

Novel Methods for Liposome Formulation: Advancements and Innovations

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REVIEW

Abstract

Liposomes are nanoscale spheroidal vesicles, predominantly composed of phospholipids, that offer a hydrophilic core surrounded by a hydrophobic bilayer. Their unique structure endows them with excellent biocompatibility and the ability to house both hydrophilic and lipophilic agents, making them exceptionally suited for targeted delivery applications. This versatility has seen liposomes gain widespread use in various industries, including medicine, pharmaceuticals, and nutraceuticals. This comprehensive review meticulously examines the spectrum of liposome fabrication techniques, providing insight into their respective advantages and limitations. Specifically, we scrutinize methods such as reverse phase evaporation, freeze drying, the Bangham method (also known as the thin film hydration technique), ethanol injection, the emulsion method, and ultrasonication. Each of these methodologies contributes to the array of liposomal forms, influencing their final characteristics and suitability for different applications. The present review categorizes liposomes based on the fabrication technique, offering a comparative perspective that is instrumental for researchers and practitioners in selecting the optimal method for their specific delivery system needs.

Keywords: liposome formulation; drug delivery; encapsulation; lipid bilayers; nanotechnology

INTRODUCTION

Liposomes are spherical phospholipid vesicles, nanoscopic or microscopic, that are made up of one or more concentric lipid bilayers that enclose an aqueous core. They were first manufactured by Bangham and colleagues in 1965 at Cambridge University and are composed of phospholipids and cholesterol, making them easily biodegradable (Enaru et al., 2021). Liposomes have the ability to incorporate hydrophilic substances inside the aqueous nucleus, and lipophilic compounds will be incorporated in the membrane region. Over the years' various compounds such as proteins, peptides, enzymes, anticancer agents, antimicrobials and DNA have been encapsulated in delivery systems that use liposomes to observe their effectiveness (Has and Sunthar, 2020). Liposomes have a great flexibility in terms of composition, size, and drug release properties. They are manufactured to be multifunctional with various components that can control specific parameters, such as half-life, permeability, biodistribution and specificity (Enaru et al., 2021). Liposomes, as nanoscale vesicular structures, possess an impressive degree of flexibility in their composition, size, and drug release properties, attributes that render them highly versatile in a wide spectrum of applications. Their primary structure, comprised of a phospholipid bilayer encapsulating a hydrophilic core, can be engineered to exhibit distinct characteristics tailored to specific applications (Nakhaei et al.,

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2021, Elizondo et al., 2011).

The compositional flexibility of liposomes allows for the incorporation of various components, each influencing a specific parameter. For instance, the incorporation of specific lipids or cholesterol can modulate the fluidity and stability of the liposomal membrane, subsequently influencing the release kinetics of the encapsulated drug. Similarly, the incorporation of PEGylated lipids (polyethylene glycol conjugated lipids) can enhance the half-life of liposomes in systemic circulation by evading the mononuclear phagocyte system, a common issue in nanomedicine known as opsonization (Mitchell et al., 2021). Size is another critical parameter that can be controlled during the fabrication of liposomes. Liposome size has a substantial impact on their biodistribution and clearance from the body. Smaller liposomes (under 200 nm) tend to have prolonged circulation times and can passively target tissues with leaky vasculature, such as tumor tissues – a phenomenon known as the Enhanced Permeability and Retention (EPR) effect (van der Koog et al., 2022).

Furthermore, the surface of liposomes can be modified with various ligands to achieve active targeting, improving the specificity of drug delivery to the intended site. These ligands could be antibodies, peptides, or small molecules that bind to specific receptors overexpressed on the target cells. This customization potential allows for the creation of 'smart' liposomes that can deliver their payload with high specificity, reducing off-target effects and enhancing the therapeutic index (van der Koog et al., 2022, Tenchov et al., 2021).

In the scientific domain, it is noteworthy to emphasize that liposomes have carved out a significant niche for themselves as the inaugural nanodelivery systems to have achieved successful clinical application. Their groundbreaking contribution to the field of nanomedicine has revolutionized therapeutic strategies, particularly for afflictions that necessitate precise drug targeting and meticulously controlled release mechanisms.

Liposomes offer an inherently versatile platform for drug delivery due to their unique ability to encapsulate both hydrophilic and hydrophobic therapeutic agents. This has substantially widened their applicability spectrum, enabling the treatment of a diverse array of diseases such as cancer, fungal infections, and genetic disorders (Tenchov et al., 2021).

Particularly in the field of oncology, liposomes have been instrumental in improving the delivery of chemotherapeutic drugs. They have been employed successfully in the treatment of various types of cancers, including Kaposi's sarcoma, multiple myeloma, and ovarian and breast cancers, among others (Koning et al., 2010, Lorusso et al., 2007, Li et al., 2022, Gottlieb et al., 1997). The incorporation of chemotherapeutics into liposomes has demonstrated significant advantages such as reduced systemic toxicity, increased drug stability, and improved therapeutic indices (Nel et al., 2023). Additionally, liposomal formulations have been applied in combating fungal, viral, and bacterial infections (Kim and Jones, 2004). For fungal infections, the liposomal encapsulation of antifungal drugs, such as Amphotericin B, has transformed the therapeutic approach to these diseases. The liposomal delivery system minimizes the drug's systemic toxicity while enhancing its efficacy, a vital advancement for treating invasive fungal infections that are often life-threatening and more prevalent in immunocompromised populations (Stone et al., 2016).

The versatility of liposomal formulations extends to antiviral therapies, where they facilitate targeted delivery and controlled release of antivirals, ensuring a higher concentration of the drug reaches the infected cells. This methodology is particularly beneficial for treating chronic viral infections, where sustained drug release can aid in managing viral replication and resistance (Bilek et al., 2011).

In the realm of bacterial infections, liposomes have been instrumental in delivering antibiotics directly to the site of infection, including intracellular targets. This targeted approach not only increases the local concentration of antibiotics but also reduces the likelihood of systemic side effects and the emergence of antibiotic-resistant strains.

The inclusion of liposomal formulations in the antimicrobial arsenal embodies a strategic response to the pressing challenges of drug resistance and therapeutic efficacy (Kim and Jones, 2004, Ferreira et al., 2021, Haggag et al., 2021). Similarly, in antibacterial therapy, liposomal formulations have been used to enhance the bioavailability of antibiotics, especially in the treatment of intracellular bacterial infections (El-Hammadi and Arias, 2019).

A study examined the use of cationic liposomes to enhance the delivery of benzyl penicillin (pen-G) to *Staphylococcus aureus* biofilms. The liposomes were more effective than free pen-G, particularly at low concentrations and brief exposures, extending the time for biofilm growth to maximum rate by about four times. The findings suggest that liposomal encapsulation can significantly improve pen-G's effectiveness against bacterial biofilms (Kim and Jones, 2004). The ability to encapsulate a broad range of antimicrobial agents and deliver them directly to the site of infection has enhanced the therapeutic efficacy and reduced off-target toxicity. For instance, liposomal Amphotericin B, an antifungal agent, has markedly reduced nephrotoxicity compared to its conventional counterpart (Tenchov et al., 2021, Wang et al., 2023).

Given these applications in multiple therapies, it becomes essential to understand the manufacturing processes of liposomes, as the method of production can significantly influence the characteristics and efficacy of the liposomal formulation. This review aims to provide a comprehensive overview of the most prevalent techniques for liposome preparation, highlighting the advantages and limitations of each method. The methodologies covered will range

from conventional techniques such as thin-film hydration and sonication to more advanced and precise approaches like microfluidics and supercritical fluid technology.

Furthermore, the review will discuss the various types of liposomes that can be produced, each with distinct properties and suitability for different therapeutic contexts. The classification of liposomes based on their size, charge, and composition is critical, as these factors determine the biodistribution, cellular uptake, and release profile of the encapsulated drugs. This review will serve as a guide through the landscape of liposome manufacturing, aiding in the understanding and selection of appropriate production techniques for creating the most effective liposomal formulations.

MATERIALS AND METHODS

Materials

This paper is an overview of the novel methods for liposome formulation. The literature search took place in PubMed, Web of Science, Scopus, and the academic search engine Google Scholar databases. The following keywords were used: liposomes* AND formulation, liposomes * AND bioavailability, liposomes * AND therapeutic effects, liposomes * AND antimicrobial, applications and liposomes * AND antiviral, applications and liposomes *. The results were screened based on their titles, abstracts, and full-text availability. All non-English publications were excluded from the present review. Filter limits (such as text availability, article type and publication date) were not applied. The time window was up to 15 May 2023.

Methods of liposomes preparation

Liposome preparation methods have been greatly improved over the years, and currently, there are several methods by which liposomes can be obtained? In order to obtain liposomes by conventional methods, four stages are required: (a) drying of lipids from organic solvents, (b) dispersion of lipids in an aqueous medium, (c) purification of liposomes, and (d) eventual post-processing steps such as sonication or extrusion (Trucillo et al., 2020). On the other hand, due to recent developments in technology, new methods of obtaining these vesicles have been characterized to overcome the limitations faced by conventional methods of liposome preparation. These include microfluidic techniques (e.g. micro hydrodynamic focusing, microfluidic droplets for the formation of giant vesicles), supercritical fluids method, or modified electroformation methods for obtaining giant vesicles. However, it is important to remember that the new liposome manufacturing methods have just been tested in the lab and are still being improved for industrial application (Patil and Jadhav, 2014). Further, we will focus on some of the conventional methods of obtaining liposomes, reviewing the advantages and disadvantages of each technique.

The reverse phase evaporation (RPE) method

This process necessitates the initial formulation of a lipid amalgam, which is subsequently relocated to a round-bottomed flask to facilitate the removal of the solvent through the utilization of a rotary evaporator. The resulting compound is then purged of any extraneous impurities *via* a nitrogen stream. Subsequently, the lipid constituents are dissolved within an organic phase, initiating the formation of reverse-phase vesicles. Post redispersion of the lipids, the intended drug for encapsulation is introduced under the aqueous phase.

This system mandates constant exposure to a nitrogen atmosphere, followed by sonication of the two-phase system until it manifests as a homogeneous, clear single-phase dispersion. Upon reaching this stage, the resultant solution must be returned to the rotary evaporator to facilitate the extraction of the organic solvent until a gel is achieved. The final step involves the exclusion of any residual unencapsulated material (Choudhury, 2011). This methodology employed for liposome preparation presents certain drawbacks, one of which includes the potential retention of a substantial quantity of solvent within the final form of the compound. Additionally, the scalability of reverse-phase evaporation liposomes (RPE-liposomes) represents a significant challenge, rendering large-scale production a complex endeavor (Catala A. , 2020).

Reverse-phase evaporation liposomes (RPE-liposomes) are employed in a variety of medical applications due to their ability to enhance drug bioavailability and control drug release (Pradhan et al., 2015). They are especially beneficial in the delivery of a diverse array of therapeutic agents, encompassing hydrophilic, hydrophobic, and amphiphilic drugs, protecting these compounds from premature degradation. In the field of oncology, RPE liposomes target the delivery of chemotherapeutic agents directly to cancer cells, which helps to reduce the systemic toxicity associated with cancer treatments and improves the therapeutic outcomes (Bothun et al., 2009). They also offer a promising vector for gene therapy, encapsulating nucleic acids like DNA and RNA to treat genetic disorders (Lu et al., 2012).

Beyond drug delivery, these liposomes have found a niche in vaccine administration, where they can encase antigens and adjuvants to heighten immune responses while reducing side effects (Turánek et al., 2012, Lu et al., 2012). For conditions involving enzyme deficiencies, RPE liposomes facilitate enzyme replacement therapies by

delivering functional enzymes to the required sites within the body. Their use extends into imaging and diagnostics as well, where they carry contrast agents to specific targets, aiding in the detection and monitoring of a range of health conditions.

In ophthalmology, the specificity of RPE liposomes is exploited to administer drugs to targeted areas within the eye, presenting a marked advantage over traditional methods like eye drops and injections (López-Cano et al., 2021). Additionally, the characteristic features of RPE liposomes are harnessed in transdermal drug delivery systems, offering a non-invasive route of administration that can transport drugs across the skin barrier. Collectively, the multifaceted applications of RPE liposomes underscore their significance in advancing therapeutic delivery and diagnostic methods across a spectrum of medical disciplines

Freeze drying method

In this case the protocol is based on the formation of a dispersion of lipids in water-soluble carrier materials (Li and Deng, 2004). This method is used to produce sterile and small liposomes. In this process, lipids and aqueous soluble carrier materials (such as sucrose) were dissolved in tertiary butyl alcohol to form an isotropic monophasic solution. Next, the product obtained will be sterilized by filtration and then collected in containers for lyophilization. After this last step, the compound will be hydrated with an aqueous solution to obtain a homogeneous suspension of liposomes. In addition, several studies on the lyophilization of liposomes have been conducted to determine the extent to which this process protects the vesicles formed against fusion and leakage to increase the stability of their storage (Has and Sunthar, 2020, El-Nesr et al., 2010, Glavas-Dodov et al., 2005).

Freeze-dried liposomes, known for their enhanced stability and shelf life, are used extensively in the pharmaceutical industry for the long-term storage of liposomal drugs (Franzé et al., 2018). The freeze-drying process, which removes water by sublimation, renders the liposomes less prone to oxidative degradation and hydrolysis, making them more durable and easier to transport without the necessity for refrigeration. This attribute is particularly advantageous for shipping to regions where refrigeration is not consistently available.

In pharmaceutical formulations, these lyophilized liposomes can be conveniently rehydrated prior to administration, enabling precise dosage and simplifying the handling of dry powders. They have a significant role in targeted drug delivery, especially in oncology, where they ensure controlled release of chemotherapeutic agents directly to tumor sites. Moreover, their application extends to vaccine delivery, where they contribute to the stability and enhanced immune response of liposomal vaccines. Lastly, in the burgeoning field of gene therapy, freeze-dried liposomes serve as protective vessels for nucleic acids, enabling the efficient delivery of genetic materials for therapeutic purposes. Overall, the versatility of freeze-dried liposomes is crucial in advancing drug delivery systems, offering stability, ease of use, and a wide range of applications in modern medicine (Engel et al., 1994, Lee et al., 2020, Daraee et al., 2016).

The Bangham method also called “thin film method”

This is the oldest, simplest, and most commonly used technique for obtaining liposomes. However, it has a relatively low degree of encapsulation. The first step in producing liposomes by this method is to obtain a thin film of lipids on a glass wall of the rotary evaporator flask. Before the second stage, hydration, the lipid film, water, and the buffer to be used for hydration must be heated above the lipid transition temperature to obtain a thin layer of the lipid film. After hydration, a mechanical stage follows, with vigorous agitation and sonication in the ultrasonic bath, so that the lipid film peels off the wall of the balloon and forms liposomes. The vesicles obtained by this method have different dimensions and are of the MLV (multilamellar) type. Depending on the nature of the substance that will be incorporated into the liposomes, it can be introduced with lipids, before the formation of the thin film (if the compounds are lipophilic) or with the water/buffer solution (if the compounds are hydrophilic). As a result, the thin film method has a very high degree of reproducibility even when working with small amounts of compounds, making it the most widely used method of obtaining liposomes since the 1970s (Šturm and Poklar Ulrih, 2021).

Thin-film liposomes are utilized across various sectors of medical and scientific research due to their unique ability to encapsulate both hydrophilic and lipophilic drugs within their bilayer structures. The formation of thin-film liposomes involves the evaporation of solvents from a lipid solution, leaving behind a film that, upon hydration, forms the liposomes. This method is fundamental in the creation of liposomal formulations for targeted drug delivery, particularly in the treatment of cancer, where they help to direct chemotherapeutic agents to tumor cells, minimizing the impact on healthy tissue and reducing side effects.

Additionally, these liposomal structures are instrumental in the development of vaccines, where they can act as adjuvants to enhance the immune response to antigens. In the realm of gene therapy, they are pivotal in safely transferring genetic material into cells, offering new avenues for treating genetic disorders. Their application is also seen in the delivery of cosmetic actives, where they provide a controlled release of compounds to improve skin health and appearance. Furthermore, thin-film liposomes are key in the field of diagnostics, where they aid in the targeted delivery of imaging agents, improving the accuracy and efficiency of imaging techniques. Their role in

enhancing the bioavailability and efficacy of oral drugs cannot be overlooked, as they protect active pharmaceutical ingredients from degradation in the digestive tract. This wide range of applications makes thin-film liposomes a cornerstone technology in both therapeutic and diagnostic interventions

The ethanol injection (EI) technique

This method involves the dissolution in an organic solvent (ethanol) of phospholipids and other lipophilic compounds. Then, the obtained solution is quickly injected in a large amount of aqueous buffer, thus forming small unilamellar vesicles (SUV) liposomes spontaneously. It is essential to note in this mechanism that the formation of liposomes is not due to the injection system but to the extension of a minor water-miscible organic phase in a major aqueous phase triggering the self-assembly of phospholipids to form liposomes (Sala et al., 2017)). Also, the size of the obtained liposomes depends on the concentration of lipids and the injection speed. The disadvantages of this method are the low encapsulation efficacy of hydrophilic compounds, the relatively low solubility of lipids in ethanol and the limited concentrations of lipids in the final solution due to the high ethanol content in it (Šturm and Poklar Ulrih, 2021).

Emulsion method

In the context of the emulsion method for liposome synthesis, it is essential to initially dissolve the phospholipids in an organic solvent. This solution is subsequently combined with a second solution to generate a water-in-oil (W/O) emulsion. This composite is then introduced into an additional aqueous solution, resulting in a double emulsion (W/O/W). Finally, the organic solvent is subjected to evaporation, culminating in the formation of a liposomal aqueous suspension (Trucillo et al., 2020). A significant benefit associated with the emulsification technique is the resultant superior encapsulation efficiency of the liposomes, particularly when contrasted with those produced *via* the injection method (Šturm and Poklar Ulrih, 2021).

Ultrasonication

Ultrasonication is a simple and frequently used method for the production of liposomes. This process is a green technology that utilizes ultrasonic waves to prepare emulsions and gives the user more control over the physical properties of the emulsions. The most important factors that determine the size of the vesicles obtained are the intensity of the pressure waves and the time of administration. Exist two types of sonication technic, bath sonication and probe sonication, but liposomes produced by both sonication techniques have similar qualities. On the other hand bath sonication, is superior in terms of controlling operatinal parameters (Ajeeshkumar et al., 2021). Some of these methods of obtaining liposomes are outlined in Figure 1.

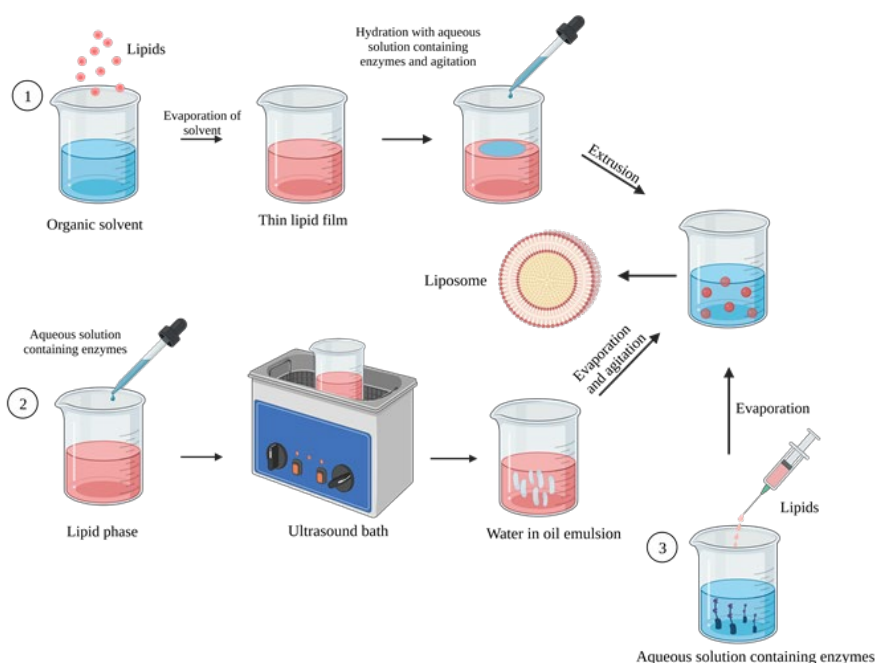


Figure 1. Schematic representation of methods for liposome preparation 1) Lipid film hydration method. 2) Ultrasonication method. 3) Injection method. Adapted after (Mohammadi et al., 2020), (Mohammadi et al., 2021). Created with BioRender.com

These methods presented above are only a part of those currently used for the production of liposomes. The advantages and disadvantages of the methods mentioned earlier for liposome preparation are further summarized in Table 1. (Maja et al., 2020, Meure et al., 2008).

Table 1. Advantages and disadvantages of methods for liposome preparation

Methods	Advantages	Disadvantages
Reverse phase evaporation	Simple design, reasonable encapsulation efficiency	It is not ideal for encapsulation of fragile molecules due to the huge amount of organic solvent use, time-consuming, sterilization problems
Freeze drying	Dry liposome preparation is possible, with low organic solvent residue and better stability	Time-consuming, sterilization problems
The Bangham method or Thin film method	Simple process	Huge amount of organic solvent use, requires vigorous agitation, large vesicles with no control on particle size, time-consuming, sterilization problems
The ethanol injection	Simple process	Organic solvent residue, nozzle blockage in ether system due to pre-evaporation, time-consuming, sterilization problems
Emulsion method	Simple, with the ability to make multivesicular liposomes for the transport of numerous substances that aren't stable together.	Huge amount of organic solvent use, requires vigorous agitation, sterilization problems
Ultrasonication method	Simple and fast method, with high efficiency	Limited processing capacity, and lipid degradation may occur

Using different methods of liposome preparation will result in liposomes with different morphologies and properties. These characteristics are essential during the selection of the type of liposome to be used in a particular experiment. Liposomes obtained have different sizes, shapes, morphology, surface characteristics, electrical charges, and lamellarity. Classification of liposomes based on their sizes is given in Table 2.

Table 2. Classification of liposomes according to size

Name	Size Range [µm]	References
Single Unilamellar Vesicles (SUV)	0.02–0.20	(Fu et al., 2020)
Medium Unilamellar Vesicles (MUV)	0.20–0.50	(Laouini et al., 2011, Li and Deng, 2004)
Large Unilamellar Vesicles (LUV)	0.50–10	(Barenholz et al., 2011)
Giant Unilamellar Vesicles (GUV)	100–200	(Walde et al., 2010)

On the other hand, the lamellarity in Table 3 indicates the number of double layers of phospholipids surrounding the inner aqueous core containing the hydrophilic drug (Table 3) (Trucillo et al., 2020). As we see in Tables 2 & 3, different types of liposomes can be prepared, each with specific characteristics, depending on their use. Therefore, the most commonly used types of liposomes are small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs), huge unilamellar vesicles (H), multilamellar vesicles (MLV/MLVs), and multivesicular vesicles (VMVs) (Šturm and Poklar Ulrih, 2021). SUV-type liposomes present as a single phospholipid bilayer sphere comprising the aqueous solution, while MLV liposomes have several layers that look structurally like an onion (Ajeeshkumar et al., 2021).

The most effective liposomes are single unilamellar vesicles (SUVs) that could be used in any type of human

tissue where nanosomes can concentrate and be taken up by cells. Medium unilamellar vesicles (MUVs), on the other hand, could be employed to entrap medicines and target specific sites (Trucillo et al., 2020).

Table 3. Classification of liposomes according to lamellarity

Name	Number of layers	References
Oligo Lamellar Vesicles (OLV)	<5	(Laouini et al., 2011)
Multi Lamellar Vesicles (MLV)	5-20	(Patil and Jadhav, 2014)
Multi Vesicular Vesicles inner core disjointed vesicles (MUV)	>50	(Liu et al., 2018)

Development of liposomes

The liposomes were first described in 1964, as self-assembling closed vesicular structures formed by phospholipids in an aqueous environment. These artificial vesicles, coined from the Greek words for "fat" (lipo) and "body" (soma), are composed of an aqueous core encapsulated by one or more concentric lipid bilayers, with the ability to carry both water-soluble and fat-soluble drugs (Samad et al., 2007). Liposomes, often larger than 400 nm, are utilized for the delivery of a diverse array of substances, including hydrophilic and lipophilic drugs as well as proteins and macromolecules, for both topical application and systemic treatment of localized diseases (Wang et al., 2023).

Building upon the conventional liposomal design, our discussion now shifts to the innovative domain of deformable liposomes, which marks a substantial advancement in transdermal drug delivery systems (Nayak and Tippavajhala, 2021). This next section will explore their distinctive flexibility, mechanisms of skin penetration, and their capacity to revolutionize the delivery of therapeutic agents, offering a potential paradigm shift in non-invasive treatment modalities.

Deformable liposomes, also known as elastic or flexible liposomes, are a novel form of liposomal drug delivery system that has been designed to enhance the transdermal and topical transport of active pharmaceutical ingredients (Nayak and Tippavajhala, 2021). Unlike conventional liposomes, which have a rigid structure, deformable liposomes possess an ability to squeeze through tight junctions in the stratum corneum, the outermost layer of the skin, due to their elastic nature. This property significantly improves the penetration of encapsulated drugs, making deformable liposomes a promising vehicle for non-invasive transdermal drug delivery, with potential applications in localized therapy, systemic drug delivery, and targeted treatments for a variety of conditions. Deformable liposomes are engineered with an intrinsic flexibility that sets them apart from their traditional counterparts. This flexibility is chiefly attributable to the inclusion of edge activators—components that disrupt the lipid bilayer's regular structure, thereby imparting the elasticity needed for the liposomes to deform and pass through the narrow intercellular spaces within the stratum corneum. As they traverse these barriers, deformable liposomes maintain their integrity and encapsulated contents, emerging on the other side ready to release their drug payload into deeper skin layers or systemic circulation (Ternullo et al., 2018, Nayak and Tippavajhala, 2021).

The mechanism by which these liposomes penetrate the skin involves a combination of processes including adsorption to the skin surface followed by penetration *via* the transepidermal or transfollicular routes. Their deformability allows them to squeeze through the skin's tight junctions without the need for enhancers that disrupt the skin's barrier, thereby minimizing potential irritation and preserving the skin's natural defense mechanisms.

The capacity of deformable liposomes to efficiently deliver a wide range of therapeutic agents through the skin opens up new avenues for non-invasive treatment strategies (Nayak and Tippavajhala, 2021, Ternullo et al., 2018). They hold the promise of enhancing the clinical efficacy of drugs, particularly those with poor skin permeability, while also improving patient compliance by providing a more convenient and less invasive route of administration compared to injections or oral medications. With their potential to deliver both hydrophilic and lipophilic drugs effectively, deformable liposomes could indeed represent a paradigm shift in how treatments are administered across various medical fields. Deformable liposomes can be classified based on their composition, the method used for their preparation, and their intended application (Nayak and Tippavajhala, 2021). Here's a brief overview of the different types of deformable liposomes:

Transfersomes: these are highly flexible liposomes that contain at least one inner aqueous compartment. Their flexibility is due to the inclusion of surfactants like sodium cholate or span 80, which make the lipid bilayer more fluid. Transfersomes are specifically designed to transport drugs across the skin barrier.

Ethosomes: comprising a high concentration of ethanol, ethosomes have enhanced skin permeation qualities. The ethanol provides the liposomes with a soft, malleable character and also contributes to the disruption of the skin's lipid bilayer, facilitating deeper penetration.

Mentosomes: these are a type of liposome that include menthol as a penetration enhancer, which disrupts the stratum corneum to allow the liposome and its cargo to penetrate more effectively.

Invasomes: invasomes are vesicles containing phospholipids, ethanol, and terpenes, which work synergistically to enhance skin permeation. The terpenes serve as penetration enhancers by disturbing the skin's lipid matrix.

Transethosomes: these are a hybrid of transfersomes and ethosomes, designed to contain both the edge activators of transfersomes and the high ethanol content of ethosomes. This combination makes them especially effective at penetrating the skin barrier.

Each type of deformable liposome is tailored to optimize the delivery of active substances through the skin, with the choice of liposome depending on the drug's characteristics and the desired therapeutic outcome. Their classification reflects the diversity and specificity of this advanced drug delivery technology (Nayak and Tippavajhala, 2021).

The categorization of liposomes into generations is informed by the evolution of their production techniques and functional sophistication, culminating in four distinct types. Each generation represents a milestone in liposome technology, characterized by unique features and intended uses. First, we have the conventional liposomes, which are basic vesicles formed by phospholipid bilayers that serve the primary purpose of drug solubilization and protection. Advancing from this point, we encounter the second generation, which branches into stimulus-responsive liposomes, engineered to discharge their cargo in reaction to specific environmental triggers, and stealth liposomes, which are surface-modified with polymers like polyethylene glycol (PEG) to evade immune detection, thereby prolonging their systemic circulation. Progressing to the third generation, ligand-targeted liposomes emerge, which are designed with surface ligands that bind to specific cell receptors, allowing for targeted drug delivery. Lastly, the fourth generation encompasses theranostic liposomes, which are a hybrid system capable of simultaneous therapy and diagnostics, representing the pinnacle of this nanotechnological hierarchy (Sogut et al., 2021). The ensuing sections will delve into a detailed discourse on each of these innovative liposomal generations.

Conventional liposomes

Conventional liposomes are the foundational class in the family of liposomal formulations. These are spherical vesicles that have at least one lipid bilayer composed of naturally derived phospholipids and cholesterol. The structural simplicity of conventional liposomes belies their significance in the realm of drug delivery systems. They are designed to encapsulate and transport both hydrophilic and lipophilic drugs, offering several advantages over free drugs, including improved solubility, reduced toxicity, and protection from rapid degradation.

A key feature of these vesicles is their biocompatibility, owing to the use of non-toxic and biodegradable materials that mimic cell membranes. Conventional liposomes can be passively targeted to sites of disease or inflammation simply because of their size and the enhanced permeability and retention (EPR) effect commonly found in tumor tissues. However, they do present limitations such as relatively quick uptake by the body's mononuclear phagocyte system (MPS), also known as the reticuloendothelial system (RES), leading to a shorter circulation time in the bloodstream.

Despite this, conventional liposomes have seen widespread use in the clinic with several liposomal drugs approved for use in treatments for cancer, fungal infections, and pain management. Their development set the stage for later generations of liposomes, which have built upon the fundamental design to improve efficacy, specificity, and the ability to deliver a wider range of therapeutic agents.

Briefly, this type of liposome is most often composed of natural phospholipids incorporating water molecules (Bueno et al., 2018). Thus, obtaining them is done by the classical method, mixing phospholipids with an organic solvent so that this solution is later subjected to evaporation to obtain a dry lipid film (Sogut et al., 2021). The method is straightforward, and studies based on this type of conventional liposome have shown that they have improved the therapeutic index and reduced the toxicity of drugs administered (Sercombe et al., 2015). However, it possesses several disadvantages, such as rapid elimination from blood, aggregation, coalescence, or degradation (Sogut et al., 2021).

Stimulus-responsive and stealth liposomes

Stealth liposomes are obtained by coating the surface of conventional liposomes with hydrophilic polymers, such as polyethylene glycol. Thus, parameters such as stability and circulation time in the blood can be improved, and protein adsorption can be reduced (Kang et al., 2017, Moghimi and Szebeni, 2003). Stimulus-responsive liposomes are produced by adding a chemical constituent to conventional liposomes to trigger structure. Therefore, stimulus-responsive liposomes can cause a higher concentration of extract, faster release at target sites, and protection against degradation. A significant advantage is that the incorporated active compounds are released faster, and they will accumulate more efficiently in the targeted organs; hence, the side effects of drugs are considerably reduced (Sogut et al., 2021).

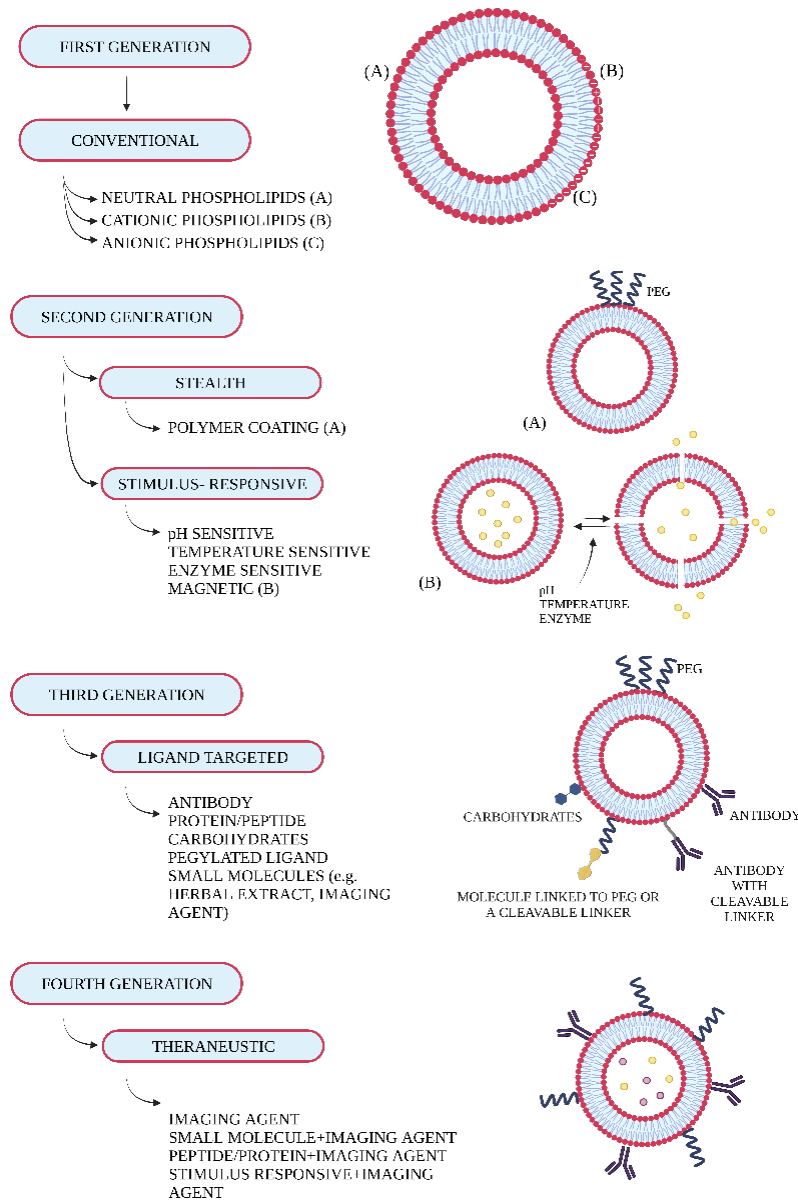


Figure 2. Differences between the four generations of liposomes (Sogut et al., 2021)(Sogut et al., 2021). Created with BioRender.com

Theranostic liposomes

In this case the protocol is based on the formation of a dispersion of lipids in water-soluble carrier materials. Ligand targeted liposomes are so named because they are conjugated with targeted molecules to improve the targeted drug delivery system (Sercombe et al., 2015). Consequently, different types of targeted molecules such as vitamins, proteins, or enzymes responsible for the target site's affinity can be added to the surface of the liposomes (Sogut et al., 2021). These types of liposomes can target multiple cells/tissues while improving the therapeutic efficacy by causing a more extensive accumulation of the carriers to the targeted site (Islam Shishir et al., 2019). This type of liposome with active therapeutic substances can precisely target the specific place and respond to external and internal stimuli through controlled release mechanisms (Sercombe et al., 2015). To produce theranostic liposomes, therapeutic agents are added to the hydrophilic nucleus or lipophilic bilayer through adsorption, encapsulation, conjugation, or entrapment. Diagnostic agents that can be encapsulated in the hydrophobic core or conjugated on the liposome surface are simultaneously added with this process so that the circulation time of the liposomes increases and the release of substances is controlled (Sonali et al., 2016). Figure 2. represents spherical vesicles to observe their composition and better understand the differences between the four generations of liposomes mentioned above (Sogut et al., 2021).

CONCLUSIONS AND FUTURE PERSPECTIVES

Liposomes stand out as highly adaptable and functional drug delivery systems due to their compatibility with biological systems and capability to encapsulate diverse therapeutic agents. The composition and size of liposomes are critical parameters influencing their interaction with biological environments, dictating drug release rates, distribution within the body, and overall therapeutic success.

The clinical relevance of liposomes is underscored by their proven effectiveness, particularly in cancer treatment and managing infectious diseases. The incorporation of drugs into liposomes has been shown to enhance treatment efficacy while minimizing the adverse effects typically associated with these drugs.

Surface modifications allowing for targeted drug delivery represent a significant stride forward, increasing the precision of treatments and sparing non-target tissues, which is of particular importance in the treatment of cancer and other specific diseases.

The production methodology of liposomes is a determinant of their final properties. Cutting-edge techniques such as microfluidics and supercritical fluid technology are paving the way for more sophisticated and precisely engineered liposomes.

The review process described demonstrates a methodological rigor, ensuring a thorough survey of the current landscape of liposome formulation research.

Clinical applications highlighted in the paper, particularly the use of liposomal Amphotericin B and its impact on reducing nephrotoxicity in fungal infections, exemplify the potential of liposomes to significantly improve therapeutic outcomes. Concluding from the outlined methods and their respective advantages and disadvantages, it appears that no single technique can be universally designated as the best for liposome fabrication without considering the specific requirements of the encapsulated agents and the intended application. However, a method that frequently stands out is the thin film hydration technique, also known as the Bangham method.

The thin film hydration method is esteemed for its simplicity and wide applicability, making it a standard in liposome preparation. Despite the use of a significant volume of organic solvents, it is a robust and adaptable technique that has been extensively refined over decades. It allows for the formation of multilamellar vesicles with reasonable encapsulation efficiency and has been successfully scaled up for industrial production.

This method's adaptability is one of its strongest points, enabling modifications to accommodate a wide variety of lipophilic and hydrophilic drugs. It also allows for the adjustment of vesicle size and membrane characteristics by varying hydration time, temperature, and the composition of the lipid mixture. These customizable parameters are crucial when designing liposomes for different therapeutic targets.

Moreover, the thin film hydration method can be combined with other techniques, such as extrusion or sonication, to further refine the size and lamellarity of the vesicles, providing an additional layer of control over the final product. This versatility is invaluable in research and clinical settings where the needs can vary widely from one application to another.

In summary, while newer methods continue to emerge, the thin film hydration technique remains a cornerstone in liposome fabrication due to its simplicity, flexibility, and the extensive knowledge base available from its long-standing use in the field. It provides a strong balance between practicality and performance, with the potential for further optimization in accordance with the evolving demands of liposome technology.

The discovery of liposomes in 1965 heralded a new era in drug delivery systems, inspiring a plethora of production methods over the years. Despite this progress, there remains a significant disparity between the methods suitable for meticulous laboratory experiments and those viable for mass production. This gap underscores the critical need for research aimed at refining liposome manufacturing processes, which, at present, are fraught with high costs that inhibit their widespread application. Although the advantages of liposomes as delivery vehicles are well documented, their practical use is limited by the profitability margin, which is severely impacted by current production expenses. Consequently, advancing our research to overcome these economic and scalability barriers is essential to unlock the full potential of liposomes across various industries, including medicine, cosmetics, and biotechnology. Addressing these challenges not only holds promise for enhancing therapeutic outcomes but also for broadening the accessibility of this technology to benefit a larger population.

In light of these discussions, the review serves as an essential resource for both researchers and practitioners, providing insights that will help inform the development of liposome-based treatments. Understanding the manufacturing processes and their impact on liposome characteristics is fundamental to advancing drug delivery technologies and ultimately enhancing patient care.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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