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# Development of liposomal cosmeceuticals

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## ABSTRACT

*A liposome is a tiny bubble (vesicle), made out of the same material as a cell membrane. Membranes are usually made of phospholipids, which are molecules that have a head group and a tail group. The head is attracted to water, and the tail, which is made of a long hydrocarbon chain, is repelled by water. Since then, liposomes have made their way to the market. Today, numerous lab scale but only a few large-scale techniques are available. Today, they are a very useful reproduction, reagent, and tool in various scientific disciplines, including mathematics and theoretical physics, biophysics, chemistry, colloid science, biochemistry, and biology. Liposomes with modified surfaces have also been developed using several molecules, such as glycolipids or sialic acid. Liposomes are used as model for artificial cells. Liposomes can also be designed to deliver drugs in other ways. The use of liposomes for transformation or transfection of DNA into a host of cell is known as lipofection. Liposome are widely used as carriers of active ingredients to human tissue and as lipid transfer vesicles to the skin. Liposomes find applications in pharmaceutical, cosmetic and other industrial field.*

**Keywords:** Liposome, cosmeceuticals, carrier, topical application.

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## INTRODUCTION

Liposomes are sphere-shaped vesicles consisting of one or more phospholipids bilayers. The story of success of liposomes was initiated by Bangham and his colleagues in the early 1960s who observed that smears of egg lecithin reacted with water to form quite intricate structures. They were analyzed by electron microscopy. The name liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body. A liposome can be formed at a variety of sizes as uni-lamellar or multi-lamellar construction, and its name relates to its structural building blocks, phospholipids, and not to its size. Their size range from 25 to 500nm. [1,2]

**TABLE 1: Types of liposomes [1,2,3]**

| S. No. | Types of liposome according to structural features | Liposome classification based on method of preparation |
|---|---|---|
| 1. | MLV: Multilamellar large vesicles | REV: Single or oligolamellar vescile made by reverse phase evaporation method. |
| 2. | OLV: Oligolamellar vesicles | MLV / REV: Multilamellar vesicles made by reverse phase evaporation method |
| 3. | UV: Unilamellar vesicles | SPLV: Stable plurilamellar vesicles. |
| 4. | SUV: Small unilamellar vesicles | FATMLV: Frozen and thawed MLV |
| 5. | MUV: Medium sized unilamellar vesicles | VET: Vesicles prepared by extrusion method. |
| 6. | LUV: Large unilamellar vesicles | FUV: Vesicles prepared by fusion |
| 7. | GUV: Giant unilamellar vesicles | FPV: Vesicles prepared by french press |
| 8. | MVV: Multivesicular vesicles | DRV: Dehydration- rehydration vesicles |
| 9. | | BSV: Bubblesomes |

The main goal of an ideal method of liposome formulation is to obtain efficient drug entrapment, narrow particles size distribution and long term stability of liposome products. The general procedure for all methods of liposomes preparation involves hydrating of the lipid, followed by sizing of the particles and removing of the non encapsulated drug. There are two types used for the preparation of liposomes: passive loading mechanical dispersion methods and active loading methods. The most common used methods in the preparation for liposomes are: thin-film hydration method, microemulsification, sonication, membrane extrusion, freezethawed method, ether injection method, ethanol injection method, reverse phase evaporation method, dehydration-rehydration, and calcium-induced fusion method. In the passive loading method the drug is encapsulated by introducing an aqueous phase of a water-soluble drug or an organic phase of a lipid-soluble drug before or at some stage during the preparation of the liposomes. The high drug encapsulation efficiency can be achieved by using passive loading method for lipid-soluble drugs with a high affinity to the lipid membrane. In the active loading method, the drugs can be loaded by creating diffusion gradients for the ions or drugs across the external and internal aqueous phases.

**1. Thin-Film Hydration Method**

The thin-film hydration procedure is the most common and simple method for preparation of MLV by dissolving the phospholipids in the organic solvents: dichloromethane[9], chloroform[10,11], ethanol and chloroform-methanol mixture (2:1 v/v; 9:1 v/v; 3:1 v/v). A thin and homogeneous lipid film is formed when solvent is evaporated under vacuum at the temperature: 45-60ºC. Nitrogen gas is involved in order to completely remove the residual solvent. A solution of distilled water, phosphate buffer[**12**], phosphate saline buffer at pH 7.4 and normal saline buffer are used in hydration step. The time for the hydration process varied from 1 h to 2 h at the temperature 60-70ºC. In order to obtain full lipid hydration, the liposomal suspension is left overnight at 4ºC[11]. The thin-film hydration method can be used for all different kinds of lipid mixtures. The main drawbacks of the method are related to low encapsulation, difficulty of scaling up and the size distribution is heterogeneous.[11]

Liposomes were reared from almond oil phospholipids using the thin lipid film hudration method.[12]

**2. Injection Methods**

**a. Ether Injection Method**

In ether injection method a solution of lipids is dissolved in ether or diethyl ether/methanol mixture which is slowly injected to an aqueous solution of the material to be capsulated. The subsequent removal of the organic solvent under reduced pressure leads to the formation of liposomes.[13,14] The main disadvantage of the method is heterogeneous population and the exposure of compounds to be encapsulated to organic solvents or high temperature.

**b. Ethanol Injection Method**

In ethanol injection method the ethanolic lipid solution is rapidly injected to a vast excess of preheated distilled water or TRIS-HCl buffer. The incorporation of the drug in liposomal vesicle depends on its hydrophilic/hydrophobic character. Nimesulide as lipid soluble component incorporates better in liposomes than 5-fluorouracil which migrates to external aqueous phase. The main advantage of ethanol injection method is including of non harmful solvent as ethanol, as well as easy scale up of the method. The possibility of formation of azeotrope with water reduces its applicability.

**3. Sonication Method**

The sonication method is based on size transformation and involves the subsequent sonication of MLVs prepared by thin-film hydration method, using sonic energy usually under an inert atmosphere including nitrogen or argon. The sonication method enables homogenous dispersion of small vesicles using bath type or probe type sonicator with a potential for greater tissue penetration. The probe tip sonicator delivers high energy to the lipid suspension. The possibility of overheating of the lipid suspension causes degradation[15,16]. Sonication tips tend to release titanium particles into the lipid suspension which must be removed by centrifugation prior to use. The bath sonicators are the most widely used instrumentation for preparation of SUV[17,18]. They are used for large volume of dilute lipids. The oxidation of unsaturated bonds in the fatty acid chains of phospholipids and hydrolysis to lysophospholipids and free fatty acids, as well as denaturation of thermolabile substances and very low encapsulation efficiency of internal volume are the main drawbacks of the method.

**4. High-Pressure Extrusion Method**
MLVs prepared by thin-film hydration method are repeatedly passed through filters polycarbonate membranes reducing the liposome size in high-pressure extrusion method[19]. The liposomes are prepared using thin-film hydration method subsequently using an extruder for ten cycles to obtain extruded liposomes with uniform diameters.

**5. Reverse-Phase Evaporation Method**
The reverse-phase evaporation method is used with the organic solvents such as diethyl ether/isopropyl ether or mixture of diethyl ether and chloroform (1:1 v/v) and a mixture of chloroform: methanol (2:1 v/v) containing phospholipids. The organic phase should be immiscible with aqueous phase, thus an oil/water emulsion is created. Phosphate buffer saline or citric-Na2HPO4 buffer is added to aqueous phase with aim to improve the efficiency of liposome formulations. The formation of liposomes is allowed by continued rotary evaporation of the organic solvents under vacuum. The main advantage of the method is a very high encapsulation rate. The main drawback of the method is the possibility of remaining the solvent in the formulation and it has difficulties to scale up.

**6. Calcium-Induced Fusion Method**
The calcium-induced method is based on adding of calcium to SUV. The formation of multilamellar vesicles is as result of fusion. The addition of ethylenediaminetetraacetic acid (EDTA) to the preparations results in the formation of LUV liposomes[19]. The preparation of LUV liposomes can be obtained only from acidic phospholipids.

**7. Dehydration-Rehydration Method**
The method of dehydration-rehydration is used as method for the preparation of liposomes, also[20]. The small unilamellar vesicles which are composed of phosphatidylcholine, 1, 2-dioleoyl-3- (trimethylammonium) propane, cholesterol and plasmid DNA are prepared by sonication method. The obtained formulation is frozen and left freeze-dried overnight. The formation of multilamellar dehydration-rehydration vesicles containing DNA in their structure due to the bound of the cationic charges of the inner bilayers is as a result of a controlled rehydration of the dry powders.

**8. Freeze-Thaw Method**
The method of freezing and thawing is introduced for increasing the trapped volume of liposomal preparations. The freeze-thaw method is dependent on the ionic strength of the medium and the phospholipid concentration. It influences to a physical disruption of lamellar structure leading to formation of unilamellar vesicles. The unilamellar vesicles are rapidly frozen followed by slow thawing, while the freeze and thawing cycles are repeated. The preparation of MLV propranolol liposomes by freeze-thaw method is described in the literature[20]. The liposomal propranolol formulation is prepared by using distearoylphosphatidylcholine and dimyristoylphosphatidylcholine as phospholipids in phosphate buffered saline buffer, followed by six freeze-thaw cycles.

**9. Microfluidization**
A method based on microfluidization i.e. microemulsification is used for the large scale manufacture of liposomes. The preparation of antibiotic liposomes by thin-layer hydration method followed by sonication with a bath-type sonicator and microfluidization in order to achieve partial homogenization was described by Boltič et al[20]. The process of microfluidization is reproducible and yield liposomes with good aqueous phase encapsulation. Supercritical Fluids (SCF) in the Preparation of Liposomes Supercritical fluids are introduced in the preparation of liposomes to overcome existing problems with conventional methods such as requiring a high amount of toxic organic solvents and limited laboratory scale production. The most common used supercritical fluid in the preparation of liposomes in pharmaceutical field is supercritical carbon dioxide. It has several advantages: non-toxicity, non-flammability, recyclable and easy removal from the solvent, operation at moderate temperatures and avoiding degradation of the product in an inert atmosphere. The use of SCF allows controlling of extraction condition by variation of temperature, pressure or adding modifier solvents as cosolvents: acetone, ethanol, methanol, dichloromethane and ethyl acetate. A comparison between thin-film hydration method and SCF method is reported by Karn et al. A mixture of phosphatidylcholine, cholesterol and cyclosporin A is dissolved in ethanol followed by pumping supercritical carbon dioxide to the reaction vesicle in SCF method. Distilled water in hydration step in thin-film hydration method is used.

**Advantage of liposomes** [21]
- Liposomes increased efficacy and therapeutic index of drug (actinomycin-D)

- Liposome increased stability via encapsulation
- Liposomes are non-toxic, flexible, biocompatible, completely biodegradable, and non-immunogenic for systemic and non-systemic administrations
- Liposomes reduce the toxicity of the encapsulated agent (amphotericin B, Taxol)
- Liposomes help reduce the exposure of sensitive tissues to toxic drugs
- Site avoidance effect
- Flexibility to couple with site-specific ligands to achieve active targeting

**Disadvantage of liposomes** [21]

- Low solubility
- Short half-life
- Sometimes phospholipid undergoes oxidation and hydrolysis-like reaction
- Leakage and fusion of encapsulated drug/ Molecules
- Production cost is high
- Fewer stables

**Applications of liposomes** [21]

- Topical application of liposomes has great potential in dermatology.
- Liposomes have been used to deliver anticancer agents in order to reduce the toxic effects of the drugs when given alone or to increase the circulation time and effectiveness of the drugs.
- Liposome designs are leading to new applications for the delivery of new biotechnology products, for example antisense oligo-nucleotides, cloned genes, and recombinant proteins.
- Recent improvements include liposomal formulations of all-*trans*-retinoic acid and daunorubicin, as first-line treatment of AIDS related advanced Kaposi's sarcoma. Examples are vincristine, doxorubicin, and amphotericin B.
- Gene therapy
- Liposomes as carriers for vaccines
- Liposomes as carrier of drug in oral treatment
- Liposomes for pulmonary delivery
- Against Leishmaniasis
- Stability
- Sterilization
- Encapsulation efficiency
- Active targeting
- Tuberculosis of bladder

**Marketed liposomal formulations**

- Capture was the first anti-ageing liposomal cream launched by Christian Dior in 1987 with claims that it can reduce the sign of wrinkles.
- The first liposmal pharmaceutical product-Doil approved in 1995 to the latest Marqibo in 2012.
- The product Liposome-annamycin are used in the treatment of Breast cancer.
- Paclitaxel liposomes is a potent anti-cancer drug used in various tumors, including ovarian, breast and non-small-cell lung carcinoma.
- Liposomal vaccines, also known as virosomes, are constructed with viral surface antigens and synthetic lipids such as DOPC,DOPE or DPPC, with simulate viral membrane for vaccine delivery.

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