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General properties and production technologies of liposomes

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Abstract

With its general definition, liposomes are lipid vesicles with a bilayer membrane structure consisting of two parts, hydrophilic and hydrophobic. Considering the similarity with the cell membrane of liposomes, which are mostly composed of phospholipids, it is seen that their biological compatibility is high. The liposome beads are capable of simultaneously encapsulating the hydrophobic and/or hydrophilic active. By delivering the active ingredients added to the liposome structure to the targeted area with high efficiency, they have widespread use in medical applications for therapeutic purposes. Liposomes can provide active and remote loading while protecting the active ingredients in their structure from the undesirable effects of external conditions. When the clinical usage areas of liposomes are examined, it is seen that they are one of the successful delivery systems and they are actively used by different disciplines. It has widespread usage areas especially in pharmaceutical applications due to its advantages such as not showing toxic tendencies and easy determination of chemical contents. Liposome production is commonly carried out by two different methods. The first method is carried out by forming a thin lipid layer in film hydration, and the second method is by adding lipids to the aqueous phase at transition temperature during appropriate hydration and mixing. This review article, it is aimed to examine the production technologies of liposomes and their common usage areas and potential usage areas. However, considering the general use of liposomes, physical and chemical stability problems limit their use and lay the groundwork for the development of new technologies.

Introduction

Liposomes have led to the emergence of Liposome Technology because they are water-filled vesicles with an amphipathic structure and their compatibility with the cell membrane. Phospholipid membranes, which are assigned as active substance carriers, are evaluated in the repair of the relevant cells when the process is completed. Thus, the liposome, which acts as both the targeted ethene and the carrier, remains in the body and becomes a part of the biological process [1]. The intravenous effect of oral use of liposome-based products has allowed the formation of irreplaceable product groups (Figure 1). The general structure of the liposome is shown. Figure 2 shows an example of a liposome used as a drug delivery system. Advantages of the pharmaceutical use of liposome technology; It can be summarized as the absorption of the active ingredient with high efficiency, high stability, low possibility of side effects, high biocompatibility, and low toxic effect. Liposome sizes vary between 20nm and 20 µm.

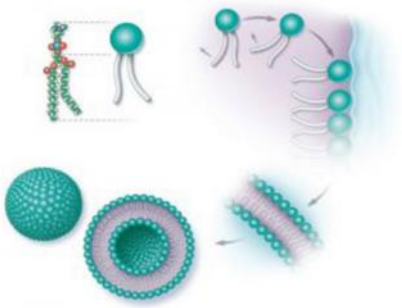

Figure 1. Lysosome hydrophilic-hydrophobic structure [1]

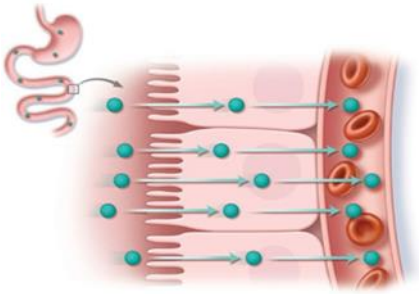

Figure 2. Drug delivery system [1]

While classifying liposomes, their size, lamellar structure and components are taken into account [2]:

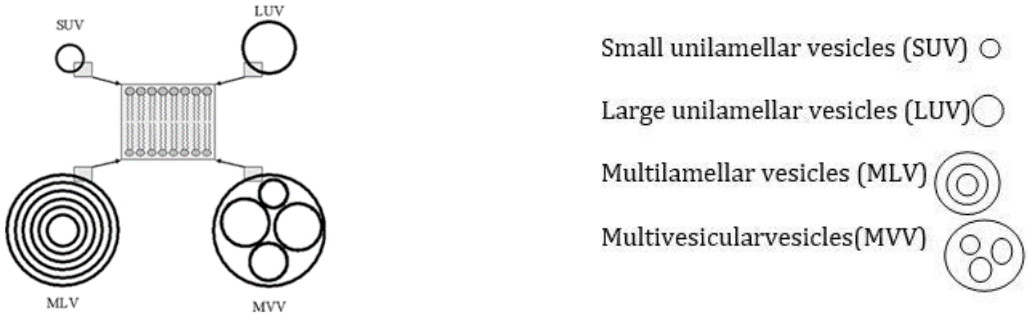

Figure 3. Classification of Liposomes [2,11]

According to their composition, liposomes are classified as follows [3,4];

- Conventional Liposomes
- Fusogenic Liposomes
- pH Sensitive Liposomes
- Cationic Liposomes
- Long Circulating Liposoms
- Immunoliposomes

The characteristics of the liposome, which is planned to be produced with liposome production technologies, are determined by the type of lipid used, the production technology used, the load, and the type of active substance used. At the same time, the size of the liposome is very important for the efficiency of its intended use. Particularly, it is observed that the biological benefits of active ingredient capsules produced orally with liposome technology are higher. The reason for this is both its structural similarity to the cell membrane and the fact that the active substance is protected from the liposome in the cell membrane and the components of the digestive system and maintains its stability until it is mixed with the blood [5,6,7,11].

Material and Method

The most commonly used type in liposomes, the main component of which is phospholipids, is lecithin. All phospholipids have specific gel liquid transition temperature (Tc) and surface charges, and before reaching this temperature, the fatty acid chains are in the crystalline phase. As this temperature increases, the chains pass into the liquid phase and expand their range of action [4]. The phospholipid types with the widest usage area and their transition temperatures (Tc) are given in Table 1.

When Table 1 of phospholipids is examined, it is seen that one of the most widely used phospholipids is DPPC. Its high Tc is considered to be important for stretching the working range and instability, which is known as its most important disadvantage [3,7,8,11].

Table 1. Phospholipid types used in encapsulation and liquid gel transition temperatures (Tc) [3]

LIPID TYPE	ABBREVIATION	Tc (°C)
Egg Phosphatidylcholine	Egg PC	-15,-7
Dioleoylphosphatidylcholine	DOPC	-22
Dilaurylphosphatidylcholine	DLPC	0
Dimyristoylphosphatidylcholine	DMPC	23
dipalmitoylphosphatidylcholine	DPPC	41
Distearoylphosphatidylcholine	DSPC	58
Bovine Brain Sphingomyelin	Brain SM	32
Egg Phosphatidylethanolamine	Egg PE	-
Dimristoylphosphatidylethanolamine	DMPE	48
Dimyristoylphosphatidylglycerol	DMPG	23
Dimristoylphosphatidic Acid	DMPA	52
Bovine Brain Phosphatidylserine	Brain PS	5
Dycetylphosphate	DCP	
Stearylamine	SA	
Ps/Dspc/Dppc (1:4,5:4,5)		43

Liposome Production Methods

Liposome production can be carried out by many different methods. Traditional methods with the most common usage; It is known as Thin Film Hydration (TFH) Method (Bangham Method), Reverse Phase Evaporation Method, Detergent Dialysis Method, Electroformation, Solvent Injection Method (Ethanol, ether injection method). However, since these methods are based on bulk production, additional processes such as membrane extraction, sonication, and high-pressure homogenization are required to obtain homogeneous and small-sized particles after production is completed. By eliminating the shortcomings of these methods, microfluidization Methods were developed for the production of more homogeneous liposomes in fewer sample sizes, and as a result of the method, no additional processing was required to reduce the particle sizes.

The developed methods of liposome technology are grouped into two parts with the most basic stages;

- Drying of lipids dissolved in the organic solvent
- Formation of liposomes in aqueous medium

The process is completed by analyzing the resulting liposomes. Liposome production methods continue to be developed and their usage areas continue to be expanded.

MLV Preparation Method

The lipid film is formed by dissolving the lipids that will be included in the liposome structure in organic solvents such as chloroform and then evaporating this solvent with nitrogen gas. At room temperature, the formed lipid film is hydrated with a buffer solution. The hydrated lipid film is immersed in water at a temperature higher than its anaphase transition temperature (Tm) (Tm + 20°C) for one minute before being removed and shaken for one minute with the aid of a vortex. These steps are repeated for 15 minutes. Multilayered liposomes are produced as a result of these processes [9-11].

SUV and LUV Preparation Method

Various processes are applied to change the size or properties of the layers of multilayer liposomes formed by dry lipid film hydration. MLVs are large and heterogeneous. Therefore, MLVs can be converted into SUVs or LUVs using methods such as sonication, extrusion, and vortexing. The liposome extruder, which is one of the devices used during these processes, is used to separate liposomes according to the pore diameter it has. Liposomes with a smaller diameter than the pore dissociate by passing through the pore. With the sonication device, a high level of energy is applied to the MLVs to obtain an SUV.

LUV and SUV can also be made in a variety of ways. The detergent used as a solvent in this method ensures that proteins are separated from the lipid-protein mixture. In the use of detergent, colloidal solutions serve as buffer solutions. To remove the detergent, centrifuge, gel filtration, or accelerated controlled dialysis methods are used, yielding SUV or LUV [9-11].

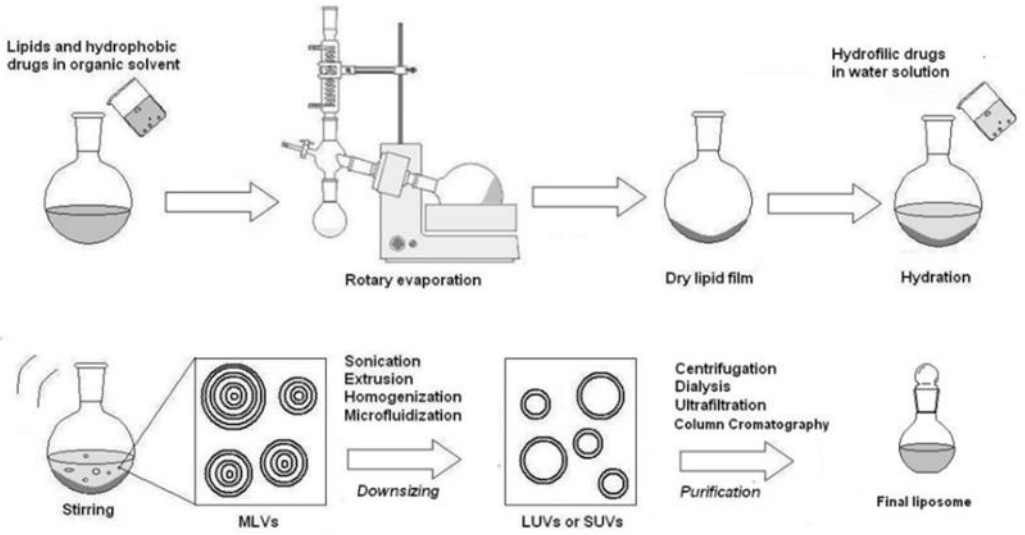

Figure 4. MLV Preparation Method [11]

Results

In the first method of liposome production, a thin lipid layer is formed in the hydration of the film, and the film is mixed above the transition temperature with the addition of water with a preprocessor providing high speed or high shear force. During appropriate hydration and mixing, lipids are added to the aqueous phase at the transition temperature in the other liposome production pathway. The stability problem is one of the issues that must be identified and resolved as a result of the production studies. To address the stability issue, new solution-oriented studies were conducted. Among these studies are lyophilization, appropriate particle size, layering, combined ratio, and so on.

Proliposomes were first developed in 1986; they are dry, granular products with good flow properties made up of active substance, phospholipids, and a water-soluble carrier material that transforms into a multilayered liposomal suspension when exposed to water [9].

In 1991, the concept of pro-liposome was expanded to include liquid phospholipid formulations capable of forming liposomes by adding an aqueous phase. Concentrated solutions of phospholipids in ethanol are the liquid formulations. As a result, pro-liposomes can be defined as a powder or liquid lipid formulations that can be converted into liposomes by adding an aqueous phase [10,11].

Conclusion

When liposomes are evaluated in terms of usage areas, it is observed that they have a very wide range. Liposomes will continue to serve especially drug delivery systems by eliminating their stability problems or reducing them to a very small amount. Although pro-liposomes are produced for this purpose, they are new-generation drug delivery systems that aim to solve the stability problems of liposomes.

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