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Liposome—A Comprehensive Approach for Researchers

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Abstract

Bangham was first to develop these spherical-shaped nano-vesicles called liposomes in the early 1960s. Today, liposomes have emerged as crucial tools for bettering the delivery of drugs that majorly includes-antifungal drug, peptide hormones, enzymes, vaccines antimicrobial agents, drugs against cancer, and genetic materials. Following the different manufacturing practices and versatile properties liposomes can be categorized in various parameters of size, charge, poly-dispersity index, encapsulation efficiency, solubility properties, and lamellarity. Alteration in such parameters elevates the loading and bioavailability of a drug by giving more clear target specification, desired or controlled release. This bibliographic chapter provides a comprehensive overview of methods for the preparation of liposomes with other perspectives that majorly includes—physio-chemical characteristics, dosage regimen, advantages over other delivery systems, approved liposomal based drugs and other ongoing drugs in clinical trials. It will help researchers to break-through more structurally successful delivery vehicles depending upon their various physic-chemical properties.

Keywords: liposomes, particle size, zeta potential, polydispersity index, encapsulation efficiency, methods of preparation and bioavailability

1. Introduction

Liposomes can be microscopically examined as the vesicle with spherical structure that comprises one or more bilayer lipid in the aqueous core part of a shell. Liposomes are widely used in the delivery of variety of drugs depending upon its various physic-chemical characteristics. Design and development of liposomes are classified in many ways among which thin film hydration method is the most globally accepted procedure. Liposomes formation occurs when lipids are incorporated into water or buffer solution under continuous stirring, that in return forms the spherically shaped vesicles termed as liposomes. There are many methods to develop liposomes among which thin film hydration method is most common. Recently, lipid film hydration method was used to develop a multilamellar vesicle (MLV) loaded with curcumin (CUR) and Rhodamine B (RhB), [1] as a successful drug delivery approach. Phospholipids and cholesterol are the major components used in the development of liposomes (**Figure 1**). Where bilayer lipid composes of a hydrophilic head group, i.e., phospholipid and a hydrophilic tail group. Where phospholipids can easily penetrate and localize in the skin thus increases the overall

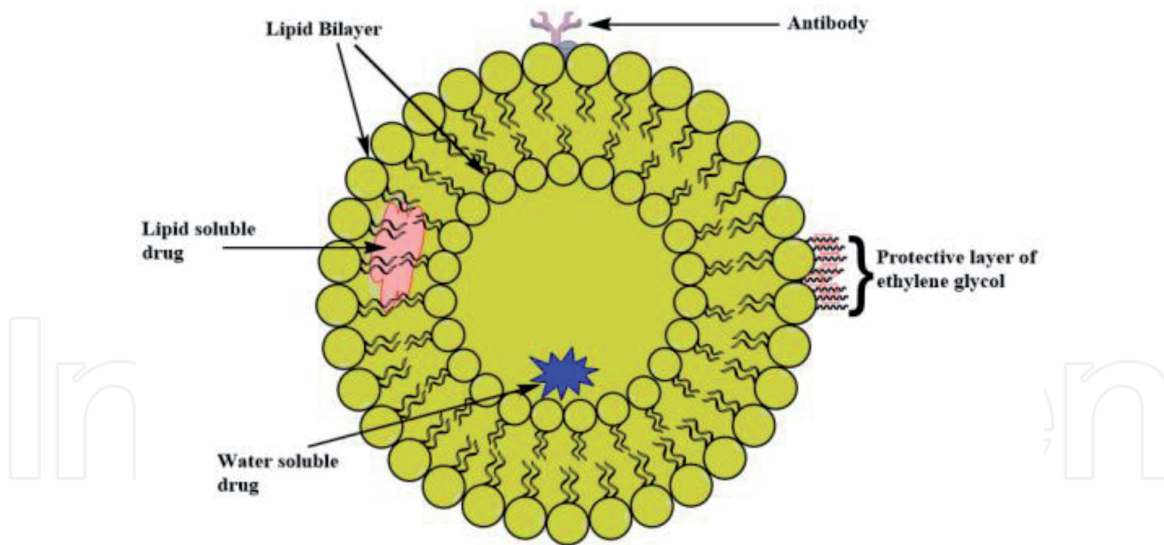


Figure 1.
Liposome molecule with lipid bilayer.

bioavailability in case of many dermal formulations whereas, cholesterol not only increases microviscosity of the bilayer but also defines the stability and rigidity of the formulation [2].

There are many routes to administer liposomes containing drugs, i.e., pulmonary, ocular, intramuscular, intravenous, topical, nasal and oral. Liposomes can be delivered in many ways involving sprays, capsules, ointments, creams, solutions, etc. for curing various diseases: bacterial, fungal, ocular, vaccines, fibrinolysis, endocrine, arthritis, asthma, diabetes, diseases of immune system, herpes, analgesics, topical anesthesia and even cancer [3].

Based on different parameters, liposomes are further classified depending upon method of preparation, structural parameters, biochemistry, cosmetics and medicine composition, and application in biology. Phospholipids can be from natural sources such as soya bean, egg yolk and olive oil. Depending upon various characteristics liposomes can be categorized on the basis of various physical parameters such as—pH, temperature, ionic charges, immunogenicity and stability.

In a recent study performed in 2019, it is revealed that the concentration of phospholipids and cholesterol varies the protein binding of the formulation [4].

Most commonly employed phospholipids in the formulation of liposomes are: phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylethanolamine (PE), dipalmitoyl phosphatidylserine, 1,2-dioleoyl-sn-glycero-3-phosphoserine, dipalmitoyl phosphatidylcholine (DPPC), distearoyl phosphatidylcholine (DSPC), dioleoyl phosphatidylethanolamine (DOPE) [5].

1.1 Composition of liposomes

A. Phospholipids

1. Derived from natural sources:

- Phosphatidylcholine
- Phosphatidylserine
- Phosphatidylethanolamine

2. Synthetic phospholipids:

- Disloyal phosphatidylethanolamine
- Disloyal phosphatidylcholine

B. Cholesterol

Cholesterol are optimized to be used in the formulation of liposomes up to a wide range with a molar ratios 1:1 or 2:1 against phospholipids. Cholesterol defines a strategic role in liposome composition; although, the adequate quantity to be used in the formulation has not been yet clarified. Thus, we can optimize lipids and cholesterol ratio, to prepare stable and controlled drug release vehicles (**Figures 2 and 3**) [6].

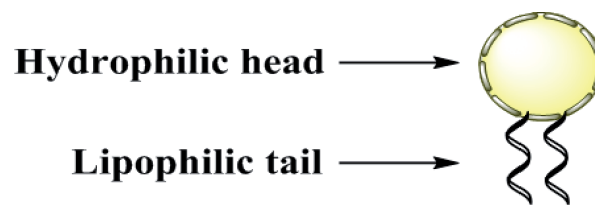


Figure 2.
Hydrophilic and lipophilic terminals of lipid.

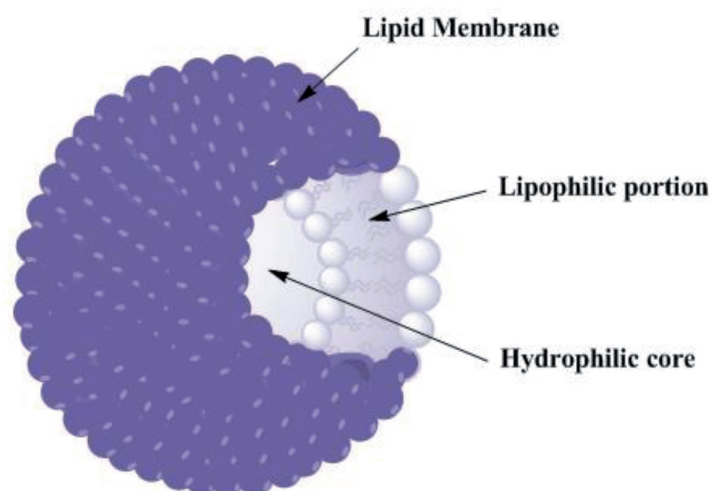


Figure 3.
Inner and outer structure of liposome.

2. Physio-chemical properties

See **Tables 1–3**.

3. Applications of liposomes

Role of liposome in drug delivery:

- Selective & passive targeting.
- It increases the overall therapeutic index and efficacy of a liposomal formulation.

Characterization parameters	Analytical method/instrument
Mean vesicle size and size distribution (submicron and micron range)	Zetasizer
Vesicle shape and surface morphology	Transmission electron microscopy
Electrical surface potential and surface pH	Zetasizer & pH measurement device
Surface charge free	Flow electrophoresis
Phase behavior	Differential scanning calorimetry (DSC)
Lamellarity	Freeze-fracture electron microscopy
Percent of free drug/percent capture	Minicolumn centrifugation, ion-exchange chromatography, radiolabelling

Table 1.
Physical characterization [6].

Characterization parameters	Analytical method/instrument
Phospholipid concentration	Barlett assay, Stewart assay, HPLC
Concentration of cholesterol	By HPLC
Phopholipid peroxidation	UV absorbance, iodometric, GLC

Table 2.
Chemical characterization [6].

Characterization parameters	Analytical method/instrument
Sterility aerobic or anaerobic cultures	Sterility aerobic or anaerobic cultures
Pyrogenicity	Limulus amebocyte lysate (LAL) test
Animal toxicity	By pathology and histology

Table 3.
Biological characterization [6].

- Due to the encapsulation of drug, overall stability is increased and reduced the adverse effects of encapsulated drug.
- It helps to improve the pharmacokinetic processes by increasing the circulation lifetime, decreasing elimination and toxic effects thus elevating the overall bioavailability of a drug [7].
- Active targeting can also be achieved by coupling with the site-specific ligands.

3.1 Other advantages of using liposomes

- Biodegradability
- Efficient control of the release
- Resemblance to natural membrane structures
- Increased targeting prospects

- Biocompatibility
- Biodegradable
- Liposomes are able to provide both aqueous “milieu interne” and the lipophilic environment in a single system
- It helps in protecting the encapsulated drug.
- Method of preparation is easy and has no such complicated or expensive procedures involved
- Facilitates both active and passive targeting.
- No toxicity in heart as it does not accumulate in the heart.
- Intercepts the oxidation of drug
- Chelation therapy in case of heavy metal poisoning
- Diagnostic imaging of tumors
- In enzyme replacement therapy
- Study of membranes
- In gene delivery
- As drug delivery carriers
- In multidrug resistance
- In immunology
- In cosmetology (**Table 4**)

Category	Application utilized
In parasitic diseases	After IV injection liposomes are comfortably digested by phagocytic cells in the body and hence considered as one of the best vehicle to dispatch cargo into macrophages
Anticancer therapy	Liposomes are effective for the cells not only in tumors but also in the gastrointestinal mucosa
Other medical applications	These liposomes are sterically stabilized vesicles and are long circulating micro-reservoirs or tumor (or site of inflammation and infection) targeting vehicles
In bioengineering	Fragments of siRNA and DNA are delivered with the help of modern genetic engineering and gene recombinant technology
In vaccination	Liposomes are considerably used in proper vaccination due its fine active targeting
In agro-food industry	Due to its versatile physio-chemical properties lipids are extensively manufactured and used in large scale up sectors

Table 4.
Applications of liposomes [6].

4. Methods of preparation

See **Figure 4**.

4.1 Thin film hydration method

This is one of the widely used methods for the preparation of liposomes. As it has no such complicated steps involved in it. Multilamellar vesicles (MLV) are prepared by solubilizing natural or synthesized phospholipid in chloroform, dichloromethane, ethanol or in a mixture of chloroform and methanol in a ratio of 3:1 v/v; 2:1 v/v or 9:1 v/v. A homogeneous thin film forms when this mixture is revolved and dried in a rota-evaporator under vacuum at a temperature around 45–60°C. Layer is kept under nitrogen drying for overnight. Next, comes the hydration process where completely dried thin film is hydrated using aqueous phase—phosphate buffer solution of pH 7.2 for 1–2 h at 60–70°C.

This kind of procedure can be applied to almost any kind of lipid mixtures, but has some drawbacks that majorly includes—low encapsulation space, a bit difficult to scale up and layer formed are not always homogeneous thus shows heterogeneous size distribution during later physio-chemical examination of liposomes through zetasizer.

4.2 Injection methods

4.2.1 Ether injection method

Here, the lipid mixture is dissolved in ether or diethyl ether under continuous stirring that is later injected into a PBS or aqueous phase. Which under injection pressure causes the removal of almost all organic solvent that ultimately forms liposomes. This method also suffers with the heterogeneous liposomal formulation defect.

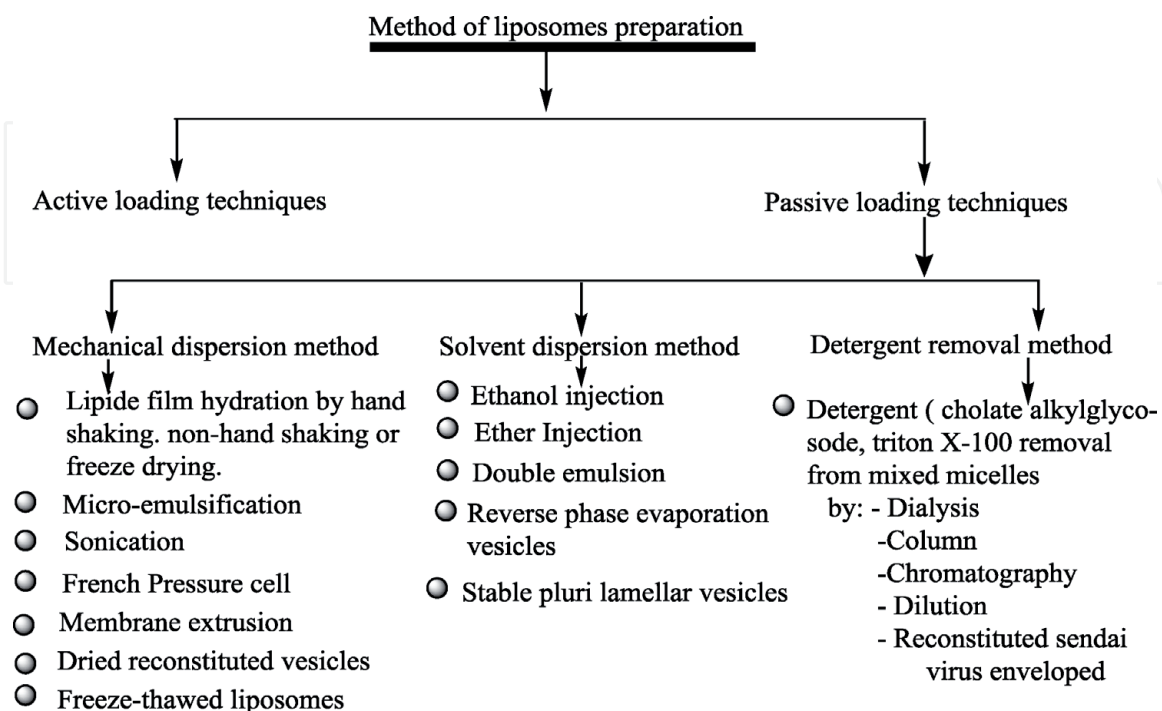


Figure 4.
General representation for method of preparation of liposome.

4.2.2 Ethanol injection method

In ethanol injection method the lipid mixture is dissolved in ethanol under continuous stirring that is later injected into a preheated TRIS-HCl buffer or distilled water. Hydrophobicity and hydrophilicity of a drug accounts for drug intake in a liposomal vesicle. It has an advantage of using non-toxic and ethanol and is also easily scalable.

4.3 Sonication method

It is the most widely accepted method to develop small unilamellar vesicles (SLV). SLV are prepared by solubilizing natural or synthesized phospholipid in chloroform, dichloromethane, ethanol or in a mixture of chloroform and methanol in a ratio of 3:1 v/v; 2:1 v/v or 9:1 v/v. A homogeneous thin film forms when this mixture is revolved and dried in a rota-evaporator under vacuum at a temperature around 45–60°C. Layer is kept under nitrogen drying for overnight. Next, comes the hydration process where completely dried thin film is hydrated using aqueous phase—phosphate buffer solution of pH 7.2 for 1–2 h at 60–70°C. Further the bath sonicator is used to transform the size of vesicles. Lastly, liposomes are centrifuged in order to remove the titanium particles that might get added due to overheating in sonication process. Less encapsulation space is the major drawback of such vesicles.

4.4 High-pressure extrusion method

Liposomes are prepared by solubilizing natural or synthesized phospholipid in chloroform, dichloromethane, ethanol or in a mixture of chloroform and methanol in a ratio of 3:1 v/v; 2:1 v/v or 9:1 v/v. A homogeneous thin film forms when this mixture is revolved and dried in a rota-evaporator under vacuum at a temperature around 45–60°C. Layer is kept under nitrogen drying for overnight. Next, comes the hydration process where completely dried thin film is hydrated using aqueous phase—phosphate buffer solution of pH 7.2 for 1–2 h at 60–70°C. In addition, these liposomes are passed through high pressure extruder for 10 cycles in order to obtain more uniform and stable liposomes.

4.5 Reverse-phase evaporation method

Here, the lipid mixture is dissolved in organic solvents ether or diethyl ether or a mixture of diethyl ether and chloroform (1:1 v/v); a mixture of methanol-chloroform (1:2 v/v) under continuous stirring that is later injected into a PBS or aqueous phase comprising citric- Na_2HPO_4 to improve the overall efficacy of a formulation. Which under injection pressure causes the removal of organic solvent that ultimately leads to the formation of liposomes. This method also suffers with the heterogeneous liposomal formulation defect. Organic solvent is then dried using rota-vapor instrument thus forming homogeneous liposome. The major disadvantage of this procedure is the leftover of remaining organic solvent in the final formulation also faces difficulty in scale up procedures.

4.6 Calcium-induced fusion method

Here acidic phospholipids are used to prepare SUV by following the thin film hydration process followed on with the addition of calcium that causes fusion to form MLV. Final addition of ethylenediaminetetraacetic acid (EDTA) to MLV results in the formation of large unilamellar vesicles LUV.

4.7 Dehydration-rehydration method

Liposomes are prepared by using the sonication method as explained in Section 4.3. Developed liposomes are freeze dried overnight where the formation of multi-lamellar vesicles occurs when dry powder gets controlled rehydration.

4.8 Freeze-thaw method

Liposomes are prepared by using thin film hydration method as explained in Section 4.1. Developed liposomes are freeze dried overnight and is then thawed

Drugs liposome formulation	Method	Type of liposome
Antifungal drugs		
Amphotericin B	Thin-film hydration method	MLV
Clotrimazole	Rotary evaporation method	MLV
Fluconazole	Thin film hydration method	MLV
Analgesic drugs		
Ketorolac tromethamine	Thin-film hydration method	MLV
Antibiotic drugs		
Amikacin	Reverse phase evaporation method	MLV, LUV
Mafenide acetate	Solvent evaporation and microencapsulation	MLV SUV
Antifibrinolytic drugs		
Tranexamic acid	Chloroform film and sonication method	SUV
Drugs against cancer		
5-Fluorouracil	Lipid-film hydration method, extrusion, ethanol injection and reverse phase evaporation method	MLV, LUV, SUV MLV, LUV
Vinblastine sulphate	Thin-film hydration method and sonication	MLV SUV
Tamoxifen	Thin-film hydration method	MLV
Bis-demethoxy curcumin analogue	Thin-film hydration method and sonication	MLV SUV
Doxorubicin	Lipid-film hydration method and extrusion	MLV
Hormone drugs		
Cyproterone acetate	Thin-film hydration method	MLV
Immunosuppressive drugs		
Sirolimus	Thin-film hydration method	MLV
Tacrolimus (Fk-506)	Thin-film hydration method	MLV
Ophthalmic drugs		
Brimonidine tartrate	Thin-film hydration method and sonication	MLV SUV
Acetazolamide	Reverse phase evaporation and thin-film hydration method	MLV, LUV MLV
Potential drugs as oral insulin		
Sodium glycocholate and metformin hydrochloride	Reverse phase evaporation and thin-film hydration method	MLV, LUV MLV
Vaccines		
Tetanus toxoid diphtheria toxoid	Reverse phase evaporation method	MLV, LUV

Table 5.
Methods for the preparation of liposomal formulation to deliver drugs [2].

in order to govern the ionic strength and phospholipid concentration of the final liposomal formation. Physical disruption of lamellar structure occurs due to freeze-thaw of liposomal formulation giving it a final ionic structure.

4.9 Microfluidization

Boltic et al. was the first to introduce such method for the preparation of liposomes. Here liposomes are prepared using thin film hydration method as explained in Section 4.1, which is then sonicated and microfluidized in order to obtain partial homogenization. This method has its wide application in industrial formulation of liposomes.

4.10 Supercritical fluids (SCF)

Supercritical fluids (SCF) were introduced to replace toxic organic solvents for the preparation of liposomes. Supercritical carbon dioxide is the most widely used supercritical fluid as it has many advantages over conventionally used organic solvents such as—it is not flammable, can be recycled, non-toxic, can be comparatively easily removed from the solvents, requires moderate temperature and also exclude the product degradation in inert surroundings. Karn et al. experimented and explained the comparative study between thin film hydration method and supercritical fluids using method evaluating the non toxicity and better field approaches in term of using super critical fluids for the formulation of liposomes (**Table 5**).

5. Mechanism of liposomal formulation

- Phospholipids shows affinity for polar molecules as well as for **aqueous** phase due to a hydrophobic tail, that has 2 fatty acids which are made up of 10–24 C atoms comprising of 0–6 double bonds in every chain [8].
- In a phospholipid molecule the polar portion connects with a polar environment of a aqueous medium.
- Phospholipids arrange layers of lipids in close alignment in a planer bilayer sheet. Sufficient amount of energy is required for this planar arrangement (sonication, homogenization, heating, etc.) (**Figure 5**).

6. Evaluation

6.1 Morphological and physicochemical characterization of liposomal-formulation

The average size, size distribution, and zeta potential shall be determined by zetasizer.

Transmission electron microscopy is used to study the shape and surface morphology of a liposomal structure.

6.2 In vitro performance evaluation and stability studies

Stability studies: stability studies shall be conducted to assess the shelf-life of product as per ICH guidelines.

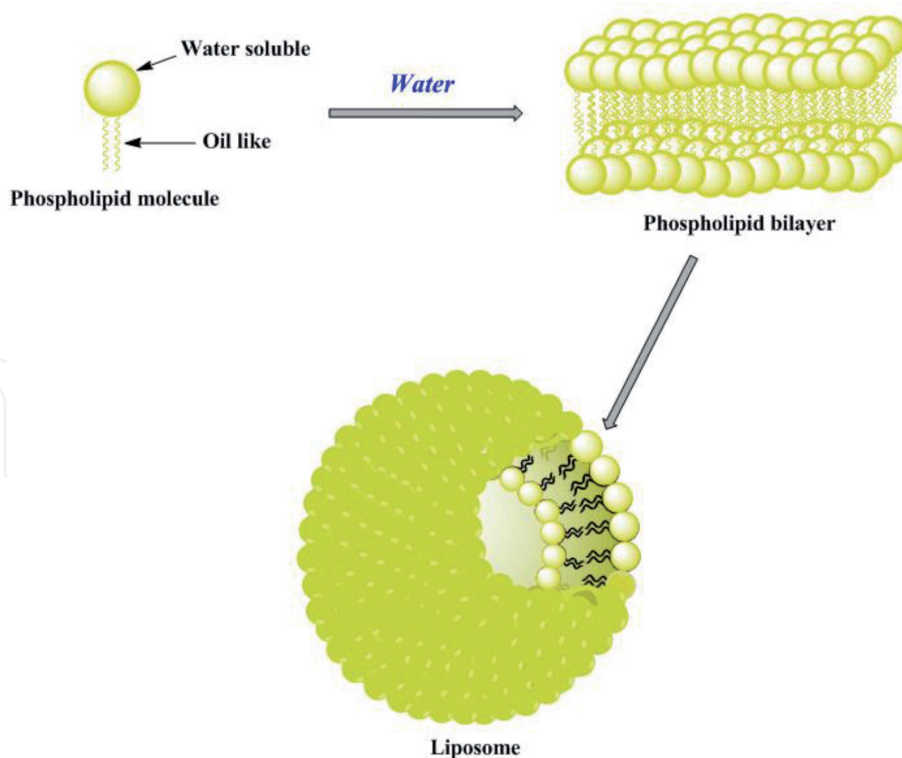


Figure 5.
Mechanism of liposome formation.

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay to evaluate the in-vitro cytotoxicity of the developed formulation.

FACS (fluorescence assisted cell sorting) is used to quantify the cell uptake study.

7. Marketed liposomal formulations

See **Tables 6** and **7**.

Marketed product	Drug used	Target diseases	Company
Alec™	Dry protein free powder of DPPC PG	Expanding lung diseases in babies	Britannia Pharm, UK
Ventus™	Prostaglandin E1	Systemic inflammatory diseases	The liposome company, USA
Topex Br	Terbutaline sulphate	Asthma ozone	USA
Doxil™ or Caelyx™	Doxorubicin	Kaposi's sarcoma	SEQUUS, USA
Novasome	Smallpox vaccine	Smallpox	Novavax USA
Evacet™ Doxorubicin	Doxorubicin	Metastatic breast cancer	The Liposome Company, USA
Fungizone®	Amphotericin B	Fungal infections	Leishmaniasis
Depocyt	Cytarabine	Cancer therapy	Skye Pharm USA
Doxil®	Doxorubicin HCl	Refractory ovarian cancer	ALZA, USA

Marketed product	Drug used	Target diseases	Company
Amphotec™	Amphotericin	B fungal infections, leishmaniasis	SEQUUS, USA

Table 6.
Liposomal formulations present in the market [9].

Product	Manufacturer	Liposomes and key ingredients
Formule Liposome Gel	Formule Liposome Gel Payot (Ferdinand Muehlens)	(Thymoxin) hyaluronic acid
Symphatic 2000	Biopharm GmbH	Thymus extract vitamin A palmitate
Niosomes	Lancome (L'Oréal)	Glyceropolyether with moisturizers
Inovita	Pharm/Apotheke	Thymus extract, hyaluronic
Future Perfect Skin Gel	Estee Lauder	TMF, Vitamins E, A palmitate, cerebroside ceramide
Flawless finish	Elizabeth Arden	Liquid make up
Eye Perfector	Avon	Soothing cream to reduce eye
Nactosomes	Lancome (L'Oréal)	Vitamins
Efect du Soleil	L'Oréal	Tanning agents in liposomes niosomes lancome
Natipide II	Nattermann PL	Liposomal gel for do-it yourself

Table 7.
Liposomal cosmetic formulations present in the market [10].

8. Conclusions

Liposomes evolved as an extraordinary tool or micro-engineered membranes for the delivery of drugs because of their minimum toxicity and flexibility that can be tailored for various desirable intentions. This unparalleled delivery approach can be used for almost every drug or active pharmaceutical ingredient despite of its varied physicochemical properties and route of administration. Extensive uses of liposome in the delivery of drugs can be starded further by researchers, medical representatives and in scale-up processes in order to develop desired modification and better delivery approaches by holding the promising physio-chemical properties and pharmacokinetics (absorption, distribution, metabolism, and elimination) involved with liposomes, as described in the chapter.

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Conflict of interest

We wish to declare that there are no known conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

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