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REVIEW PAPER

Physical and chemical properties of nano-liposome, application in nano medicine

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Abstract

The liposome is derived from two Greek roots: 'Lipo' meaning fat and 'Some' meaning structure. Liposomes can be made from natural phospholipids and cholesterol and, if necessary, other additives. Liposomes were first discovered by Bangham in 1961 due to their simple, self-fulfilling structure and low cost. Liposomes are spherical vesicles with a membrane composed of bilayer phospholipids that are used to release drugs or genetic material into the cell. Current research is focused on liposome technology based on the preparation and development of long-circulating liposomes, lipid components' changing, and vesicles' charge amount. Liposomes, in addition to pharmaceutical carriers, are used in cutaneous, respiratory, food industries, injectable, and in genetic engineering and diagnostic applications. This paper reviews the physical and chemical characteristics, structure, construction methods, and applications of nanoliposomes in various uses as drug carriers, including the treatment of specific diseases.

Keywords: Nanoliposomes, Nanoliposomes fabrication methods, Nanoliposomes applications, Characteristics of nanoliposomes.

1. Introduction

In the last few decades, nanotechnology has been the focus of researchers [1-6]. various types of drug delivery systems have been extensively studied and nanoliposomes are one of the most popular types of nanoparticles that have been potentially used as bioactive molecules' carriers [7, 8]. The liposome is derived from two Greek roots: 'Lipo' meaning fat and 'Some' meaning structure. Liposomes are small hollow spheres about microns to nanometers in size that contain a small volume of an aqueous solution. A liposome is a colloidal unit of phospholipids that accumulate itself in bilayer vesicles [7, 9-11], that first discovered by Bangham and et al. in the 1960s [12]. Bangham and et al. found that when egg lecithin is dispersed in water, they can be accumulated spontaneously in closed bilayer structures [12]. Subsequently, closed bilayer structures were named "liposomes" in 1968 [13]. Liposomes can be made from natural phospholipids with different lipid chains [8]. The polar portions of phospholipids are located on the liposomes' surface, and the fatty acid chain portions are separated from the water which is consists of a bilayer hydrophobic nucleus [8]. Nanoliposomes are nanometer versions of liposomes that can provide both lipophilic and hydrophilic regions, and they can trap drugs in lipid bilayers with different lipotropic (i.e. aqueous nucleus, or bilayer interfaces) [14-16]. The size of spherical lipid vesicles can vary from a few nanometers to a few micrometers, and nanoliposomes that are used for medical purposes are generally between 50 and 450 nanometers [17]. Nanoliposomes are considered as an ideal drug delivery system due to their similar nature to cytomembranes and their excellent ability to trap different drugs. As a result, nanoliposomes have been extensively studied over the past 60 years. In addition, nanoliposomes can preferably accumulate in tumors by relying on improved permeability and insolubility effect, which can improve the efficiency and reduce systemic anti-cancer side effects of drugs [18]. Due to the biological and technological advantages of liposomes as delivery

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systems in vitro and in vivo, nanoliposomes are currently considered as the most successful drug delivery systems [19]. The liposomes' membranes are made up of two lipids (amphiphilic) paired against each other. The same property can be used for drug delivery in drug delivery systems because it is a body material kind and therefore when it lies in a body there is no resistance to it [20]. Amphipathic lipids, like lecithin, are converted to vesicular forms by appropriate methods. These spherical structures have the ability to enclose hydrophilic materials within themselves and lipophilic materials in the membrane [21-23]. This paper reviews the physical and chemical characteristics, structure, construction methods, and applications of nanoliposomes in various uses as drug carriers, including in the treatment of specific diseases.

2. Liposomes

Liposome technology is one of the rapid scientific development fields and has led to significant advances in medicine, cosmetics, the biological membrane's structure and function, the study of life's origin, and so on. This is due to several beneficial properties of liposomes, such as the ability to mix in aqueous and non-aqueous environments, accurate targeting, adaptability to fluid conditions, the size of liposomes, the ability to load material, and multilayered globules production [24-27]. The discovery of liposomes took place in early 1965 by Bangham in England. He observed that when high concentrations of phospholipids are present in the environment, these phospholipids form liposomes spontaneously on a microscopic scale without the need for energy. He also found similarities between the structures of cellular liposomes. During a study by Leininger in 1967, this structure was named the Bangzom. This structure was named in 1968 by Wiessman and Sessa [12]. Before 1985, research on liposomes focused more on the physicochemical properties, modeling of biological membranes, assessment of their stability and interactions within the system. Since 1985, they have been used practically in various fields such as physics, chemistry, biology, agriculture, nutrition, food industry, etc. Liposomes or lipid vesicles are masses composed of aqueous dispersions of amphiphilic molecules such as polar lipids, which tend to form bilayer structures. Liposomes have a spherical shape and for this reason, they are called spherulites [28]. The formation mechanism of liposomes is such that when amphiphilic molecules such as phospholipids are placed in the aqueous medium, they form mass complexes to protect their hydrophobic parts from water molecules while still are in contact with the aqueous phase through hydrophilic heads. If the right amount of energy is provided for the phospholipids, they can be arranged in regular closed bilayer forms [29]. Liposomes inside are inherently aqueous, unlike surfactant micelles, and this is due to polar lipids that are oriented in such a way that the polar heads are located on the inner and outer surfaces of the solvent phase. Thus the liposome's inner region consists of an aqueous solution that has the same chemical composition of the environment, which they were originally formed. The membrane inner core, in which the hydrophobic tails of polar lipids interact, is non-polar in its nature. And there, the attachment of lipophilic materials can occur within the bilayer [28]. Thus, liposomes can trap dissolved hydrophilic substances in the hydration medium. Lipophilic molecules with lipid-soluble compounds such as vitamins, nutrients, and drugs can also be attached to the liposomal bilayer via dissolving these molecules with lipids. Lipid-soluble compounds may also be complexed with cyclodextrin and then encapsulated in the liposomes and nanoliposomes' aqueous portion [29-31]. The ability to produce on a large scale and the targeting ability are important advantages of liposomes [32]. Targeting bioactive molecules to the position where their effect is required is essential to achieve optimal performance for a sufficient concentration of active compounds at the target site. Timely and targeted release increases the effectiveness of bioactive compounds, expands their range of applications, ensures optimal dosage, and thus improves production cost efficiency. Lipid vesicles targeting is mainly achieved through active mechanisms (e.g., antibody attachment) or inactivation (e.g., by targeting by particle size) [29].

3. Liposomes' composition

Another salient feature of liposomes is that they are obtained from natural sources. They and nanoliposomes can be prepared using safe raw materials obtained from natural sources such as eggs, soy, or milk, and thus can obtain legal approval for use in food-grade products. Studies have shown that lipid vesicles are present in our most basic natural food, breast milk. Milk phospholipids are derived from the fat cells' membrane and are commercially available, now. The unique combination of phospholipids derived from milk fat globule membranes and the low cost of their sources, i.e. buttermilk, butter may provide benefits in the production of nanoliposomes [30, 33]. On the other hand, the phospholipid components of liposomes and nanoliposomes have some benefits for human health, such as protecting the liver and improving memory. Also, sphingolipids are needed for cellular signaling and are involved in controlling cancer cell proliferation and inflammation. In relation to sphingomyelins, the ability to inhibit intestinal absorption of cholesterol and fat has been reported, in which milk sphingomyelin is more effective than egg sphingomyelin [29]. The main chemical components of nanoliposomes are speed molecules or phospholipids. Among phospholipids that can form a liposomal structure, phosphatidylcholine, sphingomyelin, phosphatidylserine, and phosphatidylinositol can be mentioned. Depending on the number of charged lipid

molecules present in the bilayer structure, the space between the lamellae changes, and the physical properties of the vesicles are only affected. The degree of saturation and length of the fatty acid chain can also change the physical properties of the bilayer membrane, including its curvature, stability, and permeability [30]. Nanoliposomes may also have other components, such as sterols, in their structure. Sterols are important constituents of most natural membranes, and their incorporation into nanoliposome bilayers can cause fundamental changes in the properties of these vesicles. Cholesterol is the major sterol used in the preparation of lipid vesicles. Cholesterol itself cannot form bilayer structures but can attach to phospholipid membranes at very high concentrations, for example to a 1: 1 or even 2: 1 molar ratio of cholesterol to phospholipids. The use of cholesterol in the structure of nanoliposomes can increase the stability of vesicles by modulating the lipid bilayer fluidity. Cholesterol generally modulates the fluidity of phospholipid membranes by preventing the crystallization of acyl phospholipid chains and providing a steric barrier to their movement. This contributes to the stability of the nanoliposome and reduces the permeability of the lipid membrane to solutes [29]. Cholesterol also dries the water-lipid interfacial region and increases the chemical stability of the liposomal membrane against peroxidation and hydraulic estracyl [30]. Also, for different applications of liposomes and obtaining liposomes with distinct and desired characteristics, their structure can be modified. One of the important factors in the development of a nanoliposomal product is the stability of the produced vesicles. The stability of liposomes can be improved by coating the surface of the vesicles with special polymers such as polyethylene glycol or chitosan, or by using anionic and cationic components in the structure of liposomes, or by using stabilizing agents such as cholesterol and glycerol [29]. It has been shown that nanoliposomes have short release times despite their various advantages. To overcome this limitation, coverage with chitosan can be used by adding drops of chitosan to disperse liposome solution. Chitosan coating changes the surface charge of the liposome and slightly increases its particle size. At the same time, the liposome profile shows longer release and greater stability. The use of long-saturated acyl chains or hydrogen soy phosphatidylcholine and the presence of an appropriate amount of cholesterol in the liposome membrane minimizes the membrane defects. Also, polyethylene glycol-coated liposomes are resistant to salt digestion and can be useful for increasing the bioavailability of encapsulated compounds [29]. Studies also show that heat-sensitive liposomes can be produced by modifying lipid bilayers with specific polymers. The polymer must have a critical temperature that is soluble in the water below and dissolves above that temperature. Polymer-encapsulated liposomes above the critical temperature become unstable following the interaction between the liposome membrane and the hydrophobic polymer chains, which cause releasing of the encapsulated material. These types of carriers are suitable for releasing flavors by increasing the cooking temperature in prepared foods. PH-sensitive liposomes can also be prepared using amphiphilic lipid molecules such as phosphatidylinositol, unsaturated ethanolamine, and oleic acid, which make the liposome unstable under acidic conditions and release the encapsulated bio component. PH-sensitive polymers can also be added to liposomes by mixing lipids and polymers during the preparation of vesicles, so the response to stimulation of these liposomes will largely depend on the structural properties of the outer surface of the vesicles [30, 34-37].

4. Types of liposomes:

Liposomes can be structurally divided into different types based on the number of bilayers in the structure according to their size, Figure 1. Based on the bilayer structure, vesicles can be divided into unilamellar (ULV), oligolamellar and multilamellar (OLV and MLV) types, which consist of one or more concentric lipid bilayers, respectively. The difference between MLV and OLV liposomes is in the number of their layers so that more layers are seen in MLV liposomes. Another type of liposome is called a multivesicular -vesicle (MVV) vesicle, which consists of several small decentralized vesicles trapped within a lipid bilayer. In addition, vesicles can be classified into small unilamellar vesicles (SUVs) and large unilamellar vesicles (LUVs) with diameters between 20 and 100 nm (narrow size distribution) and several micrometers, respectively. SUVs generally are generated from the layering of outer layers of MLV (Figure -1) [32]. SUVs have a low water-to-lipid volume ratio and therefore effective encapsulators are not large for nutritious and beneficial food compounds. Although, liposomes have the ability to simultaneously entrap hydrophilic molecules in the internal volume and hydrophobic compounds in the hydrophobic fraction of the lipid bilayer. On the other hand, LUVs and MVVs, which have a large water-to-lipid volume ratio, are capable of carrying large volumes of water-soluble compounds in their inner nucleus, and therefore, are suitable for encapsulating large hydrophilic compounds, which compared to ULVs and MVVs, they provide a continuous release profile [32]. MLVs are also suitable for covering some lipophilic materials due to their large lipid phase [29].

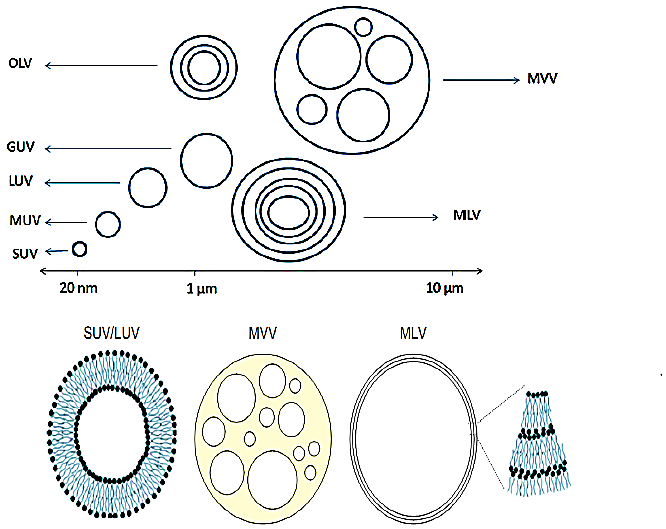

Figure-1: Schematic view of liposomes classification based on structural parameters [38].

5. Liposomal Feature:

Researches show that the methods used to prepare liposomes have a significant effect on physical properties such as size and encapsulation efficiency of bioactive compounds [39].

5.1. Particle size and distribution:

The researchers have shown that the liposomes' average particle size is affected by the composition and preparation method, and these two characteristics are parameters that change the expected performance of a liposomal system. The average particle size of liposomes aqueous dispersions can be measured with a dynamic light scattering device (DLS) [40-42].

5.2. Temperature Phase Transition:

Amphipathic molecules such as phospholipids have important properties. They can enter the thermotropic phase change at much lower temperatures than the melting point. The phase transition temperature depends on the hydrocarbon chain nature, the molecule polar part, and the environment ionic nature and degree. Chain length reduction, acyl chain saturation degree, the presence of branched chains, and the presence of bulky side groups reduce the phase transition temperature. Unsaturated chain hydrocarbons have lower phase transition temperatures rather than the chain with trans conjunction and unsaturated bonds [29].

5.3. Surface charge:

The liposomes' surface charge can be changed so that it can be neutral (by using phospholipids such as phosphatidylcholine or phosphatidylethanolamine), negative (acidic phospholipids such as phosphatidylserine, phosphatidylglycerol, and phosphatidic acid or diethyl phosphate). Acetyl phosphate) or positive (by using lipids such as acetylamino). Liposome charge is an important feature that does not determine liposome stability and encapsulation efficiency. The electrostatic attraction between the charged active substance and the liposome is a means of increasing encapsulation efficiency [43].

5.4. Zeta Potential:

The surface charge density of liposomes and the tendency to adsorb different ions on lipid vesicles can be measured by measuring a parameter called the zeta potential. The zeta potential is a function of the lipid vesicles' surface charge of each layer adsorbed on the interfacial layer and the nature and composition of the environment in which the liposome is suspended. Zeta potential cannot be measured directly and can be measured using theoretical models and electrophoretic mobility [44]. The zeta potential makes the liposomal suspension more stable, as the charged vesicles repel each other and thus overcome the natural tendency to accumulate [45].

5.5. Fluidity:

The fluidity of the bilayer membrane reflects the order and dynamics of the alkyne phospholipids chains. The presence of cholesterol in the membrane structure weakens the hydrocarbon chains interactions and prevents

liposomes from crystallization [46]. Researchers have shown that mixing some liquid fats in the liposomes bilayer membrane reduces the phase transfer temperature and increases fluidity [47]. The encapsulated drug release from the liposome depends on the number of layers and the cell membrane permeability and the bilayer membrane fluidity [48].

5.6. Number of layers:

The most important characteristics of lipid vesicles are generally the number of layers and their size. The number of layers around the inner aqueous space is the fat vesicle [48].

5.7. Encapsulation efficiency:

Bioactive substances can interact with liposomes in various ways, which depend on their specific properties such as solubility and polarity. Liposome lipid composition and preparation method affect the performance of the encapsulation. Adding cholesterol significantly alters the performance of the encapsulation. The encapsulation efficiency of liposomes depends on the strength of the bilayer membrane. Many experimental methods reported to determine the encapsulation efficiency require the separation of bioactive material and encapsulated bioactive free materials by one of the methods of column chromatography, exclusion chromatography, ultracentrifugation, dialysis, and ultrafiltration before measurement of the encapsulated material by analytical techniques such as spectrometry, high-performance liquid chromatography and spectrofluorometry [48].

5.8. Loading capacity:

The loading capacity of liposomes varies depending on whether the encapsulated compound is water-soluble or lipid-soluble. If water-soluble compounds are encapsulated, a very high loading ratio is possible. In contrast, if lipid-soluble substances are encapsulated in the shell material, only relatively small concentrations of the active substance can be encapsulated. For single lamellar liposomes, the shell is usually 4 to 5 nm thick. Thus, for a 100-nanometer liposome, the internal volume is approximately 7 times larger than the volume occupied by the shell, which decreases as they become smaller. In multi-lamellar liposomes, the internal volume can also be smaller because most of the volume is occupied by lipids. Therefore, for concentrations less than 10% of polar lipids, the volumetric component can increase rapidly above 50%. In viscosity, liposomal dispersion can further limit the increase in polar lipid concentration. If a 50% load is thought, which is unrealistically high, a single lamella system that occupies more than 50% of the volume of the food system will contain less than 5% of the active ingredient. However, the loading ratio can be significantly increased in multi-lamellar vesicles in which concentrated bilayers are formed [49].

5.9. Maintenance efficiency:

The maintenance efficiency of encapsulated material in liposomes depends on the properties of the encapsulated substance, the liposomal shell substance, and environmental conditions. If hydrophilic compounds are encapsulated inside the capsule, their survival may improve following electrostatic interactions if they have a similar charge to the constituent polar lipids. On the other hand, the membrane is flexible and is exposed to natural fluctuations that can lead to the substance's release over time. In the case of attached lipid compounds, the situation is more complex and depends on the interaction of the lipid material with the polar lipids that make up the liposome shell [48].

5.10. Chemical stability of phospholipids:

Oxidative decomposition and hydrolytic decomposition are two destructive methods that limit the liposomes retention time. Oxidation of phospholipids in liposomes occurs mainly in its unsaturated fatty acyl chain. But saturated fatty acids also oxidize at higher temperatures. Fatty acyl of the phospholipid molecules is oxidized by a free radical chain mechanism. In the presence of oxygen, the oxidation process is developed more and can occur through the decomposition of hydroperoxides in the chain. The use of high-quality raw materials free of hydroperoxides and metal ions reduces the possibility of phospholipid peroxidation in liposomes. The addition of antioxidants to the liposome formulation, the preparation of liposomes in an oxygen-free atmosphere, preservation at low temperatures, and protection against light and oxygen eliminate the possibility of oxidation [38].

5.11. Physical stability of phospholipids:

Changes in the average particle size and particle size distribution that occur due to their aggregation and fusion, and the loss of the drug or bioactive substance in the capsule due to its leakage out of the liposome are factors that determine the physical stability of liposomes. Changes in the average particle size and particle size distribution are strongly influenced by phospholipid compositions, environmental composition, and pH [38].

5.12. Physical stability of liposomes:

Liposomes are colloidal systems and, like all colloidal systems, may undergo changes over time (due to the presence of gravitational forces between colloidal particles such as van der Waals and preparation forces) such as aggregation, agglomeration, and fusion, which cause instability in size and the persistence of the active substance in liposomes. Therefore, these adverse interactions must be countered and the colloidal system stabilized for a longer period for the intended purposes. Polar phospholipids in the system form charged colloids, which form liposomes with the same charge in the system. These same charges cause electrostatic repulsion between particles at the surface of the liposomes. Depending on the pH and ionic strength of the environment, this repulsive force can be reduced or increased. Also, the purpose of using compounds such as cholesterol, glycerol, and polyethylene glycol in the structure of the liposome is to create a spatial barrier or stereo repulsion force, which is placed in the structure of the liposome as the liposomes are connected to each other. The overall stability of this colloidal system depends on the magnitude of the repulsive and gravitational forces between the colloidal particles [46]. From the stability point of view, liposomes are more stable than emulsions against separation by gravity, because the density difference between the two phases is much smaller than the stimulus density difference for the separation of oil-in-water emulsions. However, in contrast to microemulsions, liposomes are not thermodynamically stable. They tend to mix over time because the electrostatic charges on polar lipids are often small and not sufficient to prevent liposomal intersection. External parameters such as pH, ionic strength, and temperature and internal parameters including phospholipid concentration, composition, and encapsulated nature can affect the physical and chemical stability of liposomes. Chemical instability may be the result of oxidation and hydrolysis of polar lipids [28]. The liposomes stability can be increased by coating the surface of the vesicles with special polymers such as polyethylene glycol or using cationic and anionic substances in the structure of liposomes, or by using stabilizing agents such as cholesterol and glycerol. Using similar charged materials in the structure of the liposome causes a reduction in the fusion accumulation and vesicles' deposition. Increased interparticle repulsion, both electrostatically and as a spatial barrier, increases the stability of liposomes [24].

5.13. Interfacial Features:

The interfacial properties of liposomes and the surface charge that determine the capabilities and applications of liposomes depend on the chemical composition of the bilayer and environmental conditions such as solvent type, ionic strength, and temperature. Two-layer structures may be exposed to complex phase transitions during heating. In this process, their interfacial properties change drastically and the liposomes become very sensitive to mechanical stress, which may lead to disintegration and release of the encapsulated compounds. The liposomes' surface charge affects the electrostatic interaction of lipid particles with other charged compounds. Charged liposomal membranes may, for example, prevent compounds of equal charge from passing into the liposomes, which is one of the reasons that the encapsulated compounds inside the liposomes can be protected from solvent phase compounds. The orientation of bisexual heads in the interfacial region of liposomes changes due to temperature and ionic strength, and indicate that the actual liposomes surface charge alone does not depend on the properties of the phospholipid molecule, and also, the membrane interaction between molecules is important [28].

6. Preparation methods of nanoliposomes:

Liposomes, unlike other surfactant masses such as spherical and non-spherical micelles or hexagonal phases, do not form spontaneously in an aqueous medium and do not have thermodynamically stable structures. The liposomes formation requires energy and depending on the type of liposome the amount of input energy can be very variable. MLVs are easily formed when the polar lipids that make up the bilayer are dispersed in an aqueous medium under gentle agitation. But producing LUVs and SUVs requires significant energy to dismantle the MLV and MVV structures and produce vesicles with the monomodal distribution. Liposomes, like emulsions, are only stable for a certain period, in other words, they are kinetically stable, and for this reason, many principles and techniques of emulsion production are used to form liposomes [28]. Thus, lipid vesicles are formed when phospholipids, like lecithin, are immersed in water, and so they form one or more bilayers. And they are separated by molecules when sufficient energy is provided. The entrance of energy leads to the arrangement of lipid molecules in the form of bilayer vesicles to achieve a thermodynamic equilibrium in the aqueous phase [50]. In this regard, the researchers suggested that they prefer flat to symmetrical membranes since the energy is required to bend them. The type of lipids used and the presence or absence of sterols are important parameters in determining the curvature of the membrane. Cylindrical phospholipids such as phosphatidylcholine, which form bilayer lamellar structures, may bend and form vesicles. Although, these structures are generally not very stable due to geometric constraints in the absence of stabilizing agents such as sterols [51]. There are several methods for preparing liposomes and nanoliposomes, and various papers have described these methods in detail. The correct choice of preparation method depends on various factors, including the physical and chemical properties of the material to be trapped, the

liposomal components, the environmental nature that is dispersed on the lipid vesicles, the effective concentration of the encapsulated material and its toxicity, the additional processes involved during the use of vesicle transfer, optimal size, polydispersity and storage life of vesicles for the intended application, reproducibility and large-scale production of safe and efficient liposomal products. Generally, the first step in preparing standard liposomes is to prepare a solution of chloroform or methanol containing phospholipids, cholesterol, and hydrophobic compounds, and then evaporate the solution to form a thin film. Then, the aqueous phase and the hydrophilic substance are added, followed by an appropriate amount of heat and mechanical energy, which results in the formation of a sheet of hydrophobic compounds that separate from the mass and form MLVs [29]. Since the water-soluble compounds of liposomes are often released via their permeability through the membrane, so MLVs can show maximum protection of the active compound, which is due to the number of lamellas that the material must pass to reach the outside of the vesicle. The main problems with MLVs are the small volume of water trapped and the low efficiency of trapping them. Therefore, different techniques have been used to encapsulate a higher proportion of the active compound. This increase in trapping efficiency can be achieved by increasing the volume of the aqueous phase trapped in the vesicles and the use of high lipid concentrations. The obtained vesicles are also suitable in size and their properties are different for different methods. Therefore, MLVs have limited use in industrial applications due to their large diameter, size heterogeneity, instability, and inhomogeneity from one preparation to another [31]. To reduce the size and prepare single-lamellar vesicles consisting of bilayers phospholipid molecules surrounding an internal aqueous nucleus that have a greater capacity to encapsulate hydrophilic compounds, use such methods that require more energy, e.g. extrusion sonication, polycarbonate filters, or homogenization [29, 31]. The most common methods used in the preparation of liposomes are based on the entry of mechanical energy into the system and include high-pressure ultrasonication, high-pressure homogenization, extrusion, and membrane homogenization. And non-mechanical methods include reverse-phase evaporation, detergents remove from lipid/detergent mixed media, freeze-drying, and water reabsorption, freezing and thawing cycle [28], heating method or Mozaffari method [29], The Bingham method (thin layer hydration) [52], and the solvent injection method (ether or ethanol) [52] [53].

6.1. Lipid thin-film hydration:

Bangham method: Thin layer hydration is the primary method used to prepare liposomes. In this method, a mixture of phospholipids and cholesterol are dispersed in an organic solvent. Then, the organic solvent is removed by evaporation using a rotary evaporator at reduced pressure. Finally, the dry lipid film remaining on the vessel wall is hydrated by adding an aqueous buffer solution under stirring conditions at a high lipid phase transition temperature. This method is common and easy to use. Although, phospholipids dispersed in the aqueous buffer lead to multilamellar liposomes that are 1 to 5 micrometers in shape and size. Therefore, the application of size reduction methods such as sonication for SUV formation or extrusion through polycarbonate filters for LUV formation can be useful for producing smaller vesicles of more homogeneous size [52].

6.2. Reverse phase evaporation:

In the reverse phase evaporation (REV) method, the polar lipids forming the bilayer are dispersed in an organic solvent with a low boiling point such as diethyl ether, isopropyl ether, croform, or methanol. An aqueous solution containing the substance to be encapsulated is added to the organic phase, and the system is briefly homogenized to form an emulsion. The structure of the emulsion is similar to an inverted micelle or water-in-oil microemulsion system in which polar lipid hydrophilic heads in contact with aqueous droplets contain active components. While fatty acid chains interact with organic solvents, as the solvent evaporates under reduced pressure, the system becomes an aqueous dispersion of the vesicles. While the encapsulation efficiency is relatively high, complete removal of the organic solvent is often impossible and the particle size distribution of the vesicles is wide. Therefore, liposomes produced by this method are often subjected to another stage of extrusion to achieve smaller and more homogeneous dispersions [28].

6.3. Depletion of mixed detergent-lipid micelles:

The detergent dialysis method is based on the production of small mixed micelles of polar lipid surfactant, which are formed if an excess of surfactant compared to lipid is present in the system. There is a dynamic equilibrium between the concentrations of dispersed monomers in the aqueous phase and the surfactant molecules in mixed micelles. When surfactant molecules are removed from the aqueous phase by dialysis, the surfactant molecules in mixed micelles will be gradually removed. As a result, the mixed micelles first turn into a polar lipid surfactant mixed vesicle and finally into a surfactant-free liposome. Dialysis detergent vesicles are usually highly homogeneous and suitable for a wide range of polar lipids. Since any mechanical force and solvent are not used in this method, the activity of encapsulated compounds is usually preserved in comparison with liposomes produced by ultrasound. The disadvantage of this method is that it is time-consuming and a small concentration of surfactant may

remain in the system. The final particle size in this method depends on the type of used surfactant, surfactant to lipid ratio, and the surfactant removal rate from the system. Hence, it is difficult to control the production process [28].

6.4. Freeze drying - rehydration and freeze - thawing:

Another non-mechanical method of producing liposomes is freeze drying-rehydration and freeze-thawing method. Instead of producing liposomes, these techniques are mostly used to purify liposomes and improve their properties [28]. The freeze-drying method is based on the formation of an equilibrium dispersion of lipids in water-soluble carriers. In one method used, lipids forming liposomes and water-soluble carriers such as sucrose in tert-butyl, alcohol-water systems were dissolved in appropriate ratios to form a clear isotropic monophase solution. The monophase solution was then sterilized by filtration and filled into freeze-dried tubes. In the used method, freezing at -40 ° C for 8 hours, primary drying at -40 ° C for 48 hours, and secondary drying at 25 ° C for 10 hours was performed. The tower pressure was maintained at 20 Pascals during the drying process. By adding water, the lipophilized product spontaneously forms homogeneous liposomes [31]. Water absorption above the liquid-gel crystalline phase transition temperature leads to the melting of small vesicles and produce the MLVs. While MLVs are significantly larger than primary liposomes, the concentration of trapped material is very high, and the trapping efficiency is reported up to 45%. The encapsulation performance may also be improved by freezing and repetitive freezing at high phase transfer temperatures. It is thought that the thawing process after freezing leads to a reduction in the number of microscopic liposomes and form the multi-vesicle vesicles with high trapping efficiency [28]. It is observed that with some lipid compounds, the freezing-thawing cycle causes the homogeneous distribution of particle size and forms the single lamellar vesicles [29].

6.5. Solvent injection method (ether or ethanol):

In the ether injection method, a solution of lipids dissolved in diethyl ether or in the ether-methanol mixture is gradually injected into an aqueous solution of the material to be encapsulated at 55 to 65 ° C or under reduced pressure. Subsequent removal of the ether under vacuum results in the formation of the liposomes. The main drawback of this method is that the homogeneous constituent particles are 70 to 200 nm and the compounds to be encapsulated are exposed to organic solvents at high temperatures. In the ethanol injection method, a lipid solution of ethanol is rapidly injected into a large amount of buffer, and MLVs are formed immediately. In this method, the vesicles are heterogeneous in size from 30 to 110 nm and the liposomes are very thin. It is difficult to remove all the ethanol because it forms an isotropic with water, and different active macromolecules may deactivate in the presence of even small amounts of ethanol [53]. The difference between the ether injection method and the ethanol injection method is that the ether is immiscible with the aqueous phase. The advantage of this method compared to the ethanol injection method is that the solvent is removed from the product, and therefore the process can be continued until the formation of a concentrated liposomal product with the encapsulation efficiency [53].

6.6. High-pressure ultrasonication:

The high-pressure ultrasonication method involves the propagation of sound waves with frequencies between 16 and several hundred kHz through an aqueous dispersion of polar lipids. The propagation of sound waves through the environment leads to the spontaneous production and collapse of small cavities. The formation and destruction of pore cycles causes a sharp increase in pressure and temperature inside the cavity and creates severe turbulent flow conditions in the areas close to the collapsing bubbles. These conditions impose a high shear flow on large liposomes, leading to rupture of liposomal structures and smaller vesicles form that are less sensitive to stress [32]. There are two types of ultrasonic homogenization systems, including direct tip sonicators and indirect bath sonicators. Direct tip sonicators consist of an ultrasonic producer that is attached to a tip made of stainless steel or titanium and immersed directly in lipid dispersion. These types of systems are able to transfer very high energies to the system and generally produce smaller vesicles. However, the energy distribution within the sonic solution is heterogeneous. Therefore, the liposomal particle size distribution is less homogeneous, so reproducibility may be low. Overheating may also occur due to high energy input, and accurate temperature control is often difficult. In addition, the subsequent decomposition of the metal tip of the prop under high-pressure ultrasound may lead to an increase in the concentration of metal ions in the system, which may interfere with functional compounds such as enzymes. Bath sonicators are the preferred systems used in liposomes production. Because they have a relatively homogeneous energy distribution in the system, which leads to the formation of liposomes with a homogeneous particle size distribution. In addition, contamination of polar lipid dispersions may be minimized because direct contact between dispersions and props is not required. Temperature control is usually easier in bath sonicators, and the bath may be equipped directly with a heating or cooling unit. Thus high-intensity ultrasound can be a very effective way to produce small amounts of stable SUVs, or the encapsulate efficiency is usually very high using ultrasound. However, three problems may arise in the production process. First, sonication of some polar lipid

dispersions can cause lipid depletion. Second, sonication can change the functional properties of the encapsulated material. In this regard, sonication has been reported to reduce the activity of enzymes incorporated into liposomes. Third, small sonicated vesicles are often semi-stable. Vesicles may grow over time to reduce the high curvature energy due to excessive mixing of the two lipid layers. However, it is recommended that ultrasound-produced liposomes be stored for 24 hours to allow relaxation to reach a more stable size [28].

6.7. Membrane extrusion and homogenization:

In membrane extrusion or homogenization, dispersed phases containing large liposomes are passed through a membrane or filter with a homogeneous distribution of pore size to obtain a homogeneous population of small vesicles. Pressure passing through narrow areas of the membrane or filter creates shear forces that cause the membranes to rupture and rapid re-sealing. Because of this, material trapped inside larger vesicles will leak as it passes through narrow areas during the extrusion process. Therefore, extrusion must be performed in the presence of the material to be encapsulated. Membrane extrusion and homogenization are affected by temperature and external properties such as pore size, pressure applied along the membrane or filter, and flow rate. The rheological behavior of the interfacial region of lipid membranes varies considerably depending on whether the temperature above or below is the liquid-gel crystal phase transition temperature of polar lipids. Membrane extrusion and homogenization often fail at low transfer temperatures. In addition, the size of liposomes decreases with decreasing pore size and increasing flow velocity and pressure. Due to the high pressure applied along the membrane, all filter materials are not suitable and materials such as polycarbonates have been used successfully [28].

6.8. Heating or Mozaffari method:

Another method is the heating and the Mozaffar method, in which liposomes can be produced in the absence of organic solvents using low shear forces. The mentioned method is economical and is able to produce bioactive carriers including liposomes and nanoliposomes and excellent stability during storage using a simple protocol. Another important feature of this method is that it can be adapted from small to industrial scales. The thermal method involves immersing the components of the carrier system for about one hour, followed by heating and stirring at less than 1000 rpm of the active components, and compounds to be encapsulated in the presence of a polyol such as glycerol at a temperature of 40-120 degrees Celsius. The process temperature is based on the properties of liposomal components, the presence or absence of cholesterol, and the properties of the material to be encapsulated [29]. It has been shown that no lipid degradation occurs at this temperature. In addition, the use of heat in this method eliminates the need for further sterilization process and thus reduces the time and cost of liposomes production. Glycerol is also a physiologically acceptable water-soluble chemical and does not need to be removed from the final liposomal product. Glycerol also acts as a diffuser and prevents damage to vesicles, thereby increasing the stability of lipid vesicles. Glycerol also increases the stability of liposomes against freezing, thawing, etc. [29]. They showed that the nanoliposomes prepared by the heating method were completely non-toxic to the cultured cells, while the nanoliposomes prepared by the conventional method using volatile solvents showed significant levels of cytotoxicity. Another method called Mozaffari method has been used by improving the heating method for targeted antimicrobial transmission of nisin. This method allows the production of the carrier system in one step without the need for initial discharge of components and the use of toxic solvents on a small to large scale and industrial scale [54]. Table 1 summarizes the basis of these methods as well as their advantages and disadvantages.

7. Nanoliposome application:

Nanoliposomes are used in genetic engineering as gene carriers, in biology as cell membrane models, and in pharmacy for the following purposes: 1- Protection: Drugs encapsulated in nanoliposomes are protected against host degrading agents. The patient is also protected against the toxic agents of the drug. 2- Slow release: In this case, the release depends on the liposomal membrane permeability and properties and the nature of the constituting lipids. 3- Controlled release: In this case, the release and control of the drug occur as a result of lipid phase change in response to external stimuli such as temperature change or pH. 4 - Targeted drug delivery: In the first case, the liposomes and nanoliposomes normal size or charge cause a tendency to a specific tissue or organ, and in the second case, using antibodies or other ligands, cause drug delivery to a specific group of cells. 5- Drug delivery into the cell: This operation is done by nano-mechanisms or fusion causing the liposome and nanoliposome carrying drug or genetic material to enter the cell membrane [55-58].

Table-1: Liposome and Nanoliposomes Producing Methods (Basis, Advantages, and Disadvantages).

	Producing Method	Method Basis	Advantages	Disadvantages
1	- Sonication - Probe sonication - Water bath sonication - Ultrasonication	Cavitation and disruption of particles due to expansion and contraction of existing gas bubbles	- Monolayer nanoliposomes preparation, Speed and ease of operation, no direct contact of materials with water in the bath, preparation of uniform liposomes, possibility of industrialization	- Final product heating, and unsuitability for heat-sensitive materials, the possibility of contamination of the final product due to separation of the surface layers of the probe, Impossibility of industrialization, expensive equipment
2	Homogenization	Liposomal suspension passes through very small pores to produce nanoliposomes	Speed and ease of operation, repeatability, uniformity of the final product, and the possibility of industrialization	Costly and use of very sensitive tools
3	Extrusion	Passing the proliposemic mixture through a membrane with uniform pores	The uniformity of the final product	High price and long preparation process
4	Microfluidics construction	Collision of two streams of water and lipids with very high pressure	Possibility of industrialization	Loss of encapsulated materials, contamination of materials, damage to the structure of sensitive materials due to the application of high pressures
5	Reverse phase evaporation	Mixing the aqueous phase in the lipid phase dissolved in the organic solvent and then evaporating the organic solvent and leaving the formed liposomes	Speed and ease of operation, high encapsulation percentage, and the possibility of industrialization	Possibility of solvent remaining in the final product
6	Discharge of micelle lipid detergent compound	Production of polar micelles using very small amounts of surfactant	Homogeneity of the prepared nanoliposomes	Time consuming and the possibility of some surfactant remaining in the final product
7	Freeze drying	Application of freezing agents in the liposomal mixture formed	Production of nanoliposomes with a long shelf life	Costly
8	Solvent injection	Replacement of organic solvents with the aqueous medium	Production of relatively homogeneous liposomes and nanoliposomes	Cost-effectiveness, the probability of the solvent remaining in the final product
9	Thin layer dewatering	Add the aqueous phase to the prepared lipid film and vertex it	Possibility of industrialization, relatively good enclosure percentage	Incomplete encapsulation, heterogeneity of particle size distribution, and low volume of liposomes
10	Thermal (Mozaffari method)	Order in the lipid molecules of the liposome membrane due to the use of heat while mixing the components of the liposomes	Homogeneous particle size distribution, no risk of organic solvent remaining in the final product and sterilization of the product	Unsuitable for heat-sensitive materials

8. Using method of nanoliposome:

The use of nanoliposomes as drug carriers has special advantages. Including, they are used for both dermal, respiratory, and injection purposes. When used as an injection (intravenously, subcutaneously, or intramuscularly), they can prevent the drug from breaking down in the body and release the drug over a long period. This reduces the side effects, increases the duration of action, and better the effectiveness of the drug by regulating the rate of free drug delivery into the bloodstream. Nanoliposomes reduce the drug administration numbers and cause greater patient comfort via changing the drugs' tissue distribution and their specific transfer to the desired site [59].

9. Food industry application:

Today, nanoliposomes are widely used in the food industry, especially in the dairy industry. Since biochemical changes in the form of glycolysis, proteolysis, and lipolysis are the main responsible for cheese formation, trapping glycolysis, proteolysis, and lipolysis enzymes inside nanoliposomes, it accelerates the formation. And reducing cheese formation is economically cost-effective [60, 61].

10. Medical application:

Nanoliposomes are used to treat some serious diseases. Among this substance application in the treatment of diseases, the following can be mentioned: 1- Cancer: By attaching anti-cancer antibodies to the surface of drug nanoliposomes, the action of cancer antigen can be stopped. 2- Chemotherapy materials: By placing small proteins molecules, peptides, genetic materials including DNA and ribosomes, in delivering liposomes to the desired location, the spread of chemotherapy materials and their effects on the body can be prevented. 3- Antimicrobials: Nanoliposomes can be used as a drug delivery system to treat bacterial, fungal, viral, and parasitic diseases. 4- Gene therapy: Nanoliposomes are used to transfer plasmids containing specific and desired genes into cells. 5- Vaccines: Today, with the advancement of biotechnology, vaccines are produced based on pure antigens production or surface proteins [62]. Types of nanoliposomal vaccines: A- Bovine albumin serum: which is administered by drug through the nose. B- Rubella vaccine: It is intramuscular that the nanoliposome of this vaccine contains phosphatidylcholine, distilphosphate, and cholesterol in a ratio of 4: 1: 5. C- Influenza virus, D- AIDS virus E- hepatitis, Influenza-based, containing 150 nm spherical nanoliposomes and 70% phosphatidylcholine, 20% phosphatidyl ethanolamine and 10% phospholipid of covered H1N1 [21]. Table 2 shows the nanoliposomal forms of various commercially available drugs.

Table 2: Nanoliposomal form of various drugs that are commercially available [45, 63]

	Drug in nanoliposomal form	**Therapeutic Application**
1	Danurobicin	Treatment of Kaposi sarcomy
2	Doxorubicin	Breast Cancer Treatment
3	Amphotericin	Treatment of fungal infections
4	Sitarabin	Treatment of lymphoid meningitis
5	Weinstein	Treatment of non-Hodgkin's lymphoma
6	Neystatin	Topical treatment of fungal infections
7	Anamaycin	Treatment of doxorubicin resistant tumors

Genetic engineering: Nanoliposomes have been used as efficient carriers in the enclosure of macromolecules, including DNA. Healthy chromosomes have also been inserted into nanoliposomes. PH-sensitive immunoliposomes have been identified as suitable for targeting tumor cells grown in mice [45].

11. Diagnostic Applications:

Radioisotope-containing nanoliposomes are used to scan and diagnose disease in the liver and lymphatic system of the body [63].

12. Hygienic & Cosmetics industries application:

The use of nanoliposomes in anti-aging products is one of the latest advances in cosmetic technology. Nanoliposomes are one of the most suitable carriers for topical drug delivery, which contains phospholipids of

appropriate size. About 10 years ago, Christian Dior launched the first nanoliposome-based anti-aging face cream called Captur. This sample contained nanoliposomes containing thymus gland extract, collagen, and elastin peptides, which were responsible for providing moisture and vitality to skin cells, regenerating new cells, and repairing tissue [45].

13. Nanoliposomes for oral use:

The use of polymerized nanoliposomes, chitosan-coated nanoliposomes, polyethylene glycol, or kangiosides to prevent digestion under acidic conditions of the stomach and intestinal lipase enzyme has been suggested so that the nanoliposomes can be transferred from the gastrointestinal tract to the blood [64].

14. Inhalation use:

By improving the nanoliposomes drying methods, it is possible to prepare inhaled liposomes for drug delivery to the lungs. For example, studies can be performed to prepare inhaled nanoliposomes in the treatment of tuberculosis and asthma [65].

15. For external use:

A special type of nanoliposome, named transphrosome, causes a significant increase in moisture in the transdermal area, or an increase in skin flexibility following the use of nanoliposomes containing peroolide. Nanoliposomes increase the effect of the drug and reduce side effects following topical application. The following can be mentioned: Improved the topical effect of steroidal and non-steroidal anti-inflammatory drugs, progesterone, and local anesthetics in the form of nanoliposomes [66].

16. For subcutaneous injection:

Lymphatic vessels in the injection site absorb the injected nanoliposomes, then these nanoliposomes are phagocytosed by macrophages, and the material carried by them is released. This condition is used for therapeutic, diagnostic purposes in the lymphatic system and vaccination [67].

17. For intravenous injection:

It is possible to remove nanoliposomes from the circulation following intravenous injection by liver and spleen macrophages. In this case, the size of the liposome, the number of layers in the phospholipid bilayers, the strength of the double layer, the charge, and their surface properties are the parameters that affect the fate of liposomes. For example, reducing the size and increasing the strength of the double layer causes the removal of nanoliposomes by macrophages [68, 69].

18. Microfluidic devices for Synthesis of nano-liposome

The behaviour of fluids at the macroscale will different from "microfluidic" behaviour in this factors like physical phenomenon, energy dissipation, and fluidic resistance begin to dominate the system. Microfluidics studies however these behaviour amendment, and the way they will be worked around, or exploited for brand spanking new uses [70-74].

At small scales (channel size of around a hundred nanometers to five hundred micrometers) some fascinating and generally unintuitive properties seem. Especially, the Reynolds number (which compares the result of the momentum of a fluid to the effect of viscosity) will become terribly low. A key consequence is co-flowing fluids don't essentially combine within the traditional sense, as flow becomes laminar instead of turbulent; molecular transport between them should typically be through diffusion [75, 76].

Liposomes are composed of lipid bilayer membranes that encapsulate a liquid volume. A significant challenge within the development of liposomes for drug delivery is that the management of size and size distribution [77]. In standard strategies, lipids are spontaneously assembled into heterogeneous bilayers in a bulk part. Further process by extrusion or sonication is needed to get liposomes with small size and a slender size distribution. Microfluidics is associate rising technology for liposome synthesis, as a result of it allows precise management of the lipid hydration method. Researchers used variety of microfluidic strategies that to synthesis micro/nanosized liposomes with narrower size distribution in a very duplicable manner, that specialize in the use of continuous-flow microfluidics [78-83]. The benefits of liposome formation exploitation the microfluidic approach over traditional bulk-mixing approaches area unit mentioned. Confinement and well-defined mixing in microfluidics makes it attractive for production of liposomes ranging from tens of nanometers to tens of micrometers in diameter. The self-assembly in microfluidics can be controlled by varying liquid flow rates, ratios of cross-flows and the composition and concentration of lipids, resulting in tunable sizes, and narrower size distributions. Recently, several studies on liposome production in microfluidics have been reported [84-91].

19. Conclusion:

Nanoliposomes are used in biology as a model of the cell membrane, in the genetically engineered environment as carriers of genes, and in pharmacy and with their spherical structure to contain and surround drugs. Many compounds, including anticancer drugs and antibiotics, have been used with nanoliposomes. Widespread interest in the use of nanoliposomes as drug carriers is due to the acceptance process of their preparation in the field of pharmacy and drug delivery because they are both scalable and economically justifiable. Nanoliposomes are potential drug carriers for many drugs, such as therapeutic proteins, diagnostic agents, and low molecular weight drugs. When phospholipids are released into the aqueous medium, they form liposomes and nanoliposomes. When phospholipids are placed in the aqueous medium, the interaction of lipids with water results in the formation of monolayer and multilayer vesicle systems. Vesicles consist of simple lipid bilayers that are shaped like spherical biological membranes. There are several types of phospholipids used to make liposomes. Liposomes and nanoliposomes are known as drug-carrying structures or vesicles because of their ability to trap drugs.

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