Chapter

Liposome-A Comprehensive Approach for Researchers

Mani Sharma, Jyoti Joshi, Neeraj Kumar Chouhan, Mamta N. Talati, Sandeep Vaidya and Abhiram Kumar

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. Liposmes 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 comprises 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
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 variates 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 phosphatidylserine1,2dioleoylsnglycerophosphoserine, dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylycholine (DSPC), dioleoylphosphatidylethanolamine (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.](image1)

![Figure 3. Inner and outer structure of liposome.](image2)

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.
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 internee” 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 accumulates in the heart.
• Intercepts the oxidation of drug
• Chelation therapy in case of 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 comfortly 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. Layers 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. Layes 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 got 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. Layes 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 passes 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$_2$HPO$_4$ 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 multilamellar 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.
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™ or 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                   |
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 starched 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

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Author details

Mani Sharma*, Jyoti Joshi¹, Neeraj Kumar Chouhan², Mamta N. Talati², Sandeep Vaidya² and Abhiram Kumar¹

1 Uttarakhand Technical University [UKTU], Uttarakhand, India

2 National Institute of Pharmaceutical Education and Research [NIPER], Telangana, India

*Address all correspondence to: mninup2015@gmail.com

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