

# A Review: A Novel of Efforts to Enhance Liposome Stability as Drug Delivery Approach

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## ABSTRACT

Drug encapsulation in liposomes has provided an opportunity to improve therapeutic drugs mainly through changes in biodistribution and targeting of drugs to certain tissues. The role of liposomes as a drug delivery system is to provide drugs in a controlled manner, reduce toxicity, and increase the efficacy of encapsulated drugs. Although liposomes have been widely investigated in the pharmaceutical field, liposomes have problems related to stability, including particles forming aggregates and the occurrence of drug leakage in vesicles during storage. Therefore, physical stability studies are needed to ensure product integrity during storage. A number of methods were obtained to maintain liposome stability including,

freeze-drying, modification with chitosan, the addition of surfactants, and incorporation with polymer gel. Therefore, this article is intended to discuss liposome stabilization and various characterization parameters as a benchmark for liposomal stability.

**Keywords :** Liposome, Stability, phospholipid.

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## INTRODUCTION

Liposomes are drug delivery systems in the form of spheric vesicles consisting of a phospholipid bilayer membrane [1,2]. Liposomes are considered as ideal models like biological membranes, so they can be utilized to deliver drugs and other active compounds [3]. Over the past few decades, liposomes have become widely of concern as a carrier system for active compound therapy in many disciplines and their application in the pharmaceutical, cosmetic, and food industries as promising breakthroughs in new products [4,5]. Liposomes have several advantages, likely being in a position to contain drug compounds that are hydrophobic and hydrophilic, have good biocompatibility, increase the efficacy and therapeutic index, lower toxicity, and enhance drug stability in the encapsulation system [6,7].

Although liposomes have been widely studied as an active compound delivery system, in the pharmaceutical field, liposomes have several disadvantages, namely rapid degradation and the inability to achieve continuous drug administration over a long period. Also, liposome-based systems are well known for have limitations such as instability due to changes in temperature and pH [8]. Liposomes have problems related to stability which can cause damage to the storage that causes leakage of drugs from vesicles [4].

In writing this article review, the literature study method was employed in several international journals obtained from Google Scholar and online journal provider sites such as PubMed. A literature search is carried out with numerous keywords such as "Stability of liposome", "Characterization of liposome", and "Drug delivery system". The inclusion criteria established were international journals on liposomes with the publication of the last ten years (2010-2020) and exclusion criteria were journals on liposomes outside the pharmaceutical field. Some of the articles obtained, 55 articles were selected in accordance with the theme.'

## FACTORS AFFECTING LIPOSOME STABILITY

Factors that can affect the stability of liposomes include temperature and pH. It was pointed out that pH and temperature affect the level of phospholipid hydrolysis during storage. When liposomes are produced in a medium, liposome components try to balance the energy obtained by forming an appropriate structure. When contacted at a certain pH, the structure of the phospholipids will be protonated so that they can initiate the breakdown of the structure of the phospholipids due to decreased strength between bonds, it is also dependent on the composition of the lipids [9]. On the other hand, under increasing temperature conditions, the associative interaction between phospholipid molecules decreases, resulting in membrane fluidization. As the temperature increases, the hydrocarbon chains from the bilayer become random, thereby reducing membrane stiffness and forming damage such as pores or disk bilayers. This ultimately reduces the stability of the drug that is coated in the liposome [10]. Additional factors such as the surface charge of a liposome also affect the stability of the liposome [9]. Therefore, several techniques are needed in order to maintain liposome stability.

## LIPOSOME STABILIZATION

For pharmaceutical products to survive on the market, they must be able to maintain their physical-chemical properties during storage. Liposomes can be degraded chemically through oxidation and hydrolysis. Whereas physical degradation is often related to changes in the structure of liposomes. There are several methods to maintain liposome stability including freeze-drying methods that can increase the shelf life of liposomes. The addition of other ingredients such as surfactants, modification with chitosan, and special polymer gels for topical preparations [11,12].

Table 1: Various researches with liposome delivery System

| Membrane composition                | Active compound               | Lack of active compound                     | Biological activity | Stabilizator                | Result   | Ref. |
|-------------------------------------|-------------------------------|---|---------------------|-----------------------------|--|------|
| Lechitine and cholesterol           | Neuro-peptida                 | short in half live                          | Wound healing       | chitosan                    | Enhance stability and therapi index                    | [13] |
| Lechitine and cholesterol           | Budesonid                     | short in half live                          | atsma               | Trehalosa as cryoprotec-tan | Enhance stability and drugs release                    | [14] |
| Phosphatidylcholine                 | Mupirosin                     | short in half live                          | Wound healing       | chitosan hydrogel           | Extend drug release.                                   | [15] |
| Dotap , dope , dan kolesterol       | Amfote-risin                  | Toxic as side efect                         | Antifungal          | Gell poloxamer              | Enhance stability and decrease toxicity                | [16] |
| Phosphatidylcholine                 | <i>Ketorolac tromethamine</i> | Shoer in half live                          | Analgesic           | Tween 80                    | Enhance stability, drugs release, and drug penetration | [17] |
| Phosfatidil-coline And kolesterol   | Flucona - zole                | Short in half live                          | antifungal          | Trehalosa as cryoprotec-tan | Enhance stability and drugs release                    | [18] |
| Lechitin e                          | Resvera-trol                  | Short in half live and water low solubility | Anti caneer         | Polyetihlene glycol         | Enhance stability, half live and water solubility.     | [19] |
| Fosfatidil-cholin: kolesterol       | Cocktail phage                | Easily degraded                             | Wound healing       | Tween 80                    | Enhance solubility and contact time                    | [20] |
| Phosphatidyl choline and kolesterol | Docetaxel                     | Low water solubility                        | Anticancer          | chitosan, tween 80          | enhance stability and solubilty.                       | [21] |
| Phosphatidyl choline                | Triazavirin                   | Short half live                             | Antiviral           | chitosan                    | Enhance stability and half live.                       | [22] |

#### Surfactant

Surfactants are invoked as edge activators to enhance liposome stability. It was advised that if polyethylene and alkyl groups are exist on the surface of vesicles, the surfactant hydrocarbon chains can penetrate into the phospholipid bilayer, resulting in steric stabilization, which can reduce vesicle fusion. In addition, the tendency of surfactants to form micelle structures can reduce the energy needed for droplet distortion [10]. The use of surfactants makes liposomes flexible, so that vesicles have the authority to cross holes that are smaller than their size evaluated to conventional liposomes [23].

Using PEG as a surfactant participating in vesicle formation, the hydrophobic part will regulate together with phospholipids as an integral part of the phospholipid bilayer and the PEG chain surrounding the surface provides effective steric stabilization. Addition of PEG can produce vesicles with optimal size and homogeneity [24–26]. It was reported that liposomes modified with PEG, over a two month storage period at 4 - 8 C, showed no physical changes, whereas conventional liposomes showed progressive aggregation and precipitation. In addition, the adsorption efficiency for PEG modified liposomes did not decrease significantly during two month storage, with no signs of degradation. This shows that PEG surfactants facilitate the dissolution of lipophilic active compounds and thus their loading in vesicles or absorption efficiency is higher. PEG also plays an important role in controlling the rate of released [19].

On the other hand, the use of tween 80 as a stabilizer can also maximize drug loading and minimize leakage and rapid

cleaning of the body. Tween tends to form highly curved structures (micelles). Thereby reducing the energy needed for globule deformation [11,20]. However, after a 9-week stability test at 37 ° C, there remains an increase in particle size and aggregation seen in the suspension but an increase in particle size and aggregation is not as bad as liposomes without surfactants. In accordance with other findings that show elevated temperatures cause an increase in size and aggregation as well as drug leakage from liposome formulations containing active compounds [14]. The use of surfactant tween 80 causes liposomes to be more stable without significant changes in vesicle size and zeta potential after one year of storage at 4 ° C but the drug content drops [17]. It was reported that the adsorption efficiency decreased when the proportion of surfactants increased and elasticity decreased with a lower proportion of surfactants and the best molar ratio of phospholipids: surfactants was obtained (86:14). In this case, the freeze drying method becomes a solution for liposome storage to avoid drug aggregation and leakage [17].

#### Freeze Drying

Liposome formulation, such as AmbiSome® and Doxil® are commercially available. However. Its potential as a therapeutic agent still has physical and chemical limitations during the storage period in water dispersion such as hydrolysis, phospholipid oxidation, leakage, and aggregation. One method that has been investigated for liposome stabilization is lyophilization or freeze-drying method [6].

Freeze-drying is an approach that can be used to stabilize the colloidal system. However, trapped drug leakage can occur during the freeze-drying process [27]. Therefore, the freeze-drying process requires a cryoprotectant to protect the liposome system from aggregation and combining particle size. Cryoprotectants commonly utilized are carbohydrates such as saccharose, mannitol, lactose, and trehalose [28]. Freeze-drying involves removing water from the product in a frozen state at low pressure. This process is usually used to dry the thermolabile product. This method can overcome the difficulty of liposome stability in the long run by storing in a dry state [18,27].

To increase the shelf life of liposomes containing the drug, the freeze-drying method uses trehalose as cryoprotectant [14,18]. Stability of freeze dried liposomes and liposomes in water dispersion from budesonide liposomes was assessed at conditions of 2-8 ° C and  $28 \pm 4$  ° C over the resulting six month period that drug leakage was significantly less in frozen dry liposomes compared to water-dispersed liposome. The leakage of the drug corresponds to an increase in particle size were dispersed liposomes tend to form large vesicles through coalescence whereas freeze dried liposomes do not show drug crystals [14]. Trehalose cryoprotectants are more effective than other sugar in preventing fusion and leakage of drugs where trehalose can maintain biological membranes without water. The presence of trehalose during the drying process maintains the liposome-containing structure of the drug in the anhydrous state by replacing water molecules which are usually bound to the lipid head group [18].

#### Chitosan

Addition of chitosan to liposomes is efficient for trapping drugs which lead to increased bioavailability [29]. Chitosan is used as a liposome companion to regulate surface characteristics with large loading capacities. Polycationic chitosan is used to form a positive layer around a negatively charged liposomes through surface adsorption by electrostatic interactions [30]. It was noted that the chitosan layer increases liposome stability under physiological conditions and programmed modification of protein release and therapeutic peptides. Where chitosan liposomes have a low PI which shows the diagnostic of monodisperse, distribution of monomodal sizes and confirms uniform liposome size. Chitosan coated liposomes also produce a wider therapeutic window and better efficacy due to longer and sustained drug release [13]. Additionally, chitosan can increase the resistance of interactions between liposomes, thereby preventing particles from flocculation [11].

In the study of Hurler et al., 2012, chitosan liposome stabilization tests were carried out under cold storage conditions of 4 ° - 8 ° C and stability testing accelerated at 40 ° C for one month. In storage conditions for 48 hours at a temperature of 4 ° C - 8 ° C no precipitation was found. However, at 40 ° C, an increase in liposome vesicle diameter is about 15% - 20% of its original size and a small increase in the polydispersity index. This shows that vesicles tend to coagulate in forced conditions such as temperatures of 40 ° C [15]. The use of chitosan in liposomes can reduce the adsorption efficiency of positively charged compounds because chitosan and positively charged compounds compete

for negatively charged phospholipids [13]. Therefore, in addition to modifying liposomes with chitosan, it can also be done by combining liposomes in a special hydrogel matrix for topical drugs so as to provide vehicle protection and reduce drug leakage in liposomes [15]. In addition to the use of chitosan in improving liposome stability, there are various types of biocompatible polysaccharides, namely alginic acid or chondroitin sulfate, which can be used to improve the efficiency of conventional liposomes [31].

#### Gel Polymer

Poloxamer gel is said to prevent drug leakage from the administration site which can reduce the therapeutic effect. The use of poloxamer gel, can keep the structure of liposomes by trapping them into the gel through electrostatic attraction, compared to liposomes without poloxamer gel that contains AmB can not maintain the shape and structure of liposomes observed by SEM. Liposomes in poloxamer gels retain the shape of spherical particles due to the hygroscopic properties of phospholipid [16]. Additionally, stability of liposomes containing AmB without poloxamers seen through changes in particle size and zeta potential results in both increasing and loading of the drug decreasing over time due to aggregation or fusion of vesicles and cohesion. Likewise, stability can be seen visually that a supernatant turbidity has been observed after two weeks. Stability problems that still occur can be addressed by lyophilization in which the final liposome product is freeze dried with cryoprotectant and dissolved immediately before administration [14].

Other polymers such as carbomer show that carbomer changes viscosity, but does not damage the structure of the liposome, but increases the physical stability of the liposome. Therefore, it is recommended to incorporate liposomes into the gel system, especially gels made with carbomer resins. It has been reported that liposomes are quite compatible with gels made from polymers derived from cross linked poly (acrylic acid), such as Carpool® resins. Incorporation of liposomes in carbomer gels shows better stability for topical drug delivery systems. To find out whether these methods are able to maintain liposome stability, characterization is needed as a parameter of stability [32,33].

#### LIPOSOME CHARACTERIZATION

The therapeutic efficacy of drug molecules is regulated by the stability of liposomes which involve the steps of making, storing, and shipping. Stability studies, one of which is evaluation of physical parameters that ensure product integrity during storage [27, 34]. Some of the weaknesses of liposomes that have been mentioned and the challenges that still exist in most preparation methods are control over size, polydispersity, and absorption efficiency. Therefore, it is important in order to physically characterize the liposome system. Physical parameters need to be monitored to ensure that liposome preparation can be produced and optimize its function [35]. Liposome physical parameters are built on measurements of vesicle shape, surface morphology, average size of vesicles and particle size distribution, surface load, and drug absorption. Determination of liposome size distribution is usually measured by dynamic light scattering while

liposomal lamellarity is measured by electron microscopy or by spectroscopic methods [36].

Table 2: Characterization of liposom

| Active compound        | Parameter   | Method  | Results  | Reference |
|------------------------|---|---|--|-----------|
| Neuropeptida           | Efficiency of absorption; particle size; surface load   | HPLC; dynamic light scattering  | EE $66 \pm 3.5\%$ ; sized $243 \pm 24$ nm; PI $0.3 \pm 0.1$ ; zeta potential $+32 \pm 1.0$ mV                                    | [13]      |
| Budesonid              | Average particle size; surface morphology   | Laser diffraction particle size; transmission electron microscopy; scanning electron microscopy | Measuring $7.89 \pm 0.74$ $\mu$ m; ball vesicle liposomes  | [14]      |
| Mupirocin              | Efficiency of absorption; particle size   | HPLC; dynamic light scattering  | EE $57.3 \pm 0.1\%$ ; measuring $218 \pm 55$ nm; PI 0.31   | [15]      |
| Amphotericin           | Efficiency of absorption; particle size; surface charge; surface morphology                       | Spectrophotometer UV Vis; light scattering spectrophotometer; scanning electron microscopy      | EE 50-60%; size of $462.2 \pm 15.9$ nm; zeta potential $42.9 \pm 17.6$ ; spherical vesicles in poloxamer                         | [16]      |
| Ketorolac tromethamine | Efficiency of absorption; surface morphology; particle size and size distribution; surface charge | HPTLC; transmission electron microscopy; Photon Correlation Spectroscopy                        | EE $73 \pm 11\%$ ; unimellar ball vesicle liposomes; measuring $127.8 \pm 3.4$ nm; zeta potential $-12$ mV.                      | [17]      |
| Fluconazole            | Efficiency of absorption; particle size   | Spectrophotometrically; dynamic light scattering  | $56,341 \pm 0.255\%$ ; measuring $10.79$ $\mu$ m   | [18]      |
| Resveratrol            | Efficiency of absorption; surface morphology; particle size; surface charge                       | HPLC ; transmission electron microscopy; dynamic light scattering                               | EE 95%; ball vesicle liposomes; measuring $\sim 85$ nm; PI $\sim 0.2$ ; zeta potential $-20$ mV                                  | [19]      |
| Coctail phage          | Surface morphology; particle size   | Transmission electron microscopy; dynamic light scattering                                      | Ball vesicle liposomes; measuring 212 nm   | [20]      |
| Docetaxel              | Efficiency of absorption; surface morphology; particle size and size distribution; surface charge | HPLC; transmission electron microscopy; dynamic light scattering                                | EE $98.6 \pm 7.2\%$ ; ball vesicle liposomes; measuring 251.5 nm 13.8; PI $0.324 \pm 0.077$ ; zeta potential of $44.92 \pm 3.61$ | [21]      |
| Tiazavirin             | Efficiency of absorption; particle size and size distribution; liposome surface charge            | HPLC; dynamic light scattering  | EE $77.9 \pm 1.8\%$ ; measuring $188 \pm 3$ nm   | [22]      |
| Curcumin               | Efficiency of absorption; surface morphology; particle size and size distribution                 | Spectrophotometer UV; scanning electron microscopy; dynamic light scattering                    | EE $76.48 \pm 1.20\%$ to $84.92 \pm 0.19\%$ ; ball vesicle liposomes; measuring 189-395 nm; PI 0.253-0.317                       | [34]      |
| Grape Extract          | Seed Efficiency of absorption; Particle size and size distribution; surface charge                | Spectrophotometer UV Vis ; Dynamic light scattering   | EE 87%; measuring 173 nm; PI 0.4; zeta potential $+63$ mV  | [11]      |

### Particle Size and Distribution

The particle size and distribution are critical parameters especially when liposomes are intended for therapeutic use by inhalation or parenteral routes [4,37]. Liposomes have sizes of 30nm to several micrometers. The size of deformable liposomes in the size range of 300-350nm has been reported to maintain the drug in the skin layer for topical use and avoid its absorption into the systemic circulation [38]. Dynamic light scattering is one technique that is often used to determine the size and size distribution of particles that have an advantage in measuring speed, which takes 2-5 minutes. This technique is usually measured by a particle size analyzer. In measurement, the particles must be suspended to produce a refractive index [39,40].

The increase in the particle size of liposomes containing the drug compared to empty liposomes can be attributed to the amphiphilic property which can be incorporated into lamellar lipids. Various conditions have been reported to affect particle size including sonication time and the extrusion cycle [15,16,38]. It has been reported that the size of liposome vesicles decreases with increasing sonication time and the extraction cycle [17, 40,41]. However, the extrusion cycle has no significant effect on the particle size and zeta potential of liposomes containing active ingredients. From this result, the liposome condition was optimized as sonication for 90 minutes and extrusion of three cycles [16]. In contrast to the study of Ternullo et al., 2018, that extrusion of liposomes containing hydrophilic compounds results in homogeneous size distribution and a reduction in the size of liposomes with a low polydispersity index [38]. Increased particle size can also be caused by improper or unstable storage temperatures which cause particles to constitute aggregates. The polydispersity index is an indicator of particle size distribution. PI values close to zero indicate a monodisperse system and PI values close to 1.0 which indicate that the size distribution is very wide [43].

### Entrapment Efficiency

The efficiency of entrapment is a key parameter in developing liposome-based delivery. High absorption efficiency can reduce cost and increase efficacy [44,45]. To determine the efficiency of adsorption in liposomes. Free drugs are separated from liposomes. Separation methods generally used are the centrifugation method [46,47] or ultracentrifugation [48,49] and the dialysis method [34]. Then the adsorption efficiency was measured with various instruments such as HPLC [17], UV Spectrophotometer [34], UV-VIS Spectrophotometer [11,16,50] and HPLC [51,51,52] The adsorption efficiency is affected by the solution used for hydration, the ratio of the drug to lipids during preparation, and the time of sonication. Efficiency of AmB absorption increases with the ratio of the initial weight of AmB with lipids from 1: 7, 1:10, to 1:20 molar ratio. Solvents for hydration impact, wherein the study of Kang et al., 2010, two comparisons of solvent use are utilized, 9% sucrose and PBS electrolyte solution which results in high efficiency of AmB absorption (50-60%). However, hydration with PBS electrolyte solution in liposomes results in low AmB encapsulation (<1-2%). Reported long sonication time reduces absorption efficiency [16]. Additionally, the

influence of high temperatures causes aggregation as well as drug leakage from budesonide liposome formulation [20].

### Surface morphology

Another parameter in liposome characterization is visualizing the morphology of particles using a microscope. The morphology of drugs in liposomes is important since they affect the stability and kinetics of their release [19]. There are a lot of techniques for imaging liposomes which are categorized into light, electrons, or atomic force microscopes. In visualizing the morphology of liposomes commonly used electron microscopes such as transmission electron microscopy (TEM) [14,17,19–21] which provide information on morphological views and can see particles of varying sizes, homogeneity, and surface structure. However, there are some disadvantages including vesicles in direct contact with the grid which may affect the orientation and morphology of liposomes, placing the sample under vacuum can cause sample dehydration. In addition to TEM, visualizing liposomes can be used scanning electron microscopy (SEM) [14,16,34] which provides general information about the size and morphology of vesicles, but has the disadvantage of being unable to provide detailed information about vesicle lamellarities and internal structures, and liposome structure can be disturbed because of the high vacuum conditions needed [4,53].

### Surface Charge

Another parameter in liposome characterization is the surface charge of the liposome measured by the zeta potential. The zeta potential indicates the particle charge in a specific medium and the magnitude of repulsion between similar and adjacent particle loads [49]. Higher zeta potential values indicate colloidal stability and can inhibit the aggregation of liposome formulations so that the particles are more stable. Liposomes with zeta potential above 30 mV, both positive and negative, are stable in suspension as a surface charge that can prevent particle aggregation due to repulsion and electrically stabilized particle dispersion. However, if the zeta potential value of a particle is too small, there will be a force of attraction greater than the repulsion force so that it causes coagulation and flocculation which indicate colloidal instability [54,55].

## CONCLUSION

Liposomes as a drug delivery system have problems in terms of stability including damage to storage that causes drug leakage from vesicles caused by several factors such as temperature, pH, surface load, and lipid composition. To overcome this, there are numerous ways including freeze-drying methods using cryoprotectants, incorporation of liposomes in the hydrogel matrix, modification with chitosan, and the addition of surfactants such as tween and propylene glycol. In assessing the stability of liposomes several characterizations such as absorption efficiency, particle size and distribution, surface morphology, and liposome surface load can be carried out.



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