Liposomes for Herbal Drug Delivery

by Andang Miatmoko

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CHAPTER 12

Liposomes for Herbal Drug Delivery

Andang Miatmoko[1,*], **Devy Maulidya Cahyani**[1] and **Retno Widyowati**[1]

[1] *Department of Pharmaceutical Science, Faculty of Pharmacy, Universitas Airlangga, Surabaya, 60115, Indonesia*

Abstract: This chapter evaluates liposomes used as delivery carriers for herbal products, which, due to poor permeability and solubility of extract components, has become a major issue in phytotherapy for treating illnesses and human health problems. Liposomes are vesicular formations with phospholipid bilayers that possess the capability to entrap both water-soluble and hydrophobic substances. However, there are several factors that should be considered with regard to herbal drugs, especially that the preparation technique should be appropriate to the solvent solubility of the plantextracts. In this regard, the ratio of phospholipids to extracts, pH stability, otherliposomal components, and the ligand required to render liposome stability, circulation in the bloodstream for protracted periods, and targeted at specific organs should be investigated. The enhancement of phytochemical constituent stability within a context of environmental, physical, and chemical degradation, together with sustained or controlled drug release, can be achieved by incorporating extracts into liposomes. Moreover, the improved oral absorption of plant extracts by encapsulating them into liposomes indicates increased permeability and bioavailability *via* gastrointestinaltracts, thus enhancing pharmacological effects at low dose concentration as well as decreasing toxicity. However, thousands of constituents contained in plant extracts demonstrate various physicochemical characteristics that constitute significant challenges for liposomal delivery. Consequently, a comprehensive analysis of formulating and manufacturing aspects is required.

Keywords: Bioavailability, Human health, Illness, Liposomes, Phospholipids, Plant extracts, Solubility.

INTRODUCTION

Health problems involving chronic diseases such as cancer, diabetes, hypertension, stroke, cardiovascular disease, among others, are known to have a high prevalence [1]. They greatly affect people's quality of life, thus still constituting a major challenge that needs to be successfully addressed [2]. More-over, these diseases

* **Corresponding author Andang Miatmoko:** Department of Pharmaceutical Science, Faculty of Pharmacy, Universitas Airlangga, Surabaya, 60115, Indonesia; Email: andang-m@ff.unair.ac.id

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are generally slow in progression, of considerable duration, and require medical therapy [3]. Therefore, identifying appropriate treatment strategies becomes the main concern in successfully addressing them by means of both medical and non-medical therapies involving either causative or symptomatic treatments.

Medical treatment includes the use of various pharmaceutical products. Therefore, a rapid expansion in the drug product list, in addition to designing effective delivery systems to target the disease sites, is required. However, the curative effect of drugs is not the only major concern since significant attention is also devoted to avoiding severe unintended side effects on normal tissues. Minimizing the side effects of medical therapy has encouraged numerous researchers to explore various ideas and strategies for drug delivery systems [4]. In addition, pharmacological therapy-related drug development is not limited to modern synthesized pharmaceuticals but is also strongly associated with natural and biological products offering great potential within the process of new drug discovery and development [5, 6].

Natural products are compounds produced by living organisms that usually produce various pharmacological effects. Plant extract usually contains many newchemical compounds with multiple structures that may have different biological roles [7]. Between 300,000 and 400,000 plant species are known to be used as primary raw materials in the development of new compounds as active pharmaceutical ingredients, which are proven to be effective as antioxidant, antimitotic, anti-infectious, anti-inflammatory, anti-angiogenic, and anti-cancerdrugs [8, 9].

On the other hand, not all compounds extracted or isolated from natural plants or other biological sources can be synthesized into medicine. The existence of extremely complex chemical structures which are exorbitantly expensive to synthesize on an industrial scale limits their development [7]. In addition, certain problems related to poor solubility and chemical instability also constitute major limitations on their clinical uses. Complex chemical structures and high molecular weight may also affect drug absorption, thereby requiring specific drugformulation strategies [10]. The main challenge for the formulation lies in poor bioavailability [11]. For example, the use of curcumin in the treatment of various diseases, such as cancer, has limited effectiveness due to its low water solubility, limited bioavailability, expeditious systemic metabolism, and rapid elimination [12]. It has long been accepted that solubility represents a determinant factor inthe absorption of a drug and that permeability can be an important parameter for the penetration of the biological membrane by drug molecules and their entry into the blood circulation system or their diffusion into the target cells. Therefore, a modify-

cation is required to improve their physicochemical properties, increase their stability, enhance their pharmacokinetic profiles, and minimize the sideeffects of natural drug compounds.

Over the years, nanotechnology has been widely developed in various scientific fields for medical purposes [13]. Nanomedicine, which involves the use of nanoparticles, has been extensively used to diagnose, monitor, control, prevent, and cure diseases [14]. Lipid-based nanoparticles are commonly used to protect drugs from *in vivo* degradation, control drug release, modify biodistribution, target drug delivery to disease sites, and improve solubility and bioavailability [15]. Of all nanoparticles, liposomes constitute some of the most widely studied, with certain FDA-accepted formulations being used in chronic disease therapy, such as oncology, having the greatest impact on this field. Nano-sized vesicles, compatibility and degradability in a biological environment, ability to encapsulate water-soluble and lipid-soluble substances, low toxicity, and the immunogenicity of liposomes represent significant advantages for disease therapy [16].

The word 'Liposome' is derived from the two Greek words '*Lipos'* meaning 'fat' and *'Soma'* which translates as 'body' [17]. Liposomes have a spherical vesicle-shaped formation composed of phospholipids that resemble biological membranes and act as drug carriers. Liposomes have two compartments, lipophilic and aqueous phases, and can be formed when phospholipids interact with water with the result that liposomes are able to reduce unwanted drug side effects and can be used for targeted delivery systems by altering drug distribution within the body [18].

The structure of liposomes, which consists of a bilayer membrane, means that hydrophobic drugs demonstrating low water solubility can be trapped in the membrane bilayer. Meanwhile, high levels of hydrophilic compounds can also be loaded in the aqueous intraliposomal phase [19]. By encapsulating drugs inside liposomes, drug degradation related to the physiological environment and severe side effects in healthy tissues can be significantly minimized [20]. It is possible to make a drug carrier featuring the vesicular structures of liposomes with the ability to load hydrophilic and lipophilic drugs, protecting the encapsulated drugs from environmental degradation [21]. Liposomes can also possess non-toxic biomimetic characteristics capable of increasing cell penetration, thereby enhancing their therapeutic effect. Biomimetic liposomes are extremely stable, demonstrating superiority *vis-a-vis in vitro* targeting capabilities, and achieve 2.25times deeper penetration of the 3D tumor spheroid than that of their conventional counterparts [22].

As drug delivery carriers, liposomes have numerous advantages, which include increasing the efficacy and index of drug therapy, enhancing stability through encapsulation and biodistribution, and completing biodegradation in the body. Moreover, they are generally non-immunogenic in both systemic and non- systemic uses and significantly reduce the toxicity of encapsulated drugs, *e.g.*, amphotericin B and paclitaxel, by reducing drug exposure. Liposomes demonstrate flexibility in relation to specific ligand modification with the result that they can be targeted at specific tissues within the diseased site [23]. Inaddition, they can increase therapeutic efficacy and reduce toxic effects on normal cells through enhanced permeability and retention (EPR). This constitutes a passive and selective accumulation within tumor tissue because of increased permeability of tumor neovasculature and defective lymphatic drainage, thus increasing drug exposure to cancerous cells [24]. On the other hand, liposomes imbue hydrophobic drugs with hydrophilic properties that can reduce the frequency of dose administration [25]. Therefore, they offer numerous benefits in relation to increasing the bioavailability and the half-life of most drugs and delivering them to specific disease-target sites [21].

In liposomal preparation, phospholipid selection greatly influences the physicochemical and biological properties of liposomes. Phospholipids determine, to a large extent, the loading capabilities, drug-lipid molecular arrangement, membrane integrity, surface charge, steric resistance, and permeability of the lipid bilayer [26]. Moreover, they can also affect the interaction of liposomes with blood and tissue components during systemic administration [27]. Liposomes can undergo degradation or physical changes during manufacture or storage, through which the use of unsaturated lipids can cause possible oxidation. The partial substitution of phospholipids with cholesterol can decrease membrane fluidity and reduce the surface charge of the liposomes [28]. Liposomes are conventionally composed of phosphatidylcholine and cholesterol. However, after administration, these liposomes are predominantly recognized by the reticuloendothelial system (RES), which leads to rapid drug clearance and, ultimately, impairs drug-targeting efficiency. PEGylation involving the use of polyethylene glycol (PEG) can increase the hydrophilic properties of liposomes and provide a steric barrier to prevent serum opsonization, thus causing them to circulate for a protracted period in the body and improve drug accumulation in target tissues [27].

The ultimate goal of delivering drugs by means of liposomes is to achieve high drug levels at the target sites of, for example, tumor and inflammatory tissues. Selective drug accumulation can be accomplished by both active and passive targeting. High drug availability at target sites becomes an important factor in achieving a therapeutic effect. Therefore, a high drug concentration at the target site is imperative. Delivery systems with high drug loading, minimal leakage during

administration, and systemic blood circulation, which only involve complete release in the target cells, need to be produced. In this regard, liposomes differ from other controlled release systems in which drug release occurs both in plasma and at the site of administration [29]. To achieve this, a combination of lipids and cholesterol can be used [30].

From the outset of their development, liposomes undergo rapid development. Conventional liposomes, which are merely composed of phospholipids, are known to have low stability and high recognition by RES, causing faster drug excretion [27]. There are currently several types of liposomes that enhance drug delivery. Based on their composition and mechanism, they can be classified into five types, namely conventional liposome, pH-sensitive liposome, cationic liposome, long-circulating liposome (LCL), and immuno-liposome. The pH- sensitive liposome type is composed of dioleoyl-phosphatidyl-ethanolamine (DOPE), which has been designed for specific drug release by means of a rapid destabilization mechanism in response to an acidic pH environment, such asendosome [31]. The cationic liposome type is composed of cationic lipids and neutral-charged lipids (co-lipids), in which cationic lipid exhibits a positive charge triggering interaction with negatively charged nucleic acid, thus increasing nucleic acid stability during systemic blood circulation and improving cellular uptake [32, 33]. To inhibit the uptake of liposomes by RES causing rapid liposome elimination, the long-circulating liposomes can be generated by theaddition of hydrophilic layers such as PEGylation on the liposomal surface, thus inhibiting protein adsorption [34]. The recent development of an immune- liposome, which recognizes tumor cells by means of antibodies, significantly enhances specific target cell internalization and intracellular drug release [35].

Several techniques have been developed to achieve liposome surface modification with different lipids or antibodies to overcome the problems associated with conventional liposome delivery. The use of PEG-lipid conjugates, target ligands, and multifunctional antibodies can be applied to liposome surface modification [36]. All of these correlate strongly with the physicochemical properties of lipids used in liposomes to determine the liposome-protein interaction in the body during administration [37].

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In this chapter, the authors present an overview of the terminology used with liposomes, formulation, and multidimensional approaches to liposome production. There is also a summary of a number of liposome applications for herbal drug delivery.

Liposomes as Drug Carriers

Liposomes were the first drug carriers created in England in the 1960s by Bangham, who conducted research predominantly focused on phospholipids and blood clotting [38]. As seen from Figure 1, liposomes are vesicle-shaped drug carriers composed of phospholipids and cholesterol [39], which are able to encapsulate both water-soluble and lipid-soluble compounds [40]. Liposomes are of various sizes, ranging from nanometers to micrometers, which are generally between 25 nm and 2.5 μm [4]. When exposed to water, lipids will tend to self- assemble, thereby forming vesicles. The hydrophilic interaction between the two polar groups and the Van der Waals interaction between the hydrocarbon tails and with water (hydrophilic interaction and hydrophobic effect) leads to the formationof a lipid-based vesicular structure [40].

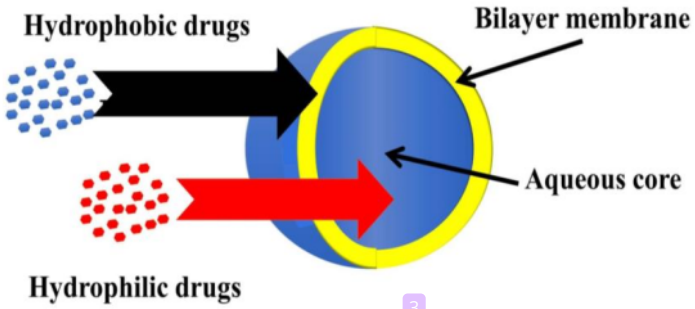

Fig. (1). The liposomal structure that enables penetration by both hydrophobic and/or hydrophilic drugs [38].

There have been numerous variations in the terminology relating to liposomes, such as ethosomes, transfersomes, and hexosomes. An ethosome, a lipid vesicle consisting of phospholipids, water, and a high concentration of ethanol, enables more efficient skin drug delivery in terms of both quantity and depth of penetration than a conventional liposome [41]. A transfersome is a highly elastic liposome composed of edge activators, *i.e.*, surfactants, enabling an improved penetration of transdermal drug delivery [42, 43]. On the other hand, a hexosome is a liposome with an internal hexagonal phase, providing superiority in formulation design, water solubility, and controlled-drug release of numeroussubstances [44].

Preparation of Liposomes

Liposomes are composed of phospholipid components, while other components such as cholesterol or PEG can be added. The characteristics of liposomes are highly dependent on the physicochemical properties of their constituent components.

1. Phospholipids are constituent components of the hydrophobic bilayer membrane.

Liposomes can be prepared with natural and/or synthetic phospholipids. Phospholipids are the main components of the cell membrane, which possess excellent amphiphilic and biocompatible properties [45]. This amphiphilic property invests phospholipids with self-assembly properties and enables them to act as emulsifying and wetting agents. When introduced into water, phospholipidscan spontaneously produce different supermolecular structures depending on their specific properties and conditions; for example, they demonstrate a tendency to form liposomes [45].

Natural phospholipids are widely derived from plant sources, such as soybean, canola, sunflower, wheat germ, and from animal sources, for instance, egg yolk and milk [45, 47]. In contrast, various types of synthetic phospholipids can be obtained from natural lipids by modifying the hydrophobic and hydrophilic regions of phospholipid molecules [40]. Synthetic and natural phospholipids have respective advantages and disadvantages. While synthetic phospholipids demonstrate comparatively high stability and purity, they are relatively expensive compared to their natural counterparts. However, obtaining consistent purity is challenging and synthetic phospholipids are relatively unstable, resulting in their metabolization into lysophospholipids during the preparation process and storage [45].

Phospholipids are amphipathic molecules composed of hydrophilic and lipophilic groups on the head and tail, respectively. The structure of phospholipids (Fig. **2**) generally consists of diglycerides, phosphate groups, *e.g.*, phosphoric acid molecules, and organic molecules such as choline. Diglycerides are glycerides consisting of two chains of fatty acids covalently bonded to a single glycerol molecule. The glycerol undergoes esterification at positions 1 and 2 with fatty and phosphoric acids at 3-position [46]. Glycerol ($C_2H_8O_3$) contains three hydroxyl groups (-OH), contributing to the solubility of phospholipid molecules in water, while fatty acid chains, whether saturated or unsaturated, possess hydrophobic properties. Thus, a phospholipid molecule has a hydrophobic tail consisting of two fatty acid chains and a hydrophilic head composed of glycerol and phosphate. A bilayer structure of liposome membrane will be spontaneously formed when the fatty acid chains of a phospholipid molecule encounter the fatty acid tail ofanother molecule with the polar head facing the water [47].

The physicochemical properties of each phospholipid are also related to the chain length of fatty acids, in which each phospholipid type has contrasting carbon atom numbers and saturation degrees, for example, hydrogenated soy phosphatidy-

lcholine (HSPC), distearoyl phosphatidylcholine (DSPC), dipalmitoyl phosphatidylcholine (DPPC), and dimyristoyl phosphatidylcholine (DMPC) [40]. For a stable liposome formulation, saturated phospholipids can be used, whereas this is rarely the case with unsaturated phospholipids. However, this selection is highly dependent on the physicochemical characteristics of the drug [48].

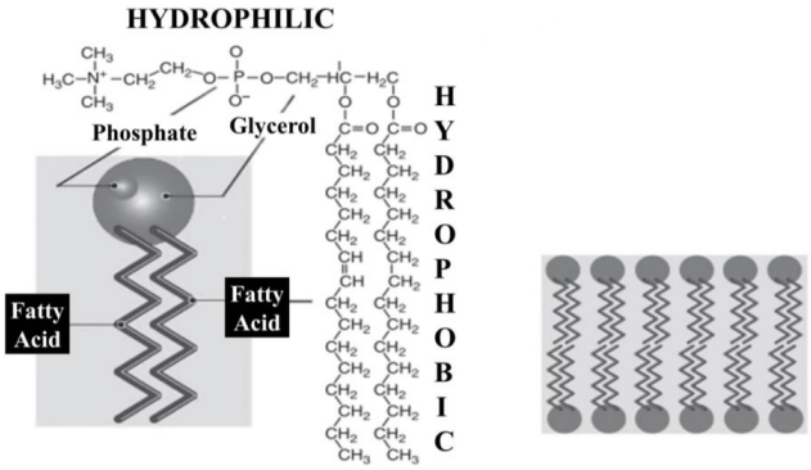

Fig. (2). The structure of phospholipids and their schematic representation in the formation of bilayer membranes of liposomes [47].

Based on the polar head group, phospholipids can be categorized as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol (PG), and phosphatidic acid (PA), while PC and PE have been generally used in the production of liposomes [40].

Liposomes are classified into three types according to their charge potential; cationic, anionic, and neutral, in which these surface charges largely determine the systemic circulation time, distribution, cellular uptake, and elimination of liposomes within the body [49].

A cationic liposome is generally prepared with lipids that can induce a positive charge on the liposome surface, as indicated in Table **1**. For the most part,negatively charged therapeutic agents can be incorporated into this liposome,forming stable complexes [49]. The general characteristics of cationic liposome include high water solubility and high cationic charge at physiological pH, thus promoting strong interaction with negatively charged cell membranes and improving cellular binding and uptakes [38], which strongly supports targeteddrug delivery. In contrast, anionic liposome, composed of phospholipids with an anionic head group, can affect cell signaling, protein-lipid interactions, and membrane trafficking

[50, 51]. The charged lipids are initially used in RNAdelivery as an application supporting successful gene delivery. On the other hand, cationic liposomes frequently have problems with systemic toxicity. The use of neutrally-charged phospholipids is generally applied to overcome toxicityproblems with high drug delivery efficiency [49].

Table 1. Phospholipid types based on the types of liposomes [49].

No	Liposome Types	Phospholipids Used as the Lipid Component
1	Cationic Liposome	1, 2-dioleoyl-3-trimethylammoniumpropane (DOTAP)
		N- [1- (2,3-dioleoyloxy) propyl] -N, N, N-trimethyl-ammonium methyl sulfate
		Dioleoylphosphatidyl ethanolamine (DOPE)
		Oleic acid (OA)
		Dimethyldioctadecylammonium bromide
2	Anionic Liposome	Phosphatidylglycerol (PG)
		Phosphatidylinositol (PI)
		Phosphatidic acid (PA)
		Phosphatidylserine (PS)
3	Neutral Liposome	1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC)

The most commonly employed phospholipid for preparing neutral liposome is phosphatidylcholine which consists of a hydrophilic group, *i.e.*, phosphocholine, together with a glycerol bridge and a hydrophobic region incorporating double acyl hydrocarbon chains [52]. Hydrogenated soy phosphatidylcholine (HSPC) is anon-ionic synthetic phospholipid obtained from hydrogenated natural phospholipid, which is produced from egg yolk or soy by hydrogenation. HSPC has a molecular weight of 837.44 and a number of saturated chains of 16-18 witha transition temperature (T_c) of 52°C [40, 47]. Its several advantages comprise being odorless and unaffected by oxidation [40, 53]. Moreover, it is easier to hydrogenate soybean PC in producing HSPC than synthesize DSPC as synthetic lipids. In addition, rather than DPPC or DSPC, HSPC is most often used to ensurethe stability of liposomes during systemic blood circulation.

In addition, the liposome bilayer membrane may contain other constituents such as cholesterol, hydrophilic lipids that are conjugated with lipids, and the water phase [38].

2. The hydrophilic aqueous core inside liposomes

The inner core of the liposome, surrounded by the polar head groups of the phospholipids, becomes the loading site for hydrophilic molecules. Therefore,

most hydrophilic drugs will tend to become entrapped in this aqueous core [55]. On the other hand, the lipophilic molecules will be enmeshed in the hydrophobic portion of the phospholipid bilayer [54].

Hydrophilic drug encapsulation can be passively induced by the hydration of a buffer or solution containing hydrophilic drugs [43]. Through this method, the drug can enter the liposomal aqueous core, although a limited amount of it will remain outside the liposome section. In addition, dehydration and rehydration methods can also be applied for DNA and protein encapsulation [56]. Drugs with good permeability coefficients that are reflected in their partition coefficient (Log P) can be efficiently loaded using an active method with the presence of pH or ammonia gradient. This loading method generally produces high encapsulation efficiency, which is, in most cases, at a level of >95% [48, 57].

3. Cholesterol as an additional component of the bilayer membrane

Cholesterol is generally used to enhance the physical stability of the membrane bilayer of liposomes, which reduces the water permeability of soluble molecules across the membrane bilayer [40], thus increasing liposome rigidity and stability [47]. Cholesterol plays a major role in the regulation, dynamics, and function of liposomal bilayer membranes by decreasing the free rotational force of the phospholipid hydrocarbon chain, thereby helping to reduce the loss of hydrophilic parts and stabilize the lipid bilayer [30]. In addition, without the addition of cholesterol, liposomes can interact rapidly with plasma proteins such as albumin, transferrin, and macroglobulin, causing physical instability of the liposomes and rapid elimination from the body. Cholesterol will occupy the gap produced by the phospholipids and render the structure more rigid, thus reducing the interaction between phospholipids and plasma proteins [58]. The use of cholesterol has also been reported to affect vesicle diameter in addition to efficient drug encapsulation [30].

4. PEG-conjugated lipids form a hydrophilic layer outside the liposomes

Targeted drug delivery systems represent a promising strategy for increasing selective drug delivery to unhealthy tissue or disease sites, thereby improving therapeutic efficacy and lowering toxicity. The use of hydrophilic polymer such as polyethylene glycol (PEG) is often associated with targeting specific cells, tissue, and even intracellular localization in organelles so as to prolong circulationtime, increase drug bioavailability, and reduce unwanted side effects [59].

Liposomes are known to be very rapidly metabolized by the mononuclear phagocyte system (MPS). PEG has been used to improve drug stability and solubility, decrease toxicity, increase half-life, and reduce clearance and

immunogenicity of liposomes [60]. PEG can be attached to the liposomal membrane surface using numerous methods, the most frequent of which is to conjugate the polymer into the membrane *via* a PEG-conjugated lipid, such as 1,2-sn-distearoyl-glycero phosphoethanolamine methoxy-polyethylene glycol 2000 (DSPE-mPEG2000) (Fig. **3**) [60].

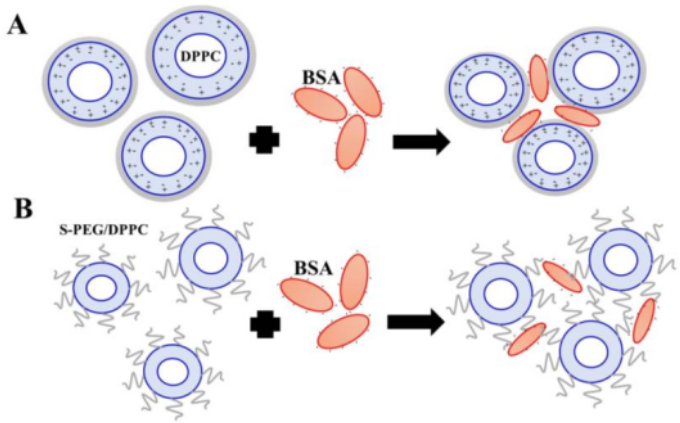

Fig. (3). Modification of DPPC vesicles by S-PEG addition on the liposome surface induces high stability in the presence of Bovine Serum Albumin [61].

The effect of PEGylation on liposomes in increasing circulation time largely depends on the length of the PEG chain and the relative density of the total lipid constitution. In general, PEG featuring longer chains represents superior steric obstacles [62]. The lipid-PEG modification sterically inhibits the interaction between liposomes and MPS, which causes rapid clearance of liposomes from the blood. Santos *et al.*'s research shows that DSPE-mPEG2000 at a concentration of ≤5 mol% levels combined with the DSPC liposome results, with the effect of extending circulation time. However, at a level between 1 and 5 mol%, it will have a different effect. The addition of DSPE-mPEG2000 also prevents liposome aggregation, thus extending circulation time [63].

Manufacturing of Liposomes

The main objectives of ideal liposome manufacture are to achieve efficient drug encapsulation, small particle size, and high stability during long-term storage. In general, liposome manufacture includes the processes of lipid hydration, particle size reduction, and separation from free drugs. Approximately ten methods are usually adopted for the preparation of liposomes, including the thin-film hydration method, micro-emulsification, sonication, membrane extrusion, the freeze-thawed

method, the ether injection method, the ethanol injection method, the reverse- phase evaporation method, dehydration-rehydration, and the calcium-induced fusion method [64]. Method selection largely determines the unilamellarity, vesicle size, and stability of liposomes. In addition to the manufacturing method, there are two main drug loading methods into liposomes; passive loading and active loading.

Of all these manufacturing methods, thin-film hydration, reverse phase evaporation, and ethanol injection have become the most commonly used in the preparation of liposomes. The following are brief overviews of the various manufacturing methods for liposome manufacture:

1. Thin Film Hydration

The thin film hydration method is the most common and simplest liposome production technique, which involves dissolving phospholipids in organicsolvents, such as dichloromethane, chloroform, ethanol, and chloroform-methanol [64] (Fig. **4**). This is the simplest original method devised by Bangham, which, if compared to those of reverse-phase evaporation and ethanol injection, canproduce liposomes with the optimum properties and stability [40].

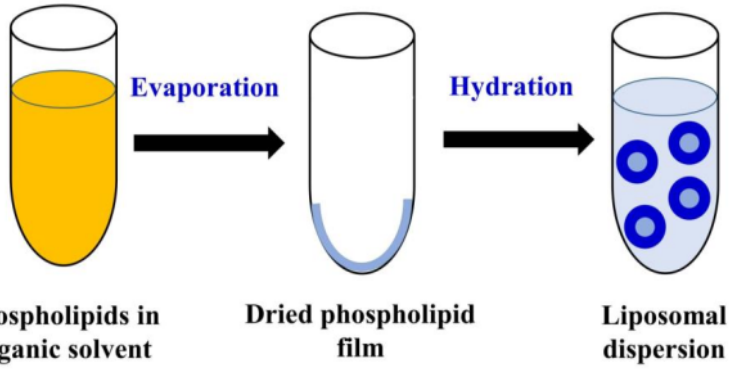

Fig. (4). The schematic representation of liposome production using the thin film hydration method [65].

At the first stage, the organic solvents with volatile properties, especially chloroform, ether, or methanol, are used to dissolve lipids. The lipid will become a thin film layer on the bottom tube wall when the solvent is evaporated using a rotary vacuum evaporator [40]. Usually, an evaporation process completed in a vacuum at a temperature of 45-60°C will result in a homogeneous thin film layer. Nitrogen can also be used to remove the remaining solvent [64]. Furthermore, the dry, thin-film layer is hydrated under agitation with a hydration solution, such as

distilled water, phosphate buffer, phosphate buffered saline (PBS) at pH 7.4, or normal saline at a temperature above the transition temperature of the lipid used [38]. The hydration process usually varies from 1-2 hours in duration [64].

This thin layer method will produce a heterogeneous multilamellar vesicles (MLV) liposome that requires a further process to reduce its size. For example, sonication will produce small unilamellar vesicle (SUV) liposomes and/or extrusion with a polycarbonate membrane to produce homogenous size distribution among unilamellar liposomes [38].

2. Reverse Phase Evaporation

In the reverse-phase evaporation method, phospholipids are dissolved in diethyl ether/isopropyl ether or a mixture of diethyl ether and chloroform at a volume ratio of 1:1 or a mixture of chloroform-methanol at a ratio of 2:1 v/v. Phosphate buffer or citrate-disodium hydrogen phosphate buffer is subsequently added at the aqueous phase in order to increase the drug encapsulation efficiency of the liposomes. The organic phase must remain distinct from the water phase to ensure that an oil/water emulsion is formed. Liposome formation will occur during continuous rotational evaporation of organic solvents in a vacuum, as seen in Fig. (**5**). This method produces very high drug encapsulation. However, thepossibility of remaining solvents and the difficulty of large-scale manufacturing constitute the main limitations of this method [64].

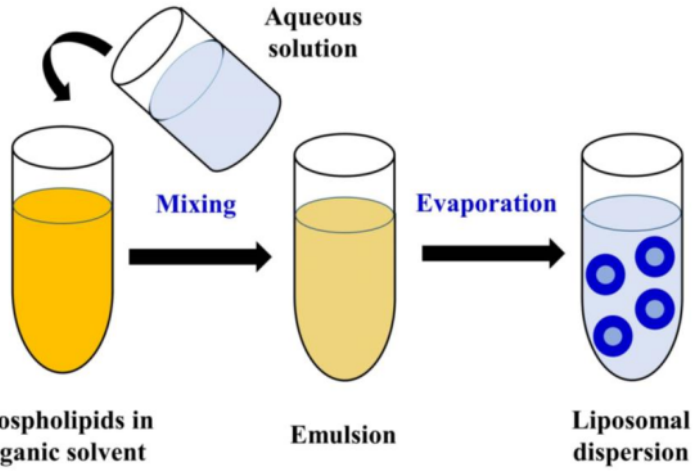

Fig. (5). A schematic representation of liposome preparation procedures involving the reverse phase evaporation method [65].

3. Ethanol Injection

In the ethanol injection method, lipids are first dissolved in ethanol, with the resulting solution being rapidly introduced into large quantities of preheated water or in a buffer to form SUV liposomes. The incorporation of drugs in liposome vesicles depends on their hydrophilic and/or hydrophobic properties. This method uses ethanol as a relatively harmless solvent, which can also be easily scaled up. In addition, no physical or chemical treatment exists that can cause lipid damage. However, the concentration of the vesicles obtained is extremely low, and there may be an azeotropic mixture with water rendering the removal of ethanol from the final product difficult [40, 64].

The use of Liposomes for Herbal Product Delivery

Liposomes have been used for many purposes, including drug delivery systems and cosmetics, among others. In this section, the use of liposomes is focused on a herbal drug delivery system since liposomes effectively enhance the drug solubility, bioavailability, and pharmacological activity of certain compounds.

1. Liposomes Increase Herbal Drugs Solubility

Liposomes can dissolve water-insoluble drugs by trapping them in the liposomal lipid membrane. Apart from increasing their solubility and biocompatibility, the liposome structure, which is similar to that of a biological membrane, can encourage its use in delivering drugs with poor permeability [65].

The use of liposomes has been applied to *Orthosiphon stamineus* (OS) extract formulation. *Orthosiphon stamineus* Benth. is a medicinal plant commonly used as an ingredient of various traditional drugs in the treatment of angiogenesis- related diseases, such as rheumatoid arthritis, tumor edema, obesity, diabeticretinopathy, and psoriasis [66]. The ethanol extract of OS contains many flavones,including sinensetin (SIN), eupatorine (EUP), and 3′-hydroxy-5, 6, 7, 4′- tetramethoxyflavone (TMF) as markers of pharmacological activity. However, these flavones exhibit low water solubility, which limits their clinical use [67]. Increasing the solubility of lipophilic flavones produced from OS extract can increase their bioavailability and pharmacological activity.

Liposomes have been prepared to increase the solubility and permeability of OS ethanolic extract, thereby enhancing their bioavailability. Aisha *et al*. reported the use of liposomes in preparing OS extract (OS-L) by the thin film hydrationmethod, which involves dissolving phospholipids in chloroform.

The optimum ratio of phospholipids to extract is selected according to the increase in the solubility of the OS extract in water. The liposomes are prepared using crude soybean phospholipids prepared in ethanol (PH-Et) at various weight-to-weight ratios of the OS extract. The crude soybean lecithin contains 62 ± 0.2% acetone insoluble phosphatides, while PH-Et has a phosphatidylcholine (PC) fraction of 13.4%, as determined by column chromatography. The solubility ofthe non-formulated OS extract (OS-E), as measured by UV-vis spectrophotometry, is 956±34 µg/ml. The results show a significant increase inthe water solubility of the formulation prepared on phospholipids at extract ratios of 50:50, 150:50, and 100:50, as shown in Table **2**. The formulation prepared at a weight ratio of 100:50 shows the greatest solubility improvement, albeit stilllimited. Consequently, the methanol is used to dissolve extract resulting in a significant increase in the water solubility of OS extract from 1,402 ± 66 µg/mLin ethanol to 3,979 ± 139 µg/mL in methanol (P = 0.000). The methanol used to dissolve extract has a similar boiling point to chloroform (65.0°C) as phospholipid solvent (61.2°C), resulting in a homogenous phospholipid-extract thin film. Onthe other hand, the higher boiling point of ethanol (78.4°C) compared to chloroform tends to produce phase separation and the precipitation of phospholipid from extract mixtures [67].

Table 2. The water solubility of OS extracts prepared at various phospholipid-extract ratios in liposomes [67].

Formula	Crude soybean phospholipids prepared in ethanol/PH-Et (mg)	OS Extract/ OS-E (mg)	Solubility in water (µg/mL)
F1	150.0	50.0	1,296.5 ± 18
F2	100.0	50.0	1,401.7 ± 66
F3	50.0	50.0	1,188.8 ± 169
F4	25.0	50.0	1,018.8 ± 15
F5	25.0	75.0	1,072.6 ± 6

The interaction of the extracts with phospholipids results in changes in the infrared absorption spectrum. The most noticeable spectral change can be observed in the phospholipid polar group and may indicate the presence of hydrogen bonds between the polar phospholipid groups and the hydroxyl groups from the OS extract. Hydrogen bonds can also occur between the keto groups of flavonoids and the phospholipid oxygen groups. In addition, hydrophobic interactions occur between the flavone's methoxy group and the carbon chain in the tail portion of phospholipids [67].

The stability study shows that the OS extract liposomes are stable at pH 5.5 and 7.4, while at a lower pH of 1.6 (gastric pH), OS liposomes experience agglomeration and precipitation. Moreover, although the OS liposomes will clump, they will re-dissolve at pH 5.5 and 7.4, which represent the pH of the intestines. No precipitation occurs at pH 1.6 by using soybean phospholipids and the OS extract as a single compound [67].

The OS extract released from the liposome is evaluated by a dialysis bag that has a molecular weight cut-off (MWCO) of 8,200 using phosphate buffer pH 6.8 media at a stirring rate of 100 rpm and temperature of 37°C. The results show thata cumulative percentage release of OS extract loaded in liposomes is 62% after 24 hours. On the other hand, the cumulative release percentage of free OS extracts is 94%. These results indicate that OS extract is stably encapsulated in liposomes, probably due to OS extract and phospholipid interaction. Consequently, results related to the slow release of flavones contained in OS extracts include rosmarinic acid (RA), sinensetin (SIN), eupatorin (EUP), and 3′-hydroxy-5,6,7,4′-tetramethoxyflavone (TMF) [67].

A further evaluation carried out on the antioxidant effect on DPPH of the extract release indicated that the OS extract loaded in liposome demonstrates stronger antioxidant activity against DPPH with an IC_{50} value of 23.5 ± 1.1 μg/ml than that of non-formulated extracts, which have an IC_{50} value of 32.4 ± 0.5 μg/ml [67]. This is because OS extract is stably encapsulated in liposomes with a slow-releaserate that enables it to maintain or increase the antioxidant activity.

A higher intestinal permeability of liposomal extracts compared to non- formulated extracts is obtained due to loading into liposomes, higher solubility, and the nano-sized vesicles that produce an increase in negative charge andenhance colloid stability. Previous studies have reported that anionic liposomes demonstrate superior colloid stability and absorption and an even higher cellular uptake rate than those of neutral and cationic liposomes. Based on the evaluation results related to the liposome formulation of the OS extract, it is clear that an increase in solubility, absorption, and antioxidant effects can intensify the pharmacological effects of the OS ethanol extract [67].

Liposome formulation to increase drug solubility has also been applied to silymarin extract. Silymarin has been known as the main flavonoid obtained from the extraction from the dried fruit of *S. marianum* and has been widely employed in the treatment of liver disease. Silybin constitutes the main and major active component of silymarin and plays a role in its pharmacological activity. Silymarinis known to be widely used in the treatment of liver disorders to reduce glutathione oxidation as a means of increasing glutathione levels in the liver, enhancing the stabilizing

effect of liver cell membranes, and the enhancement of hepatocyte protein synthesis [68]. However, the low solubility of silymarin in water produces low oral bioavailability of approximately 20-50%. Some degradations have also been reported due to the presence of gastric juice, which limits the use of silymarin [69, 70].

Based on Wang *et al.*'s research, it has been reported that silymarin prepared as pro-liposome improves encapsulation efficiency when the drug to phospholipids mass ratio or pH is increased [65]. Pro-liposomes form a dry and readily flowing powder that will convert to liposomes after hydration. Pro-liposomes offer several advantages over conventional liposomes, such as increased stability due to the dry-solid state forms during storage [65].

The pro-liposomal silymarin is prepared with soy lecithin, cholesterol, and sodium oleate dissolved in propylene glycol to produce a silymarin proliposome solution with a transparent light yellow color. The liposome is obtained by mixing proliposomes with water resulting in vesicles with multilayer membranestructures, as seen in Figure 6, with silymarin loading at a high entrapmentefficiency [71]. The hydrated pro-liposomes produce liposomal vesicles with small particle sizes, approximately 70 nm, and are homogeneous in terms of the distribution of their size. The use of sodium deoxycholate or sodium oleate in proliposomes can facilitate the formation of liposomes without any changes in their morphological structures and stable silymarin loading even after proliposome hydration [71].

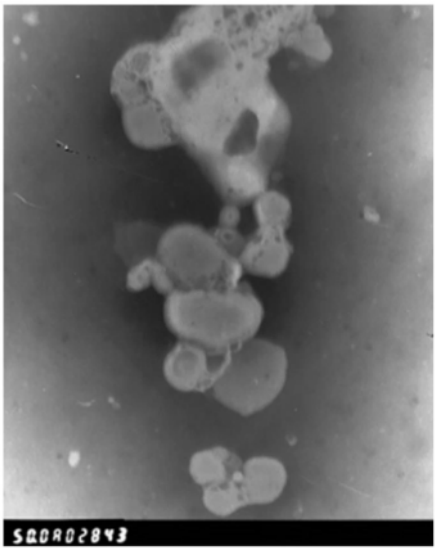

Fig. (6). Transmission electron photomicroscopy of silymarin liposome (magnification 5x1000 times) [71].

It has also been reported that liposome is successfully used to load curcumin (CUR). Liposomes that are composed of lecithin, cholesterol, and CUR at a molarratio of 60:15:1, respectively, and prepared by reverse-phase evaporation method generate a stable, homogeneous, and semitransparent liposome with an efficient encapsulation of 89.3% [12]. The liposomes successfully increase the stability of CUR at high temperatures and/or in acidic conditions.

Liposomes Improve the Bioavailability and Pharmacological Effects of Herbal Drugs

The main challenge to the development of an oral dosage form lies in managing poor drug bioavailability, which is known to be affected by several factors, including water solubility, permeability, dissolution rate, first-pass metabolism, and pre-systemic metabolism. However, poor water solubility and limited permeability are often associated with low oral bioavailability [11]. Liposomesare potentially effective drug carriers that can promote an improvement in the solubility of drugs in water, provide protection against degradation in the gastrointestinal environment, and ensure permeability through the epithelial cell membrane, thereby increasing the bioavailability of the drug [65]. In addition, liposomal drugs are known to be of low toxicity and offer improved pharmacological effects [72].

The use of liposomes has been successfully applied to increase the bioavailability of curcumin (CUR). CUR occurs as a yellow polyphenol compound derived from the turmeric plant and has been widely used in the treatment of various cancers affecting the lungs, cervix, prostate gland, breasts, bones, and liver, in addition to inflammatory diseases [12, 68, 72]. However, CUR has poor bioavailability resulting in its low blood level [74]. Therefore, a well-designed CUR formulation is necessary to improve its efficacy.

Several studies have reported that the encapsulation of CUR into liposomes improves its stability, bioavailability, targeting properties, and anticancer effectiveness. Several methods for the preparation of CUR liposomes have been reported, including thin-film, freeze-thawing, freeze-dried, solvent injection, and reversed-phase evaporation method [12].

Liposome preparation by the thin layer dispersion method increases the stability of CUR. According to the research conducted by Chen *et al*., the use of N- trimethyl chitosan chloride (TMC) in the CUR liposomes composed ofphosphatidylcholine, cholesterol, and D-α-tocopheryl polyethylene glycol 1000 succinate, increases the bioavailability of CUR. Another study by Gu *et al*. highlights that the preparation of liposomes coated with Carbopol is also reported to produce an increase in bio-

availability 2.22 times higher than that of CUR liposomes without coating.

Positively hybrid charged liposomes (PHL) prepared by thin layer hydration method, as reported in the study of Pamunuwa, Karunaratne, & Karunaratne(2016), significantly reduces the release of CUR from the liposomes compared to that of negatively hybrid charged liposomes (NHL). The use of stearylamine (SA)as an additional component of the lipid bilayer successfully produces a slow CUR release from liposomes. In another study, it is reported that the use of hydroxypropyl-β- or hydroxypropyl-γ-cyclodextrin (HPβCD or HPγCD) complexes in liposomes by the thin layer hydration method results in an encapsulation efficiency 2.02 times higher than those without cyclodextrins. The use of HPβCD shows higher stability than HPγCD. The formulation of CUR liposomes in cyclodextrin can significantly increase the solubility and stability of CUR [77].

CUR liposome formulation is also carried out using a lipid composition containing different characteristics of phospholipids, including neutrally charged phospholipids of varying rigidity, *i.e.*, soy phosphatidylcholine (SPC, a non-saturated phospholipid), dipalmitoylphosphatidylcholine (DPPC, a saturated phospholipid), negatively charged phospholipids, *i.e.*, dipalmitoyl phosphatidyl-glycerol (DPPG), and a mixture of these, *i.e.*, DPPC + DPPG (7:3 $^{w}/_{w}$), with 30% mole addition of cholesterol. The results show that the charges of lipid polar head and hydrophobicity related to saturated carbon chains of phospholipid tail greatly affect the particle size and the loading efficiency of liposomal CUR. The surface charges induced by environmental pH determine protonation of the phosphate portion of phospholipid molecules, thus affecting the hydrogen bonding between molecules leading to changes in particle size, the phospholipid liquid-crystalline stating transition temperature, and the instability of the liposomes. In acidic pH, SPC-based liposomes have the smallest vesicle size and most stable liposomes, whereas, in neutral and alkaline pH, DPPG demonstrates the highest degree of physical stability. However, the deprotonation of CUR hydroxyl groups occurring at alkaline pH causes lower entrapment efficiency for all phospholipids, specifically for DPPG, which produces repulsive interaction with CUR. The high hydrophobicity of neutral CUR form renders it highly loaded with acidic pH. The more rigid the phospholipids, *i.e.*, DPPC, with saturated hydrocarbon chains in their hydrophobic tail, the higher the entrapment efficiency and the lower the release of CUR from the liposome. The use of liposomes successfully induces a sustained and prolonged release of CUR [78].

Liposome formulation is further carried out using the freeze-thawing method, in which it has been reported that the use of 10% sucrose as a cryoprotectant can

protect liposomes from vesicle fusion and damage due to ice crystallization during the freezing process. Freeze-dried preparation itself is used to improve long-term liposome stability. The addition of sucrose demonstrates excellent maintenance of particle size as well as encapsulation efficiency, with almost 99% of CUR potentially being entrapped after rehydration [79].

Moreover, it has been reported that Hyaluronan and Eudagrite S100, both anionic polymers, can be employed as stable and effective coating substances for liposomes composed of SPC with rapid dissolution in the upper gastrointestinal tract [80]. The results show coating liposomes with these polymers producesvesicles of 220-287 nm, spherical in shape, and without any changes in dimension, even when exposed to a gastric bowel stimulated medium with high ionic strength. In addition, it produces an efficient encapsulation of CUR in the range of 78%-82%. Good water dispersion and homogeneous suspension without the presence of aggregates and precipitates being formed can be achieved. The combination of these two polymers can protect CUR from metabolism in the gastric environment. Biodistribution studies indicate that CUR levels in the liver and kidneys are low. On the other hand, there is a greater accumulation of CUR in the lower gastrointestinal tract, specifically the intestines and colon, while very low levels of CUR suspension are observed. The results show that these delivery carriers can be used to protect polyphenol compounds from the acidic gastric environment.

The solvent injection method can be performed using ethanol or ether. Through this method, phospholipids and hydrophobic drugs are dissolved together in an organic solvent as the oil phase, with the solution being rapidly injected into the water-soluble drug or aqueous phase while being stirred. Liposomes will be spontaneously formed when the organic solvent is removed. However, ether is used less frequently than ethanol because of its toxicity. CUR liposome composedof 1.0 mg of CUR with cholesterol: lecithin at a ratio of 1:3 and prepared usingthe ethanol injection method demonstrates high CUR encapsulation efficiency of up to 72% with a vesicle size of 830 nm [12]. Other research shows the use of propylene glycol liposome (PGL) as a CUR carrier prepared by the solvent injection method using ethanol produces a high CUR encapsulation efficiency of 92.74% without any aggregation or fusion, whereas conventional liposomes experience a degree of aggregation over time. It is also reported that CUR-PGL shows a gradual release up to a maximum of 46%. A 3-month stability evaluation shows that CUR-PGL has non-significant changes in the particle size, polydispersity index, and encapsulation efficiency, in contrast to the CUR liposome that experiences decreases in drug encapsulation. Therefore, the CUR- PGL formulation with the injection method is effective in increasing the efficiency of encapsulation and reducing the dosage and, inevitably, the side effects of CUR [81]. Another study by Li *et al*. on silica-

coated ethosome shows ahigh CUR encapsulation efficiency of 80.77%. The silica-coated CUR-ethosomes are stable and gradually release CUR. Moreover, these ethosomes produce 11.86 times higher CUR bioavailability than that of the CUR suspension [82]. An *in vivo* study of mice shows that the transporting of silymarin liposomes across the intestinal biological membrane is more efficient than that of silymarin solution [71]. These results highlight the successful use of liposomes to increase thebioavailability of natural products, thereby promoting their potential applicationin clinical contexts.

The Use of Nanoliposomes for Enhanced Drug Delivery

Nanoliposomes have many advantages, including controlled drug release, tumor targeting, low toxicity, high stability, extensive bioavailability, and reduced therapeutic dosages. Drug encapsulation in the nanoliposome system can be employed to improve the physicochemical stability of CUR. The use of chitosan in the formulation of CUR nanoparticles composed of CUR: Chitosan: Tripolyphosphate at a ratio of 3:24:8 w/w, respectively, produces high CUR nanoliposome stability when stored at 4°C, with no changes observed even up to ten months later. In addition, it results in greater *in vivo* bioavailability than the CUR suspension. This is because the use of chitosan can provide a positive charge, thereby extending the contact time of the drug with the absorption surface [83]. Shin *et al*. reported that the CUR nanoliposomes prepared using an ethanol injection method with chitosan coating have spherical morphology, as presented in Figure 7 and demonstrate improved physical stability with good mucoadhesive properties, thus enhancing bioavailability [84].

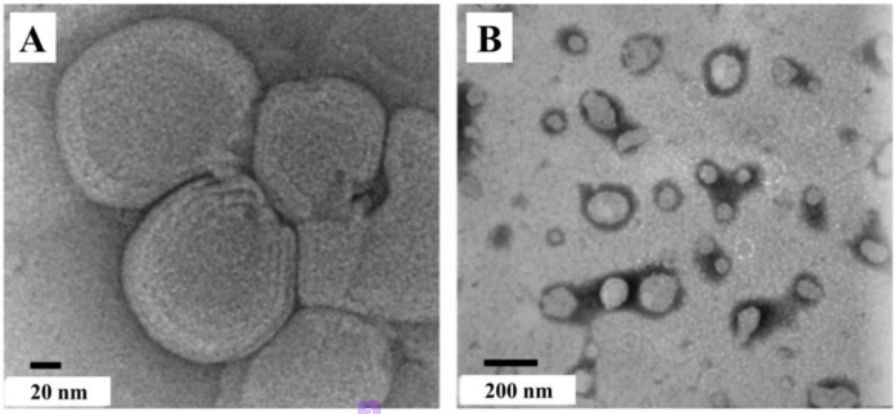

Fig. (7). Transmission electron microscopy (TEM) images of chitosan-coated CUR nanoliposomes with scales of (A) 20 nm and (B) 200 nm [84].

On the other hand, the use of nanoliposomes is unable to prevent CUR from hydrolysis at an alkaline pH of 12.0, during which it causes degradation of nanoliposomes as well as decomposition of CUR. When nanoliposome enters the circulation system, it can be rapidly phagocytized by monocyte-macrophages [85]. In addition, it can be easily and extensively accumulated in tissues with extensive vascularization in the liver, spleen, and kidneys. Consequently, thetarget specificity cannot be maximally achieved, thus reducing the effective dose delivered to the target sites. A modified liposome with PEG or ligand binding can prove as an effective strategy in solving this problem [12, 86, 87].

Long Circulating Liposomes

The conjugation of hydrophilic polymers, such as PEG, has been extensively used to obtain long-circulating liposomes through the formation of aqueous layers on the surface of liposomes. This polymer prevents plasma protein binding to liposomes through a steric protective layer, thereby reducing the excretion of liposomes by RES and prolonging systemic blood circulation. Long circulating liposomes are known to provide a homogeneous distribution without any aggregation. Therefore, the use of long-circulating PEGgylated liposomes in the CUR liposomes results in greater stability [12].

Lin *et al*. reported that TEM analysis of liposome-PEG-polyethyleneimine (PEI) encapsulated CUR shows a coarse round shape in the presence of a hair-like surface, as shown in Fig. (**8**).

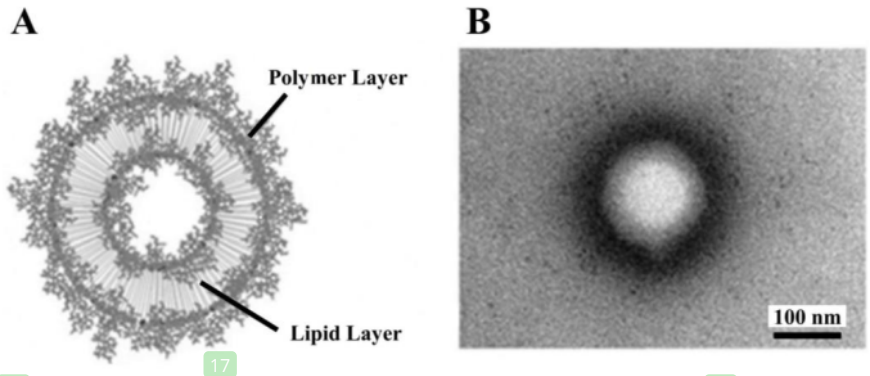

Fig. (8). Microstructures of the cationic liposome-PEG-PEI complex. **(A)** Schematic illustration of the structure of the cationic liposome-PEG-PEI complex. **(B)** TEM image showing the structure of the cationic liposome-PEG-PEI complex, consisting of the light circular area and dark corona on the surface [88].

Ligand Targeting Liposomes

Ligands can direct liposomes to bind specifically to receptors in the target cells. Therefore, the liposomes binding with ligands are generally used to enhance the therapeutic effect, produce synergies, reduce side effects, lower the dosage,

shorten the treatment period, and produce a specific targeting effect on the body with highly selective drug delivery. Encapsulation of CUR in liposomes with folic acid modification results in solubility and increased activity in tumors [12, 88].

In addition to the use of liposomes to increase the bioavailability and pharmacological effects of CUR, liposomes are also employed to enhance the pharmacological effects of *Cistanche tubulosa*. *Cistanche tubulosa* is a parasitic plant of the Orobanchaceae family that produces various pharmacological effects, including increasing immunity and endurance, nourishing the kidneys, treating impotence, and improving intelligence. Moreover, it also possesses anti-oxidant and anti-aging properties [90]. This plant contains phenylethanoid glycosides (CPhGs) as the main component that stimulates immunity. Despite their strong pharmacological effect, CPhGs have poor oral absorption [91] that severely limits their use for clinical and therapeutic applications.

Zhang *et al*. reported that liposomes composed of lecithin, DPPC, and cholesterol at a respective weight ratio of 1:2:2 are successfully prepared for encapsulating CPhGs. The liposomes have spherical shapes, uniform surface thickness, a homogenous particle size of 216.7 nm with a zeta potential of -55.6 mV, and efficient encapsulation of 38.46%. The release profile shows a release that is close to 100% after 24 hours. Moreover, CPhG liposomes greatly inhibit HSC viability and proliferation and increase the activity of concentration-dependent apoptotic cells with an IC_{50} value of 42.5 μg/mL

Based on the evaluation results, 24 hours after exposure of CPhG liposomes to hepatic stellate cells (HSCs), and lactate dehydrogenase (LDH), an enzyme present in the cell cytosol, was released, thus indicating cell membrane damage. Moreover, the study also reported that CPhG liposomes induce apoptosis of hepatocytes, which intensifies with increasing doses of CPhGs liposomes. Inaddition, CPhG liposomes induce G1 phase cell cycle arrest. These results indicate that CPhG liposomes have a high potential for preventing and treating liver fibrosis by regulating cell proliferation, apoptosis, and cell cycle arrest [91].

The results also indicate that CPhG liposomes have a significant inhibitory effect on cell proliferation in HSCs. In addition, it has been found that p-PI3K and p-Akt levels consistently decrease in HSCs after 24 hours of CPhG liposome administration, indicating that the anti-proliferative effect of CPhG liposomes on HSCs is related to the deactivation of the PI3K/Akt pathway. CPhG liposomes at a concentration of 29.45 μg/mL can reduce the expression level of phosphorylated PI3K protein and phosphorylated Akt ($p < 0.01$) [91].

The foregoing results indicate that the use of liposomes in CPhG liposome formulation significantly improves the inhibition activity of CPhGs for HSC

activation and reduces the progression of liver fibrosis by increasing apoptosis and regulating the cell cycle in HSC. This suggests that the liposome formulation in this study can be developed as an antifibrogenic agent for the treatment of liver fibrosis.

Factors Affecting the Encapsulation of Herbal Extract in Liposomes

In order to prepare liposomes with high encapsulation efficiency to improve solubility as well as bioavailability and pharmacological effects, there are certain critical parameters that should be identified.

1. The selection of phospholipids:

In the entrapping of hydrophobic substances, which will be located within the lipid bilayer membrane, the types of phospholipids will determine the entrapment efficiency. This is because the ordered structure of saturated phospholipid will limit the number of hydrophobic molecules located inside. In general, the less the crystalline structure of the lipid bilayer, the higher the loading capacity of the bilayer membrane since there is more space for hydrophobic molecules trapped inside. However, it will affect the molecular diffusion within the water, thus increasing drug release from liposomes [48, 92 - 94].

2. Extract to phospholipid ratio:

The drug and lipid ratio is a critical parameter that shows the capability of the liposomes to accommodate drug encapsulation, thus playing a critical role in the optimization process [95]. Most hydrophilic drugs will be trapped in the intraliposomal phase. Therefore, entrapment efficiency is significantly determined by the vesicular lipid volume capacity. In this case, the selection of phospholipids will also play a role in quantifying the capacity for entrapping drugs. Each phospholipid has a different surface area which determines the vesicle numbers in the dispersion media, thus affecting the volume of water available for loading drugs.

The ratio of extract to phospholipids affects the encapsulation for liposomalherbal preparation. The extract to phospholipid ratio in the OS liposome formulation greatly affects the extract solubility in the liposomes. The use of phospholipid:extract at a ratio of 100:50 w/w has the highest solubility, *i.e.*,1,401.7 ± 66 µg/ml [67].

In silymarin liposome formulation, the mass ratio of silymarin to phospholipids also significantly influences drug encapsulation and loading efficiency. A significant increase in encapsulation efficiency from 31% to 42% is observed when the

drug-phospholipid ratio is increased from 1:5 to 1:10 ($P<0.01$). However, no changes are obtained after increasing the ratio to 1:20 ($P>0.05$)while the drug loading efficiency decreases.

3. pH of liposomes:

The encapsulation efficiency also changes with variations in pH. The silymarin liposomes are composed of 1 g of silymarin, 10 g of phospholipids, 5 g of cholesterol, and 0.5 g of sodium oleate and prepared with a silymarin- phospholipid at a ratio of 1:10. Adjusting the pH of liposomes to 5.0, 6.0, 7.0, and 9.0 increases their encapsulation efficiency, indicating that the larger the amount of silymarin, the more easily it is trapped when the liposomes are present in a more alkaline environment. This may be related to a chemical reaction between silymarin and phospholipids. Moreover, the improved transport of silymarin liposomes increases bioavailability up to a 1-fold increase in AUC ($P<0.01$) [71].

4. Incubation temperature

In a drug loading process involving active loading, the fluidity of the bilayer membrane determines the ability of water-soluble solutes to permeate across the membrane and enter the intraliposomal phase. The presence of pH or ammonia gradient will drive the permeation or loading process to produce high levels of the drug inside the inner water phase, which sometimes results in high dense aggregates due to oversaturated internal drug concentration. Both the incubation period and temperature affect the gel to liquid crystalline state of phospholipids asthe main component of the bilayer membrane and determine the solute transfer process from the exterior of the liposomes to their interior. This constitutes amajor factor to be considered with regard to the loading of the active substances.

CONCLUSION

The compounds contained in plant extracts exhibit various physicochemical characteristics, which constitute a significant challenge for the liposome drug delivery system. The use of liposomes to develop natural products has several benefits, such as enhancing drug solubility and increasing the bioavailability and pharmacological effects of active substances. Based on the findings of research into the use of liposomes in herbal products, there is an increase in phytochemical stability in terms of physical and chemical degradation, with sustained or controlled drug release. Moreover, an increase in the oral absorption of plant extracts in the form of liposomes indicates increased bioavailability in the gastrointestinal tract, thereby increasing the pharmacological effect at low dose concentration and reducing toxicity.

FUTURE PROSPECTIVE

A comprehensive analysis from the formulation and manufacturing aspects to the composition of the liposome formula for the natural product drug delivery system and the appropriate manufacturing method is required. The various components contained in the plant extract, in addition to largely water-insoluble compounds, remain as challenges to the optimizing of liposomes preparation intended to improve solubility and permeability, thereby enhancing oral bioavailability. Liposomes could be beneficial, acting as potential drug delivery carriers, which enhance the biological activity of most hydrophilic and hydrophobic substances of herbal drugs due to their vesicular structures mimicking biological membranes.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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