensitive tissues to toxic drugs.
- Site avoidance effect.
- Flexibility to couple with site-specific ligands to achieve active targeting.

Disadvantages of Liposomes: There are some disadvantages of liposomes;

- Production cost is high.
- Leakage and fusion of encapsulated drug /molecules.
- Sometimes phospholipids undergo oxidation and hydrolysis like reaction.
- Short half-life.
- Problem to targeting to various tissues due to their large size.
- Low solubility, less stability [5].

Classification of Liposome: Liposomes can be classified on the bases of following parameters;

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Type 1: On structure basis, liposome classified into [6]:

Abbreviation	Vesicle type	Diameter range
LUV	Large unilamellar	More than 100 nm
MUV	Medium unilamellar	More than 100 nm
SUV	Small Unilamellar	20-100 nm
GUV	Giant unilamellar	More than 1 micrometer
OLV	Oligolamellar	0.1 to 1 micrometer
MLV	Multi lamellar	More than 0.5 micrometer
MV	Multi vesicular	more than 1 micrometer

Type 2: On preparation, liposome classified into:

REV	Single or oligolamellar vesicle made by reverse phase evaporation method
MLV-REV	Multilamellar vesicle made by reverse phase evaporation method
SPLV	Stable plurilamellar vesicle
FATMLV	Frozen and thawed MLV
VET	Vesicle prepared by extrusion technique
DRV	Dehydration rehydration method

Type 3: On the basis of composition and application, liposomes classified into:

Conventional liposomes	Neutral or negatively charge phospholipid
Fusogenic liposomes	Reconstitute sendai virus envelop
Cationic liposomes	Cationic lipid
Long circulatory liposomes	Neutral high transition temperature liposome
pH sensitive liposomes	Phospholipid like phosphatidyl ethanolamine
Immuno liposomes	Long circulatory liposome with attached monoclonal antibody

Method of Preparation of Liposomes: There are different methods of preparation of liposomes;

Passive Loading Techniques: This technique includes three different methods;

Mechanical Dispersion Method:

- Lipid film hydration by hand shaking, non hand shaking or freeze drying
- Micro-emulsification
- Sonication
- French pressure cell
- Membrane extrusion
- Dried reconstituted vesicles
- Freeze-thawed liposome

Solvent Dispersion Method:

- Ether injection
- Ethanol injection
- Double emulsion vesicles

- Reverse phase evaporation vesicles
- Stable plurilamellar vesicles
- Detergent removal method
- Detergent (cholate, alkylglycoside, Triton X-100) removal form mixed micelles
- Dialysis
- Column chromatography
- Dilution
- Reconstituted sendai virus enveloped vesicles

Active Loading Techniques [7, 8]

Marketed Formulation: There are different formulations of liposomes available in market;

S.No	Product	Drug	Company
1	Ambisome	Amphotericin B	Nexstar Pharmaceutical
2	Abelcet	Amphotericin B	The Liposome Company
3	Amphocil	Amphotericin B	Sequus Pharmaceuticals
4	Doxil	Doxorubicin	Sequus Pharmaceuticals
5	Daunoxome	Daunorubicin	Nex Pharm
6	Mikasome	Amikacin	Nexstar Pharmaceuticals
7	Epaxal	Hepatitis A Vaccine	Swiss Serum Institute
8	ELA-Max	Lidocaine	Biozone Labs
9	Depocyt	Cytarabine	Pacira(Formerly Skyepharma)
10	Myocet	Doxorubicin	Zeneus
11	Estrasorb	Micellular Estradiol	Novavax
12	DC99	Doxorubicin	Liposome Co.
13	Visudyne	Verteporfion	Novartis
14	DepoDur	Morphine	Skyepharma
15	Inflexal V	Influenza Vaccine	Berna Biotech

Therapeutic Application of Liposome: There are many therapeutic applications of liposomes;

Liposome as Drug/protein Delivery Vehicles:

- Controlled and sustained drug release
- Enhanced drug solubilisation
- Alter pharmacokinetics and bio distribution
- Enzyme replacement therapy and bio distribution
- Enzyme replacement therapy and liposomal storage disorder

Liposome in Antimicrobial, Antifungal and Antiviral Therapy:

- Liposomal drugs
- Liposomal biological response modifiers

Liposome in Tumour Therapy:

- Carrier of small cytotoxic molecules
- Vehicle for macromolecules as cytokines or genes

Liposome in Gene Delivery:

- Gene and antisense therapy
- Genetic (DNA) vaccination

Liposome in Immunology:

- Immunoadjuvant
- Immunomodulator
- Immunodiagnosis
- Liposome as artificial blood surrogates.
- Liposome as radiopharmaceutical and radio diagnostic carriers.
- Liposome in cosmetics and dermatology.
- Liposome in enzyme immobilization and bioreactor technology [9, 10, 11].

Patents of Liposomes:

- Yuanpeng Zhang *et al.* [12]: Liposome formed by cold process with active agent cold at room temperature, in the absence of heat and /or in the absence of a heating step. Liposome's particles size was 200-500 nm. It included at least about 18 w/w % vesicle-forming lipids. The vesicle-forming lipids were at least about 45-50% phosphatidylcholine (derived from soy or egg). It also included thickeners and/or emulsifiers *i.e.* xanthan gum and Tween™ 80. This method was used to treat cancer, hepatic dysfunction, malignancies, AIDS, trauma, burns, sepsis, pulmonary disease, Parkinson's disease, diabetes, Alzheimer's disease, schizophrenia, heart attack, seizures, chronic fatigue syndrome, diabetes, liver disease, inflammation, obesity, cancer and hypertension in a subject with ALA entrapped in a liposome was provided.
- Samuel Zalipsky *et al.* [13]: This method included a liposome composed of the peptide boronic acid compound bortezomib. A suspension of liposomes having bortezomib entrapped in the form of a peptide boronate ester. Liposomes were formed of a vesicle-forming lipid and entrapped in the liposomes. It further comprised with a higher inside / lower outside ion gradient. When the ion gradient was a pH gradient, the inside pH of the liposomes could be between about 7.5-8.5 and outside about 6-7. It further included between about 1-20 mole percent of a hydrophobic moiety dehydrated with a hydrophilic polymer. Hydrophobic moiety covalently linked to a hydrophilic polymer, a preferred polymer

was polyethylene glycol. A preferred hydrophobic moiety is a lipid and is preferably a vesicle-forming lipid.

- Mantripragada B. Sankaram *et al.* [14]: It included the process involved encapsulating at least one biologically active substance and optionally an osmotic spacer in a first aqueous component encapsulated within the liposomes. It disclosed that multivesicular liposomes (MVL's) containing biologically active substances which had defined size distribution, adjustable average size, adjustable internal chamber size and number and a controlled and variable release rate of the biologically active substance. The rate of release of the active substance introduced liposomes, decreased by increasing the osmolarity of the first aqueous component or increased by decreasing the osmolarity.
- Michael B. Chancellor *et al.* [15]: The present invention composed of liposomes containing sphingomyelin or sphingomyelin metabolites to prevent, manage, ameliorate and/or treat disorders involving neuropathic pain and aberrant muscle contractions etc. It also provided liposome-based delivery of drugs, e.g., antibiotics, pain treatments and anticancer agents, to the bladder, gastrointestinal system, pulmonary system and other organs or body systems. In particular, liposome-based delivery of vanilloid compounds, such as resiniferatoxin, capsaicin, or tinyatoxin and toxins, such as botulinum toxin is provided for the treatment of bladder conditions, including pain, inflammation and incontinence and voiding dysfunction.
- Luke S.S. Guo *et al.* [16]: The liposome contained about 10-40 mole percent of an amine-derivatized lipid used to improve retention ophthalmic drug delivery and dry eye treatment. The liposome preferably had a close packed lipid structure produced. The liposome might be suspended in an aqueous medium containing a high-viscosity polymer, formulated in paste form, or embedded in a polymer matrix, to enhance further the retention of liposomes on a corneal surface liposome.
- Brian C. Keller *et al.* [17]: A liposome-capsule dosage unit system formed by encapsulating biologically active materials then placed into a capsule. A less water tolerant capsule could be employed if the liposomes were dehydrated prior to placement within the capsule. Biologically active materials include

drugs, nutritional supplements, vitamins, minerals, enzymes, hormones, proteins and polypeptides. The system is suited for materials with poor oral solubility, not absorbed or poorly absorbed from the gastrointestinal tract. The system could be administered orally, intraocularly, intranasally, rectally, or vaginally.

- Leaf huang *et al.* [18] Methods and compositions were used for the delivery of bioactive compounds to a cell, tissue, or physiological site. The compositions comprised delivery system complexes comprising liposomes encapsulating a biodegradable ionic precipitate. It is used to treat disease or an unwanted condition in a subject, wherein the methods comprised administering the delivery system complexes comprising bioactive compounds that had therapeutic activity against the disease or unwanted condition to the subject.
- Eduard Babychuck *et al.* [19]: The invention related to the use of empty liposomes of defined lipid composition or mixtures of empty liposomes of defined lipid composition for the treatment and prevention of bacterial infections. It had been found that such liposomes with cholesterol, sphingomyelin, phosphatidylcholine, prevented binding of bacterial toxins to target cells and toxin-induced lysis of the target cells. Injected intravenously, liposomes mixtures prevented death of laboratory mice infected with lethal doses of *Staphylococcus aureus* or *Streptococcus pneumoniae*.
- Richard T. Proffitt *et al.* [20]: This novel composition and method used to improve liposomes with solubilizing amphiphilic drugs. A phosphatidylglycerol was acidified in a small amount of organic solvent. The amphiphilic drug, such as Amphotericin B, suspended in organic solvent was then added to the acidified phosphatidylglycerol and a soluble complex was formed between the phosphatidylglycerol and the amphiphilic drug. When the liposome composition incorporating the soluble complex was hydrated, the final pH of the hydrating aqueous buffer was carefully controlled. The Amphotericin B formed liposomes had markedly reduced toxicity.
- Edgar Sache *et al.* [21]: Heparin-based preparations wherein heparin retained in or on liposomes. The lipids of said liposomes were preferably phospholipids comprising acyl chains derived from non saturated fatty acids, advantageously essential acids. These heparin liposomes preparations had heparinic activity when administered *in vivo* by the oral route and a delayed-type action.
- Pieter R. Cullis *et al.* [22]: The present invention related to liposomes and virosomes and, more particularly, to liposomal and virosomal delivery systems for transporting materials such as drugs, nucleic acids and proteins. The present invention provided a fusogenic liposome comprising a lipid component. Fusogenic liposomes were extremely advantageous because the rate at which they became fusogenic could be not only predetermined, but varied as required over a time scale ranging from minutes to days. Control of liposome fusion could be achieved by modulating the chemical stability and/or exchangeability of the bilayer stabilizing components.
- Kaj Mahlberg *et al.* [23]: The invention related to a microencapsulated liposomal composition comprising liposomes and a modified whey protein mixture. The liposomes comprised a lipid composition originating from bovine animals and/or pigs and also to a method for the manufacture of the microencapsulated liposomal composition
- Hiroshi Kikuchi *et al.* [24]: The present invention used organic solvent for the membrane components. In accordance with this invention, any special equipment or procedure was not required for a large scale of production of liposomes. This invention related to a method for producing liposome preparations which comprised mixing physiologically acceptable membrane component.
- Shaoling Huang *et al.* [25]: The present invention provided methods of generating gas-containing liposomes where the gas was introduced under pressure, as well as gas-containing liposomes which contained a large volume of gas (e.g., 10 ul of gas per 5 mg of gas-containing liposomes). In certain embodiments, the gas-containing liposomes contained nitric oxide gas. In some embodiments, such nitric oxide containing liposomes were used to treat a medical condition that is treatable by nitric oxide gas (e.g., intimal hyperplasia).
- Orlando Hung *et al.*, [26]: The composition of liposomes was relatively uniform in size. Their compositions contained a cannabinoid or cannabimimetic agent. The composition was in a form that was suitable for pulmonary administration.
- Yechezkel Barenholz *et al.* [27]: Present invention based on a new method for preparing a bioavailable

formulation containing water immiscible carotenoids. Encapsulating liposomes substantially water immiscible carotenoids. Therefore, there were provided by the present invention formulations comprising liposomes loaded with an amount of at least one water immiscible carotenoid. Pharmaceutical compositions comprising such a formulation and a method for preparing liposomes loaded with said carotenoid.

- Michel G. Bergeron *et al.* [28]: Encapsulating liposomes were used to treat viral diseases comprising the administration of antiviral agents. Also provided formulations of liposomes for the treatment of infections caused by viruses like human immunodeficiency virus (HIV) and cytomegalovirus (CMV). These formulations of liposomes composed of specific classes of lipid components and contained an entrapped drug effective against the viral disease. These liposomal formulations of antiviral drugs allowed high cellular penetration in different cell lines, good *in vitro* antiviral efficacy against HIV and CMV replication, efficient *in vivo* targeting of HIV reservoirs and a marked improvement of the pharmacokinetics of drugs.
- Gregory Gregoriadis *et al.* [29]: Liposomes included cationic components such as cationic lipids such as DOTAP. The method of forming liposomes used the dehydration-rehydration method in the presence of the polynucleotide. The polynucleotide preferably operatively encoded an antigen capable of eliciting a desired immune response which was a gene vaccine.
- Stephane Clerc *et al.* [30]: Liposomes encapsulated with a weak acid drug at a high concentration. The method employed a proton shuttle mechanism involved the salt of a weak acid to generate a higher inside/lower outside pH gradient. By this method, reagent combination for practicing the method and a liposome composition were formed.
- Gregory Scott Retzinger *et al.* [31]: Formulated fibrinogen-coated liposomes. In this process, fibrinogen and an acylating agent were reacted in the presence of a dispersion of liposomes under specifically defined reaction conditions. The liposomes formed using this process, pharmaceutical compositions containing those liposomes and the methods of clotting blood and delivering pharmaceutically-active agents and/or other chemicals utilizing those pharmaceutical compositions were disclosed also.
- Mathew Cherian *et al.* [32]: A new method disclosed for sterilizing or heat treating liposomes. The liposomes might be empty or contained a bioactive agent; such liposomes might be administered in any pharmaceutically acceptable fashion such as parenterally or topically. The method involves subjecting liposomes to heating at a sufficient temperature and time to achieve sterilization.
- Yechezkel Barenholz *et al.* [33]: Used procedure which was simple, efficient, safe, economical and fast transmembrane lining for efficient active loading of weak amphiphatic drugs into liposomes using the transmembrane gradient. The resulting liposomes loaded with the amphiphatic drug were stable and safe. A storable form of loadable liposomes had extended period of stability. The reversed procedure is applicable for sustained release of liposome encapsulated drugs from ammonium liposomes etc.
- Lawrence Boni *et al.* [34]: Provided liposomal taxane formulations where the liposomal lipid was a phosphatidylcholine; these formulations were useful for treating animals afflicted with cancers.
- Sean M. Sullivan *et al.* [35]: A process for the encapsulation of oligonucleotides in liposomes included the suspending of liposomes containing a divalent cation in a solution containing an oligonucleotide and having an osmolarity of a less than that of the internal aqueous phase.
- Alan I. Faden *et al.* [36]: A dosage unit used for intravenous, intramuscular or subcutaneous injection which included an effective amount of magnesium compound contained in positively charged, unilamellar liposomes capable of releasing said magnesium and a pharmaceutically acceptable solution. Compositions also containing the liposome entrapped magnesium compound and methods of treating traumatic injuries and inducing calcium channel antagonistic activity through the administration of the liposome entrapped magnesium compound.
- Francis C. Szoka *et al.* [37]: This method was useful for poorly-soluble in aqueous solution, but generally for any compound or combination of compounds which could be dissolved in the aprotic solvent or aprotic solvent/lower alkanol mixture. Liposome and lipidic particle formulations of compound prepared by dissolving in a solution of liposome-forming lipids in an aprotic solvent such as DMSO,

optionally containing a lipid-solubilizing amount of a lower alkanol and either injecting the resulting solution into an aqueous solution, or the aqueous solution into the resulting solution. The resulting liposome or lipidic particle suspension might then be dialyzed or otherwise concentrated.

- Kazuhiko Suzuki *et al.* [38]: formulated haemoglobin containing liposomes encapsulating within liposomes having membranes comprising lipid material haemoglobin and a methemoglobin formation-inhibiting agent. The liposomes were used as artificial blood with high oxygen-carrying capacity. As the lipid material of the liposomes were used phospholipid materials such as, for example, lecithin, phosphatidylethanolamine, phosphatidic acid, sphingomyelin and hydrogenation products thereof. As the methemoglobin formation-inhibiting agent were used salts of ascorbic acid, glutathion and the like. The liposomes were prepared by dissolving a liposome membrane-forming lipid material and a surface-active agent, removing the solvent from said solution, adding to the residue a haemoglobin solution to which a methemoglobin formation-inhibiting agent has been added and subjecting the starting solution thus obtained to dialysis with a physiological saline solution containing the same methemoglobin formation-inhibiting agent to remove the surface-active agent from the starting solution.
- Liangfang Zhang *et al.* [39]: Control of the fusion activity of liposomes by adsorbing biocompatible nanoparticles to the outer surface of phospholipid liposomes was disclosed. The biocompatible nanoparticles effectively prevent liposomes from fusing with one another. Release of cargo from the liposome was accomplished via trigger mechanisms that include pH triggers, pore forming toxin triggers and photosensitive triggers. Dermal drug delivery to treat a variety of skin diseases such as acne vulgaris and staph infections was contemplated.
- Centre Degert *et al.* [40]: A method for preparing liposomes by mixing together an aqueous solvent, a surfactant including a hydrophilic terminal and a C2-16 hydrocarbon chain, a sterol and/or a membrane protein and /or a product to be encapsulated, to dissolve the sterol and/or the product to be encapsulated; mixing the resulting composition with a lipid surfactant to form a uniform lamellar liquid crystal phase or a liquid crystal phase suspension in water; and converting the liquid crystal phase or liquid crystal phase suspension into liposomes.
- Alain Meybeck *et al.* [41]: A composition having anti-inflammatory, anti-allergic or anti-aging activity comprising hydrated lipidic lamellar phases or liposomes containing an extract of Scutellaria and a method for treating inflammation, allergies or aging by topical administration of the composition.
- Masazumi Eriguchi *et al.* [42]: The present invention provided a liposome preparation containing oxaliplatin and derivatized with a hydrophilic polymer, as well as a pharmaceutical composition for treatment of tumor comprising the liposome preparation. The liposome preparation according to the present invention is characterized in that it is derivatized with a ligand. The ligand is preferably transferrin. In accordance with the invention, the uptake of a pharmaceutical agent in the liposome into tumor cells can be enhanced through transferrin receptors expressed on the surface of the tumor cells.
- Ander Falk Vikbjerg *et al.* [43]: The present invention provided liposomes that are useful for delivery of bioactive agents such as therapeutics. Among others, the liposomes of the invention are capable of delivering their payload at sites of increased secretory phospholipase A2 (sPLA2) activity, because phospholipase A2 (PLA2) will hydrolyse lipids of the liposome. Thus, the liposomes of the invention may e.g. be used in relation to cancer therapy. Another aspect of the invention is a liposomal formulation comprising the liposome of the invention. Still another aspect is a method of producing a liposomal formulation of the invention.
- Mark John Ernsting *et al.* [44]: In one aspect, the present invention provides a thermosensitive liposome comprising a lipid bilayer comprising 1, 2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) and a compound of formula (I): $C_{17}H_{35}(CH_2)_p(CO)_q(OCH_2CH_2)_nOH$ wherein p is an integer selected from 0 or 1; q is an integer selected from 0 or 1; p + q = 1; and n is an integer selected from about 10 to about 100.
- Ajoy Chakrabarti *et al.* [45]: The present invention relates to liposomal compositions having a concentration gradient which load amino acids and peptides exhibiting weak acid or base characteristics into liposomes. Specifically loaded into liposomes by the methods of the present invention are C-terminal substituted amino acids or peptides.

The liposomes are preferably large unilamellar vesicles. The concentration gradient is formed by encapsulating a first medium in the liposomes, said medium having a first concentration of the one or more charged species and suspending the liposomes in a second medium having a second concentration of the one or more charged species, such as for example a pH gradient. Also disclosed did pharmaceutical preparations comprise such C-terminal substituted amino acids or peptides which have been loaded into the liposomes by the method of the invention.

CONCLUSION

It is concluded that liposomes are biocompatible, biodegradable in nature with increase efficacy and therapeutic index of drug. Liposomes are stable via encapsulation. They are biocompatible and completely biodegradable in nature. Liposomes are prepared by passive loading technique and active loading techniques with different marketed formulations.

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