A COMPREHENSIVE REVIEW ON BILOSOME: A NANO-VESICULAR DRUG DELIVERY SYSTEM TO ENHANCE BIOAVAILABILITY OF THE DRUG THROUGH DIFFERENT ROUTES OF ADMINISTRATION

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Recently, several nano-vesicular delivery systems are developed to resolve the problems associated with many drugs such as liposomes, nanoparticles, and niosomes. Unfortunately, their use is limited by the oral route due to the obstacles that face them in the gastrointestinal tract (GIT) such as pH, bile salts, and metabolic enzymes. Due to these difficulties, bilosomes which are non-ionic surfactant nano-vesicular delivery systems encompassing bile salts in their membrane have represented a recent approach to entrap the drug, protect it from gastric degradation, and increase its bioavailability. The presence of the non-ionic surfactants and bile salts in their compositions affect their properties as they make them more stable chemically and impart elasticity to the vesicle that enables them to squeeze through smaller pores than their diameters and penetrate deeply through the tissue layers, respectively. With the aim to deliver the oral vaccine, biological therapeutics, and traditional small-molecule drugs, bilosomes showed their high ability to protect the entrapped peptides and proteins after oral administration.

In this review, we will clarify the unique properties of bilosomes that make them the best choice as nano-vesicular drug delivery systems, compositions, preparation techniques, and methods of characterizations representing the factors that affect their parameters. Furthermore, this review represents the therapeutic applications of bilosomes by various routes of administration and the effect of changing bilosomal components into their therapeutic efficacy. Finally, information on the future prospections of bilosomes through different routes and their marketing approaches is addressed.

Keywords:*Nano-vesicular drug delivery systems, bilosomes, non-ionic surfactant, preparation techniques, and in vitro characterizations*

INTRODUCTION

Bilosomes, the flexible and ultra-deformable niosomes, are bilayered structures formed by the self-assembly of the hydrated non-ionic surfactant monomers. Being amphiphilic in nature, they can entrap both hydrophilic and lipophilic drugs, which are encapsulated in interior hydrophilic compartments and outer bilayered membranes, respectively[1,2]. Thus, a large number of drugs can be delivered by bilosomes[3]. They are non-ionic surfactant drug delivery systems encompassing bile salts in their bilayer membrane, with or without the inclusion of cholesterol[4]. They were first expressed in the innovative work published by Conacher et al.[5] that investigated the ability of non-ionic surfactant vesicles containing bile salts (in specific sodium deoxycholate) to provoke systemic immune responses after oral administration in mice. The study showed that the presence of bile salts in the developed formulations boosted the entrapment efficiency of the drug. In addition, the first report of non-ionic surfactant drug delivery system was in the seventies and came from the cosmetic applications developed by L'Oréal[3,6,7].

Bilosomes are structurally similar to liposomes, but the critical component in the preparation of bilosomes are the non-ionic surfactants which make them more stable when compared to liposomes. As they overcome the physical instability problems associated with liposomes due to their lipid content: such as susceptibility to oxidation and difficulty in obtaining high purity levels that affect vesicles size, stability, and shape[3,8,9].

Additionally, bilosomes depend on the presence of bile salts in their structure due to the peculiar physiochemical properties that enable them to enhance drug absorption and exert their targeted action[10]. Bile salts act as both solubilizing agents and penetration enhancers due to their membrane-destabilizing properties. Therefore, they enhance the bioavailability of drugs with low aqueous solubility or low membrane permeability, so make them applicable to many routes[11,12]. A schematic representation of the typical bilosomal structure is depicted in **Fig. 1.**

Advantage of Bilosomes
Among the various advantages of the use of bilosomes as a nano-vesicular drug delivery system:
- Preparation: they do not require specific precautions and conditions during the preparation process[13,14].
- Handling: bilosomal components do not require any specific handling procedures and handling problems[8,15].
- Storage: they do not require specific storage conditions due to the presence of non-ionic surfactants[3,14,15].
- Stability: bilosomes are physically and chemically stable due to the presence of non-ionic surfactants[7,8].
- Size: bilosomal size is usually in the nm range that is suitable and well tolerated by various route, unlike liposomes and microparticulate systems[15].
- Versatility: they have the to encapsulate both hydrophilic and lipophilic drugs[13,15].
- Protection: prevent drug metabolism by the metabolic enzymes present at the tear/corneal epithelial surface and in GIT due to the presence of bile salts[3,13].
- Biocompatible, biodegradable, non-toxic, and non-immunogenic as they are made up of non-ionic surfactants[8,14].
- Effects: they offer targeted and sustained effects and release the drug independent of pH, thus resulting in significant enhancement in drug bioavailability[16].
- Patient acceptance: they have better patient compliance[1].
- They overcome challenges associated with conventional nano-vesicular delivery systems and exert a better curative outcome when compared to them[5,17].

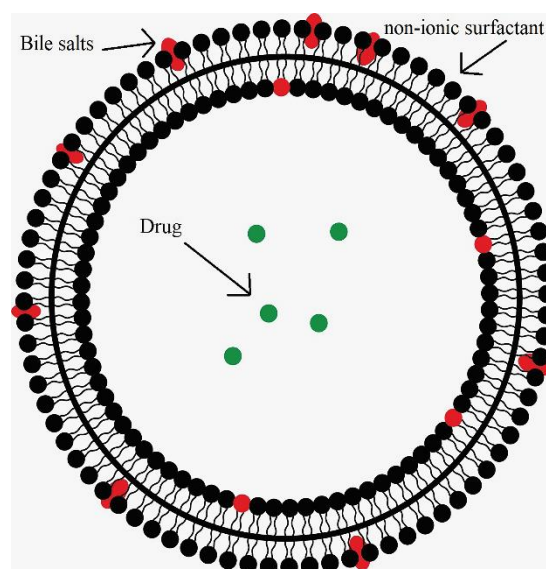Consequently, they are considered as the preferred nano-vesicular drug delivery systems for various route[18].

Fig.1: A schematic representation of the typical bilosomal structure and composition.

Bilosomes Structure and Composition

Bilosomes are amphiphilic in nature and spherical in shape. Being amphiphilic in nature, they can entrap both hydrophilic and lipophilic drugs, which are encapsulated in interior hydrophilic compartment and outer bilayered membranes, respectively[1,2]. Bilosomes consist of a non-ionic surfactant, bile salt, lipophilic materials such as phospholipids and cholesterol, and other additives such as charge inducers and hyaluronic acid, as shown in **Fig. 2**.

Non-ionic Surfactants

The commonly used surface-active agent during the bilosomal fabrication are the non-ionic surfactants due to the advantages concerning their compatibility, stability, and toxicity properties compared to the anionic, cationic, or amphoteric forms. They are safe, with low irritant effect to cellular surfaces, and help to keep the physiological pH in the solution. They have high interfacial activity and act as solubilizing agent, wetting agents, emulsifying agent, and permeability enhancing agent. Furthermore, they are P-glycoprotein inhibitors, so they can boost the absorption of the drugs and enhance the targeting of the drugs to specific sites[7]. The chemical structure of the non-ionic surfactants involves two regions, one of them is water-like (hydrophilic), and the other is lipid-like (hydrophobic). These two regions are connected by ether, amide, or ester bonds[19]. The capability of the vesicles to entrap the drug is mainly influenced by the hydrophilic lipophilic balance (HLB) value of the non-ionic surfactants from which their membranes are created. Also, the entrapment efficiency of the drug is influenced by the chain length and the size of the hydrophilic head group of the non-ionic surfactants[20]. The entrapment efficiency percent reduces as the HLB value of the surfactant declines[21]. The commonly used non-ionic surfactants for bilosomes formation are as follows[7]:

- Polyethylene glycol hexadecyl ether and octadecyl ether (Brij 58- Brij S10).
- Sorbitan fatty acid ester (Spans).
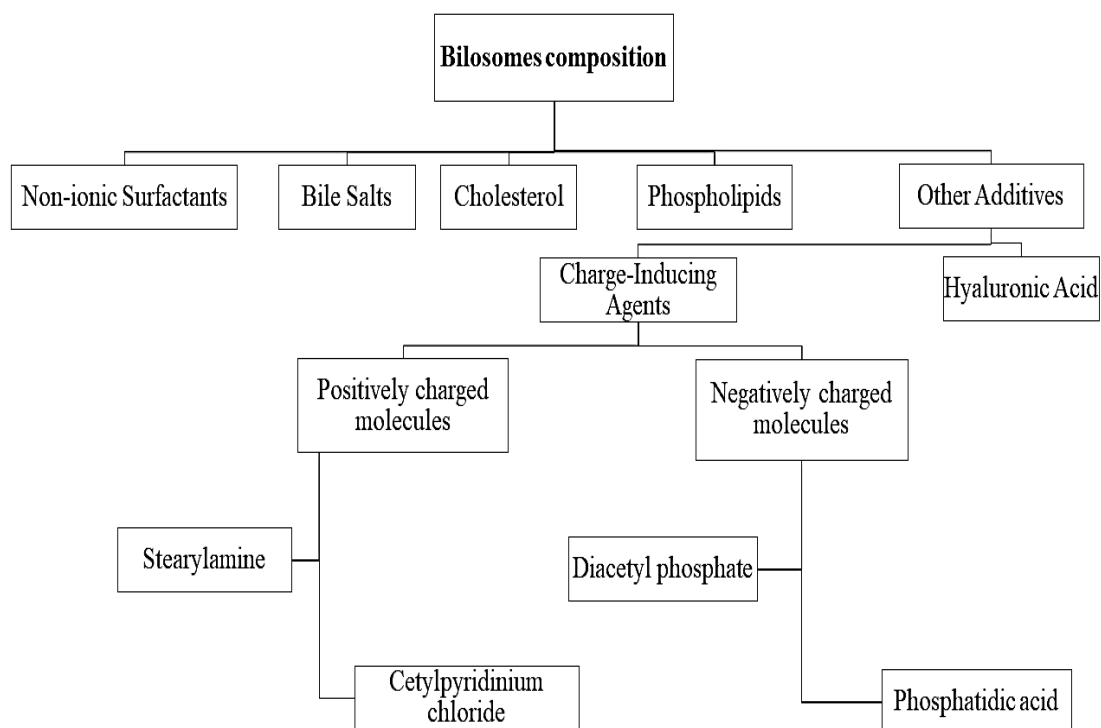- Polyoxyethylene sorbitan fatty acid ester (Tweens).

Fig.2: Composition of bilosomal vesicles .

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Bile Salts

Bile acids are amphiphilic because they possess the hydroxyl group on one side, representing the water-loving side chain, and the methyl group on the other, representing the hydrophobic side chain. Bile salts are interested in the drug delivery fields due to their peculiar physicochemical properties and biocompatibility. They can enhance drug absorption as they act as both solubilizing agents and penetration enhancers due to their membrane-destabilizing properties which make them applicable to many routes[11,12]. Therefore, bile salts may enhance the bioavailability of drugs with low aqueous solubility or low membrane permeability. They play an essential role in the fat's digestion and absorption process as they can form mixed micelles. In addition, Bile acids can incorporate themselves into the phospholipid of biological membranes, which leads to enhanced membrane permeability and the consequent improvement in the absorption of fat-loving drugs through the plasma membrane leading to increased oral bioavailability of many drugs[22]. Bile salts play an important role in enhancing oral bioavailability of the drug in the gastrointestinal tract (GIT), as they resist lysis and deformation by bile salts present in GIT[23]. Also, bile salts have been examined for their ability to enhance eye penetration for β-blocking agents with different polarities. In general, they boosted the permeability rates of the water-loving drugs more effectively than those of hydrophobic drugs without raising the hydration level of the cornea beyond the safety level and without any irritating activity[24]. Additionally, the accessibility and low expense of bile salts make them attractive building blocks for the design of novel systems for many drugs[25]. Examples of bile salts that are utilized in bilosomal fabrications are[9,26]:

- Sodium deoxycholate (SDC).
- Sodium taurocholate (STC).
- Sodium cholate (SC).
- Sodium glycocholate (SGC).
- Sodium taurodeoxycholate (STDC).

Cholesterol

Cholesterol is an amphiphilic molecule when inserted into the bilosomes formulations. The hydrophilic chains orient themselves toward the aqueous surface, while the aliphatic chains orient toward the bilayer membrane. Cholesterol has the ability to interact with non-ionic surfactants. So, it can influence the physical structure and properties of bilosomes[19]. Cholesterol increases the cohesion forces between the non-polar parts of the bilayer, thereby increasing the tightness of the bilosomal bilayer. Also, cholesterol is introduced into bilosomal formulations to impart the rigidity of their bilayer membrane and modify their mechanical strength and water permeability[27]. Furthermore, cholesterol has an impact on the capability of the non-ionic surfactants to form more vesicles and prevents the tendency of aggregates formation of the surfactant to form more vesicles, prevents the tendency of aggregates formation of the surfactant, and enhances the gel liquid transition temperature of the vesicles, which offers higher stability[7]. The permissible percentage of cholesterol is determined mainly according to the HLB value of non-ionic surfactants[28]. Increasing the HLB value of non-ionic surfactants above ten necessitates increasing the added amount of cholesterol to balance higher head groups[7]. Moreover, cholesterol prevents the leakage of the drug from the bilosomal vesicles; by increasing the viscosity of the prepared systems, which results in a high entrapment efficiency percent, high membrane hardness, and good physical stability[29].

Phospholipids

Phospholipids are the main components of the cellular membrane. They are biocompatible with the cellular membrane and have good emulsifying properties. There are a wide variety of phospholipids due to the difference in the polar head groups, non-polar acyl chains, or alcohol moieties. Based on the types of alcohol in phospholipids, there are two types of phospholipids (glycerophospholipids and sphingomyelins). Also, there are different sources of phospholipids, as natural phosphatidylcholine, either from (soybeans or egg yolk), synthetic phosphatidylcholine, or hydrogenated phosphatidylcholine. Phospholipids are essential substances for the maintenance of human life. Besides building the cell membrane, they have a role in the synthesis process of circulating lipoproteins, the main task of which is to carry triglycerides and cholesterols throughout the blood. They have an amphiphilic property that enables them to achieve wetting and emulsification processes. Also, the phospholipid is a main component of vesicle preparation and is responsible for the

hardness of the bilayer[30]. Examples of phospholipids utilized in bilosomal fabrications are[22,30]:

- Di-hexadecyl phosphate.
- L-α-Lecithin from soybean.
- 2-Palmitoylglycerol.
- 1,2-dipalmitoylphosphatidylcholine.

Other Additives
Charge-Inducing Agents

To enhance the stability of bilosomal formulations, charge-inducing agents are incorporated into the bilayer membrane. There are both positively charged molecules: such as stearylamine (SA) and cetylpyridinium chloride, and negatively charged molecules as diacetyl phosphate (DCP) and phosphatidic acid. They hinder the aggregation of developed vesicles[19]. In general, zeta(ζ)-potentials higher than |30| mV are required to ensure the electrostatic stability of the developed systems. So, the addition of charge inducers is necessary because charged vesicles (high ζ-potential) are less likely to accumulate due to the repulsive electrostatic forces between them. This regulation cannot be functionalized exactly for systems involving steric stabilizers. For the reason that the adsorption of these steric stabilizers diminishes the ζ-potential due to swinging in the shear level of the particle. Also, they increase the entrapment efficiency percent of the charged bilosomes and enhance their permeability through the eye and skin[19,31,32]. The effect of using charge-inducing agents on the ocular delivery of acetazolamide was investigated by Hathout et al.[33]. The study showed that the drug's release rate follows an order of negatively charged > neutral > positively charged liposomes, that is the opposite of the drug encapsulating efficiency results. Also, physical stability test showed that approximately 89%, 77%, and 69% of acetazolamide were kept entrapped in the positive, negative, and neutral liposomes, respectively[33]. Hence, positively charged agents (such as SA and cetylpyridinium chloride) are most preferred to enhance ocular drug delivery because it promotes electrostatic interactions with mucin (which is negatively charged), thus boosting the retention to the corneal surface[34].

Hyaluronic Acid (HA)

Hyaluronic acid (HA), a linear, sulfated, anionic glycosaminoglycan consisted of repeating units of disaccharide, is one of the main components of the extracellular matrix and can be gained by extraction from animal tissues such as the rooster comb but also via bacterial fermentation in Streptococci or Bacilli[35]. HA is a well-known mucoadhesive polymer. It is safe, non-immunological, biodegradable, and has the high-water binding capacity, pseudo-plasticity, viscoelasticity, and visual transparency[36]. HA is widely used in the ocular drug delivery systems. As it is one of the natural components of the tear fluid. It is usually positioned in CD44 receptors, located mainly in the epithelium of the cornea and lining[35]. Also, it conducts the spaces between the collagen fibers in the vitreous body, so it stabilizes the collagen fibers and averts their accumulation[37]. Recently HA has been used as a bilosomes coating agent to enhance the bioavailability of hydrophobic drugs in the eye, as stated by Fahmy et al.[38] that have developed a hybrid system that mixes elastosomes with HA for the ocular delivery of voriconazole. This study showed that HA coating increased the interaction of ultradeformable elastosomes with mucin and enhanced the ocular bioavailability of voriconazole.

Furthermore, HA is an amphiphilic molecule; thus, it is standard for transdermal drug delivery as the water-loving domain helps HA to hydrate and permeate through the stratum corneum of the skin, and the hydrophobic domain improves its permeability across the stratum corneum[39].

Different Techniques of Preparation

Bilosomes were fabricated by different techniques. Some of these techniques are suitable for large scales and others are suitable for small scales. A brief overview of some of these techniques is described hereafter.

Injection Technique

The injection technique was first introduced by Deamer et al.[40,41] for the preparation of liposomes. It is characterized by providing a high captured volume per mole of lipid. However, it has disadvantages as it is a slow process, requiring the careful introduction of lipid solution into the aqueous phase, and the entrapment efficiency percent is relatively low. In this technique, the non-ionic surfactants, lipids, and hydrophobic drugs are dissolved in an organic solvent and injected slowly into a n aqueous phase, in which the bile salts are dissolved and preheated above the boiling point

of the organic solvent. Then, the formed dispersion was exposed to continuous agitation to remove the volatile organic solvent. The appearance of turbidity in the hydroalcoholic solution indicates the formation of the bilosomal vesicles. The developed dispersion was left to cool at 25±2ºC then it was subjected to sonication by an ultrasonic water-bath sonicator to obtain a fine dispersion[20,42]. Pegylated bilosomes loaded with resveratrol (antiviral agent for coronavirus management) was prepared by Zakaria et al.[43] via the ethanol injection technique to enhance its solubility and protect it from the extensive metabolism in GIT. The optimum bilosomal formula exhibited a maximum entrapment efficiency percent of 86.1% and a small particle size of 228.9nm. Also, it showed a higher solubility and superior cellular uptake about 4.7-fold compared to the drug suspension.

Thin Film Hydration Technique

The thin film hydration technique was described by Baillie et al.[44] for the preparation of liposomes. This technique is simple and reproducible. Also, it can entrap lipophilic drugs efficiently and produces vesicles with high entrapment efficiency percent. But this method has disadvantages as it is not suitable for hydrophilic drugs, so it produces vesicles with low entrapment efficiency percent. It is a time-consuming technique and produces large multilamellar vesicles that require further size reduction procedures. In this technique, surfactants, lipids, drugs, and cholesterol are dispersed in a suitable organic solvent and evaporated by a reduced pressure to form a thin lipid film. Then an aqueous solution containing the bile salt was added to the film to hydrate it at a temperature determined according to the phase transition temperature of the used surfactants[45]. Next, the dispersion was agitated at a constant rate for a specified period[46]. This produces large multilamellar vesicles that are subjected to homogenization by using a high-pressure homogenizer to transform them into smaller ones[47]. Purification of the bilosomal vesicles is made to get bilosomal vesicles filled with the drug. The process variables to be validated include the rotation speed of the vacuum rotary evaporator, the hydration temperature, and the hydration media. The hydrated media may be water, phosphate buffer, or phosphate buffer saline[22]. Using a thin film hydration technique under reduced pressure, El-Nabarawi et al.[48]

developed pegylated bilosomes loaded with daclatasvir (antiviral agent for hepatitis C treatment) to improve its delivery to the liver. The formed bilosomes showed an entrapment efficiency percent of 95.5%, a small particle size in a nano range(200nm), and the *in vivo* study demonstrated an improvement in the hepatocellular delivery compared with drug suspension.

Reverse-Phase Evaporation Technique

The reverse-phase evaporation technique was represented by Szoka Jr et al.[49] for the preparation of liposomes. This technique gives water-in-oil emulsions. Although this technique has several advantages in terms of being suitable to entrap hydrophilic drugs and macromolecules such as RNA and enzymes effectively, there are some disadvantages, as the exposure of the entrapped materials to organic solvents and sonication during the preparation leads to the denaturation of protein and the breakage of RNA. In this technique, the drugs are dissolved in the aqueous phase while surfactants, cholesterol, and lipids are dispersed in the organic phase. The aqueous phase is added drop by drop to the organic phase. Sonication of the obtained mixture was maintained for 5 minutes(mins) until the complete formation of a water-in-oil emulsion. Next, the emulsion is rota evaporated under reduced pressure to evaporate the organic solvent and form a semi-solid gel that is hydrated with a small quantity of buffer and forms large vesicles[47,50]. The produced vesicles are sonicated to produce unilamellar vesicles that are smaller in size[47,50]. Apigenin-loaded bilosomes was fabricated by Imam et al.[51] for oral drug delivery by a reverse phase evaporation method which showed superior entrapment efficiency percent of 88.1%, improved drug release by 69.37%, and enhanced drug permeation and mucoadhesion properties.

Hot Homogenization Technique

In the hot homogenization technique, lipids are homogenized at temperatures higher than their melting points. Although, this technique suffers from the disadvantages of being temperature-dependent which leads to degradation of the drug, permeation of the entrapped drugs into the aqueous phase during the homogenization process, and difficulty of the crystallization step of the formed emulsions leading to several modifications. This technique can be utilized successfully for the delivery of

macromolecules: as proteins and antigens by including the antigens at the final phase of the hot homogenization process to reduce the prolonged contact with the homogenization effect[52]. The lipid phase of the developed bilosomes is softened at a temperature of 140°C with occasional swirling for 5 mins, then hydrated by the preheated buffer solution that leads to the formation of pre-emulsions. The mixture was homogenized for 2 mins, and the resulting dispersions were named empty bilosomes. Then a preheated bile salt solution at 60°C was putted to freshly prepared empty bilosomes, and the resulted mixture was homogenized. Finally, to load the empty bilosomal vesicles, the buffer solution of the antigen was added to the freshly prepared empty bilosomes. The mixture was homogenized again, and the resulting dispersions were named loaded bilosomes. To avoid the homogenization effect, the antigen is added at the final phase of the hot homogenization process[5]. Mann et al.[53] prepared bilosomes-loaded with influenza A antigen and tetanus toxoid by the hot homogenization technique using monopalmitoyl glycerol, cholesterol, and dicetyl phosphate in a molar ratio of 5:4:1, respectively, with the incorporation of SDC (100mg) and they showed 50% EE%[53,54]. Furthermore, Gebril et al.[55] used the hot homogenization technique to entrap GnRH immunogens inside bilosomal formulation to determine the effect of the mucosal delivery to induce anti-GnRH antibody titers. Also, Wilkhu et al.[9] prepared bilosomes-loaded with the recombinant influenza antigen (rHA or H3N2 subunit protein) by the hot homogenization technique for oral vaccine delivery with 32% EE%.

Detergent Dialysis Technique

The detergent dialysis technique was represented by Weder and Zumbuehi, 1981, for the preparation of liposomes[56]. This technique is suitable to entrap hydrophilic drugs inside the aqueous space of the liposomes. Also, it is suitable for entrapping enzymes, macromolecules, proteins, and oligonucleotides which are sensitive to denaturation in organic solvent and require gentler handling[57]. It produces unilamellar vesicles with large encapsulated volumes and are homogeneous in size. In this technique, lipids and other additives are dissolved by detergent then the resultant solution is subjected to lyophilization. After that hydration by phosphate buffer is done that

produce a clear mixed micellar solution. The obtained solution is then dialyzed through the detergent dialyzer Lipoprep® against phosphate buffer for 22 hr at room temperature to remove the remained detergent. This method produced large multilamellar vesicles of homogeneous size if dialysis of a mixed micelle is occurred from DLPC/cholate. This technique suffers from the disadvantages of the dialysis of the entrapped drugs depending on their three-dimensional size together with the detergent through the membrane and removal of the remaining detergent may require multi-step dialysis which is a time consuming and expensive for industrial scale. Also, after dialysis about 3 to 4 % residual detergent was still exist in the obtained formulation, so that the use of the hazardous solvents and detergents during liposome preparation are not be recommended[58].

High Pressure Homogenization Technique

This technique is also named a single-step liposome preparation technique depend on generating high-velocity collisions to reduce vesicle size[59]. The main advantages of this technique are producing homogenous and small unilamellar vesicles instead of heterogenous and large multilamellar ones[60,61]. Moreover, the preparation procedure occurs under mild conditions, continuous, cheap, eco-friendly, and may be scaled up easily. In this technique, the lipids and aqueous phase are homogenized directly (one-step method) unlike in the preparation method of Bangham et al. 1965[62]. Although, the homogenization technique provides high scalability and huge size reduction but it produces massive volume loss during production procedure which generate barriers for scientists with low materials[57].

There are two methods to prepare samples for high pressure homogenization technique. The first method is the hand shaken method which is utilized to produce dispersion of multilamellar vesicles by mixing lipids with water or normal saline solution (0.9% NaCl). The second one occurred by mixing powdered lipids with water or buffer solution to produce raw lipid dispersion. Then the resulted dispersion is homogenized by high pressure homogenizer[59]. In high-pressure homogenizers, the size of the obtained liposome is readily decreased by its passage through a narrow gap (Gaulin homogenizer type) or micro-channel (Microfluidizer type) without inducing

degradation of the lipids[63]. There are two types of homogenization instruments based on their efficiency: low efficiency instruments which are utilized for non-parenteral liposomal formulations. High efficiency instruments that are utilized to decrease the liposome size and lamellarity[63]. Industrial-scale high-pressure homogenizers are existed and utilized by the food industry (emulsification, microbial load reduction, and cell disruption)[64].

Low Pressure Extrusion technique

This technique includes passing vesicles through series of filters with defined pores size to produce vesicle with smaller particle size with less volume loss than occurred with the homogenization technique. This technique can be utilized to obtain product up to 10 Liter. Filter matrices made from polycarbonate enable to produce filters with uniform pore diameters up to 35 nm with low variation[65]. In this technique the lipids are extruded through a series of filters to produce small and uniform lipid particles. Commercial extruders are already existed,

including the Lipex (Northern Lipids, Burnaby, Canada), Maximator HPE 12.0–100 (CPL Sachse, Berlin, Germany), and LiposoFast (Avestin, Ottawa, Canada)[65-67]. Small scale production of liposomes with a volume of 0.5 L was produced using Lipex extruder, but large-scale production is limited due to the difficulties of controlling the temperature while using large volume products and the capability of the utilized lipids to clogged the filters pores[57].

Parameters for Bilosomal Evaluation and Factors affecting these Parameters

Bilosomal vesicles were assessed by numerous parameters as represented in **Fig. 3.** Generally, the composition of the nano-vesicular drug delivery systems is strictly affecting their therapeutic efficacy. Particularly, in the bilosomal formulations, the selection of surfactant, phospholipid, and stabilizer is a key part of obtaining the optimized parameters and targeting therapeutic efficacy.
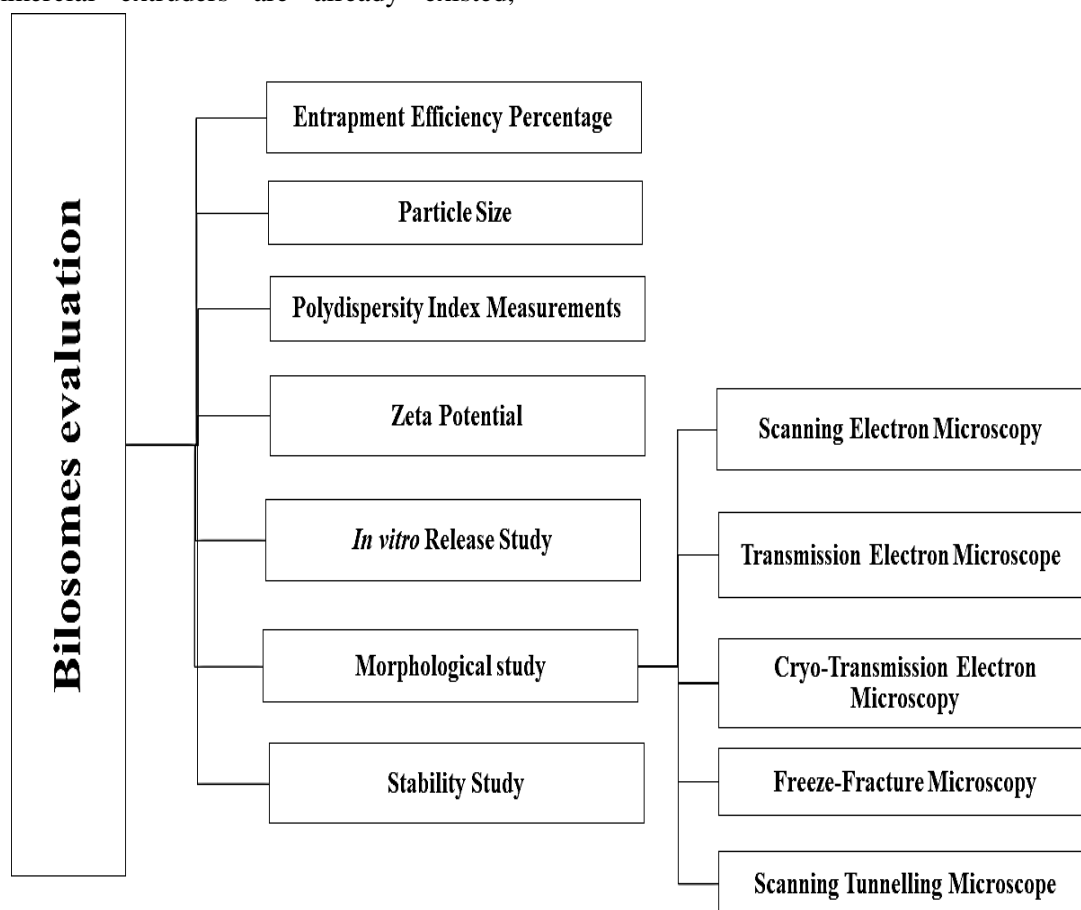

Fig.3: Parameters for bilosomal vesicles assessments .

Entrapment Efficiency Percent (EE%)

The EE% of the bilosomal formulations can be identified as the percent of the drug entrapped throughout the bilosomal vesicles. It can be calculated either by direct or indirect methods. The indirect method is done by subtracting the unentrapped amounts of the drug from the total amount68. The total amount of the drug can be identified by withdrawing a definite volume of the formulation and then analyzed by an appropriate analytical technique. While measurement of the EE% is done by rupturing the precipitated vesicles using an organic solvent then analyzed by an appreciate method of analysis. Examples of these techniques are spectroscopic or chromatographic techniques, such as UV spectrophotometry and High-Performance Liquid Chromatography (HPLC). Also, the unentrapped amounts of the drug can be separated by one of these methods' exhaustive dialysis, gel filtration, and centrifugation[29].

The percentage of the entrapped drug was calculated by indirect method using the following formula[69]:

$$EE\% = \left(\frac{\text{total amount of the drug} - \text{unentraped amount of the drug}}{\text{total amount of the drug}} \right) \times 100$$

(1)

The percentage of the entrapped drug was calculated by direct method using the following formula:

$$EE\% = \left(\frac{\text{entraped amount of the drug}}{\text{total amount of the drug}} \right) \times 100$$

(2)

Many factors can affect the EE% in the bilosome formulations as the HLB and chain length of the surfactant, the concentrations of cholesterol, the concentrations and type of bile salts, charge inducers, and the method of preparation[19].

Regarding the HLB and chain length of the surfactant, the ability of the bilosomal vesicles to entrap the drug is mainly influenced by the HLB value of the non-ionic surfactants from which their membranes are created. EE% increases as the HLB value of the surfactant decreases due to a marked reduction in the hydrophilicity of the developed vesicles[68]. Additionally, the chain length and size of the hydrophilic head group of the non-ionic surfactant affect the EE% of the drug[20]. There is a direct relationship between the chain length of the used surfactants and their ability to entrap the drugs. EE% increases when the surfactant's chain length increases, as the increased chain length is associated with an increase in surfactant hydrophobicity[70].

Also, the concentrations of cholesterol have a positive effect on EE%. Increasing the concentrations of cholesterol leads to an enhancement in EE%. This is due to the ability of cholesterol to increase the hydrophilicity of the developed systems, increase membrane rigidity, and enhance system stability, thereby decreasing drug leaching and prolonging drug retention[71].

Regarding the concentrations of bile salts, there is a negative relationship between the concentrations of bile salts and EE%. Increased concentrations of bile salts resulted in a simultaneous decrease in EE%. This is due to the fluidizing effects of bile salts on the bilayered membrane of the developed vesicles that allow the leakage of entrapped drugs. Also, bile salts can integrate within the lipid membrane, decreasing the rigidity of the membrane, and enhancing the solubility of drugs. But at higher concentrations of bile salts, mixed micelles may form and leading to an increase in the solubility of the drug in the dispersion media and a decrease in EE%.

Also, the type of bile salts affects EE%. The lower the HLB values of the bile salts, the higher the EE %, as decreasing HLB value increases the hydrophobicity of the developed systems. So, bilosomes prepared using sodium cholate (SC) exhibited the highest EE% compared to those prepared from SDC and STC. This is due to the lowest HLB value of SC compared to those of SDC and STC (21.825, 21.925, and 39.175, respectively)[69,72].

Charge-inducing agents have a positive effect on EE%. They induce charges in the vesicle bilayers that increase the inter-space between the successive layers of the vesicle structures and increase both the entrapped volume and EE%.

Furthermore, the method of preparation affects the resulting EE% and particle size. For example, the injection and reverse phase evaporation methods have high entrapping volumes, so they can entrap more drugs and produce vesicles with high EE%. On the other way, the thin film hydration method affects the particle size of the formed vesicles. It forms higher particle size vesicles[29].

Particle Size (PS)

PS is a critical factor in the evaluation process as it is used to determine the physical stability and properties of the bilosomal formulations. Also, *in vitro,* and *in vivo* performances of the bilosomal vesicles are mainly affected by their PS. Photon correlation spectroscopy is used to measure the PS and polydispersity index (PDI) of the formed bilosomal formulations[73]. Photon correlation spectroscopy measures time-dependent variations in the intensity of scattered light. Also, determining the particle diffusion coefficient, known as particle size distribution, can be obtained from these intensity variations using photon correlation spectroscopy.

Additionally, many factors can affect PS in bilosome formulations: the type of surfactants, the concentrations and type of bile salts, the concentrations of cholesterol, and homogenization or ultrasonication conditions that occur during the preparation process[74]. Firstly, there is a direct correlation between EE% and PS of the developed bilosomes. PS increases when the distance between the bilayer membrane increases due to the enclosure of more drugs within[75]. Also, there is a relationship between the PS of bilosomes and surfactant hydrophobicity, in terms of HLB, using surfactants with lower HLB values, producing vesicles with lower EE% and smaller PS. This is due to the surface-active properties of the surfactants that lower surface free energy and decrease interfacial tension, producing vesicles with smaller PS[20,76].

The presence of bile salts leads to the production of sub-micron vesicles, which lead to enhanced drug solubility, drug dissolution rate, and an increase in the bioavailability of drugs[24,77]. Increasing the concentrations of bile salts produce vesicles with a smaller PS. Because bile salts are anionic surfactants decrease interfacial tension and form smaller vesicles. Also, mixed micelles may be formed at higher concentrations of bile salts, which are smaller than the developed vesicles[78].

Regarding the type of bile salts, bilosomes prepared using SC produce vesicles with higher PS than those from SDC and STC. This could be attributed to the higher negative charge of SC that increases the repulsion force between the charged bilayers of the developed vesicles. Thus, increasing the space between them and producing vesicles with higher ZP and PS[69,72].

Also, the concentrations of cholesterol influence the PS of the obtained vesicles, as the increase in cholesterol leads to a decrease in PS. This is due to a marked reduction in the hydrophilicity of the bilayer membrane, which leads to a decrease in both surface free energy and water intake within the formed vesicles that lead to the formation of smaller PS vesicles[27].
The reduction of the PS of the developed vesicles that occur at homogenization or sonication processes leads to a significant decrease in the EE% while enhancing the solubility of the drug and the *in vitro* release profile[79].

Polydispersity Index Measurements (PDI)

PDI is a gauge of the size distribution and the homogeneity and uniformity of the particles. Small values close to zero indicate homogeneous size distribution, and large ones close to one mean heterogeneous size distribution[80]. PDI is measured using the same device utilized in the PS measurement (photon correlation microscopy/Laser Light Scattering /Dynamic Light Scattering)[77,81].

Zeta Potential (ZP)

ZP is a means of the whole charge gained by the vesicles in a specific medium. It is an important parameter that determines the stability of the bilosomal dispersions by studying the electrophoretic mobility in an electrical field using Zetasizer, dynamic light scattering technology, nanoZS-90 Zetasizer, or Malvern Zetasizer instrument[82]. Also, ZP is a marker for physical stability as the reduction in ZP is accompanied by physical stability problems such as aggregation and phase separation[83]. Higher values indicate that the higher repulsive forces between the particles[27].
Furthermore, bilosome compositions may affect the ZP values of obtained formulations: such as the type and concentrations of the surfactant, the presence of bile salts, and the concentrations of cholesterol.

Regarding the type and the concentrations of the surfactant, systems with higher absolute ZP values are obtained using surfactants with a lower chain length and concentrations. This could be attributed to the formation of a denser film of the surfactant on the surface of the formed vesicles that is happed by increasing surfactant concentrations, leading to decreasing the electrophoretic mobility and hence, decreasing ZP values.

Bilosomes usually carry a negative charge for the reason that the existence of bile salts as one of their constituents. This might be due to the presence of 2 OH groups that increase the negative value of ZP and prevents aggregation of vesicles[46,77].

Additionally, increasing cholesterol concentrations leads to a further decrease in ZP values. This is due to reduced fluidity and increased rigidity that cholesterol transfers to the membrane of the formed vesicles. With increased cholesterol concentrations, the arrangement of non-ionic surfactant chains increases, and the stability of the membrane increases[84].

In vitro Release Study

In vitro release studies are performed to predict the attitude of the drug delivery system in the *in vivo* studies. They are carried out using one of these techniques: membrane diffusion technique, dialysis technique, or Franz diffusion cell technique. All these techniques depend on measuring the circumvent of the outflow of developed bilosomal vesicles from the dialysis bags into the external dissolution medium[74,80] But the main disadvantages of these techniques are the failure to mimic the digestion of the drug *in vivo* and the lack of consideration of the likelihood of vesicular interference in physiological environments[85] Many factors can affect the *in vitro* release of bilosome formulations: such as the type and the concentrations of surfactants, the concentrations of bile salts, the concentration of cholesterol, and homogenization or ultrasonication conditions that occur during the preparation process.

The type of surfactant affects the release of the entrapped drugs from the developed formulae in terms of the HLB value and alkyl chain length of the surfactants used. Surfactants with lower HLB values and longer alkyl chains length release drugs slower than those with higher HLB values and shorter chain lengths. This is due to the increase in the hydrophobicity with surfactants having lower HLB values and longer alkyl length. Also, the concentrations of surfactants have a negative effect on the *in vitro* drug release: an increase in the concentrations of surfactants led to an increase in the viscosity of the formed vesicles, which slowed down the release of the drug[27].

Furthermore, there is a relationship between the concentrations of bile salts and both PS and the *in vitro* drug release. At higher concentrations of bile salts: smaller vesicles form that increase surface area leading to a higher dissolution power and a higher drug release. Also, at higher concentrations of bile salts: mixed micelles may be obtained that are smaller in size than the developed vesicles and exhibit faster drug release[27,86].

Moreover, the concentration of cholesterol has a negative effect on the *in vitro* release of the drug. An increase in cholesterol concentrations leads to an increase in the rigidity of the vesicle membrane, which leads to a significant decrease in the release of the drug[87].

Homogenization and ultrasonication processes positively affect the *in vitro* release of the drug from the obtained vesicles. Because they significantly reduce the PS, which leads to a higher surface area and a significant enhancement in both the dissolution power and the *in vitro* release profile of the drugs[79].

Morphological Study

The structure and the morphology of the bilosomal vesicle are commonly characterized using a microscopic approach. Bilosomal vesicle characterization can be performed by numerous electron microscopic techniques: such as Scanning Electron Microscopy, which is utilized for formulations in the solid state, and in which an image is generated by concentrating electron beam scans over a surface. Transmission Electron Microscope, preferentially for bilosomal vesicles in the liquid state[88,89]. Cryo-Transmission Electron Microscopy, in which the structure of the bilosome is maintained very close to its normal state, and bilosomes can be imagined without any staining[90]. Freeze-Fracture Microscopy is applied for bilosomal formulations with large particle sizes[91]. Atomic Force Microscopy and Scanning Tunnelling Microscope are utilized to measure the shape and surface characteristics of the vesicles[29].

Stability Study

A stability study is an important parameter to determine the stability of the developed vesicles. Bilosomal vesicles should be examined for various types of stability: such as physical, chemical, and biological stabilities[19]. The main stability problems associated with the storage of vesicles are aggregation, fusion, and leakage of drugs. So, the stability study for the developed bilosomal formulations is an important parameter[7]. For the formula to be stable, it must

maintain the characterization parameters under various storage conditions during a specified period. The storage conditions for stability study as stated in the International Council for Harmonisation (ICH) guidelines[29]:

- Long term stability study: 25±2ºC/60% ±5% RH or 30±2ºC/ 65%±5% RH.
- Intermediate stability study: 30±2º /65% ±5% RH.
- Accelerated stability study: 40±2ºC/75% ±5% RH.

Applications of Bilosomes

Bilosomes are a versatile drug delivery system. They have been extensively utilized in diverse areas to treat numerous diseases due to their inherent advantages. A brief discussion of the therapeutic applications of bilosomes through different routes are expressed below.

Oral route

The failure of the conventional drug delivery systems (liposomes and niosomes) to resist the solubilizing and enzymatic degradation effect of the bile salts in GIT necessitates the need to develop modified vesicles (bilosomes) that can counteract this effect to be effective for oral vaccines delivery. Bilosomes were initially developed for the oral delivery of antigens such as influenza A virus hemagglutinin, tetanus toxoid, and hepatitis B antigen that demonstrated significant systemic and cellular immunity to oral immunization[92]. They represent hope in oral vaccine delivery as they can stabilize the formed bilosomal vesicles and resist the drastic condition that is presented in GIT and affect drug bioavailability as GIT enzymes, PH, and bile salts. Also, they can stimulate systemic and mucosal immune responses after oral delivery[93]. The presence of bile salts plays an important role in enhancing stability, and hence oral bioavailability of the entrapped drug in GIT, as they resist lysis and deformation by bile salts present in GIT[23].

Examples of the therapeutic applications of bilosomes in the oral route are shown in **Table 1.**

Table 1: Therapeutic Applications of Bilosomes Through Oral Route.

Drug delivered via bilosomes		Route of delivery		
Insulin	Diabetic treatment	Oral Delivery	Prove the safety of the oral administration of insulin.	23
Cyclosporin A	Immunosuppressant	Oral Delivery	More effective and safer for oral delivery.	22
Tacrolimus	Immunosuppressant	Oral Delivery	More efficient and safer in comparison with free drugs.	82
Eprosartan mesylate	High blood pressure treatment	Oral delivery	Showed a nephroprotective effect after oral administration.	94
Apigenin	Anti-inflammatory And anti-carcinogenic effects	Oral Delivery	Enhanced solubility, dissolution, and oral bioavailability.	51
Acyclovir	Antiviral agent	Oral Delivery	Improved absorption and bioavailability of acyclovir through gastrointestinal tract.	17
Risedronate	Postmenopausal osteoporosis treatment	Oral Delivery	Improved stability to digestive media with enhancement in the permeation and reduced drug toxicity more than drug solution.	95

Intranasal route

The intranasal route is an alternative route for oral drug delivery to solve the problems that hinder oral drug bioavailability or to eliminate the GIT side effects. Conventional nano-vesicular systems (liposomes and niosomes) exhibited short duration time in the systemic circulation after intranasal delivery due to the effect of the reticuloendothelial cells that eliminated them rapidly. Modified nano-vesicular systems have been investigated (Bilosomes) that can prolong the circulation time and enhance the drug targeting to the brain.

Examples of the therapeutic applications of bilosomes in the intranasal route are expressed below:

Zolmitriptan-loaded bilosomes were constructed through the thin film hydration technique for intranasal drug delivery that could be used to relieve migraine pain. Zolmitriptan belongs to BCS class III with high solubility and low permeability[96]. The optimal bilosomes were incorporated in in-situ gel consisting of a mixture of poloxamer 407 and hydroxypropyl methylcellulose to enhance brain targeting efficiency and improve brain bioavailability with a sustained release effect of the drug. The formed in-situ gel exhibited an EE% of 73.63% and a PS of 417.56nm. The incorporation of the optimal formula in the gelling system revealed a higher brain bioavailability than the bilosomal dispersion of about (1176.98 vs. 835.77%, respectively) after intranasal administration[97].

Chitosan-coated bilosomes loaded with resveratrol and super magnetic iron oxide nanoparticles for Alzheimer's disease treatment were fabricated to be delivered intranasally. The particle size of the developed bilosomes was 238 and 243nm, and they exhibited a sustained release effect for one day. Then, the chosen formulae were incorporated inside sodium alginate and administrated nasally. The neurobehavior showed an improvement in cognitive and memory functions and a reduction of pro-inflammatory markers levels[98]

Bilosomes-loaded with doxylamine succinate and pyridoxine hydrochloride was formulated by Salem et al.[99] using a thin film hydration technique to be delivered intranasally. They are used for the treatment of nausea and gestational vomiting. Doxylamine succinate and pyridoxine hydrochloride-loaded bilosomes were prepared using sodium cholate (SC) as bile salts, phospholipids, and cholesterol as a stabilizer. The optimum formula was incorporated in situ gel and showed EE% of 58.18%, 41.63%, and accumulative permeated amounts of 347.92 and 195.4μg/cm^2 through one day, respectively. The intranasal delivery study ensured the superiority of the developed bilosomal vesicles by a 2.3-fold and 3.7-fold for Doxylamine succinate and Pyridoxine hydrochloride, respectively, which expressed the significant extension in the mean residence times for both drugs.

Topical routes
Transdermal route

Delivery of the drugs by transdermal route is the best choice to avoid the GIT side effect associated by the oral delivery of many drugs. Also, the failure of the conventional vesicular systems (liposomes and niosomes) to resist the destruction effect of GIT bile salts make bilosomes have the priority as the nano-vesicular systems for transdermal delivery. So, bilosomes have recently been utilized for transdermal drug delivery[25]. Examples of the therapeutic applications of bilosomes in the transdermal route are expressed below:

Bilosomes-loaded with tenoxicam (BCS class II drug) has been formulated for transdermal drug delivery. The percent of tenoxicam entrapped within the developed bilosomes was approximately 68%, the produced PS was about 242.5nm, PDI was equal to 0.387, and ZP was equal to - 41.1mV. Also, the *ex vivo* skin permeation and the *in vivo* skin deposition studies showed that bilosomes loaded with tenoxicam enhanced the penetration of tenoxicam through the skin layers and promoted skin deposition[100].

Aziz et al.[101] used a thin film hydration technique to formulate bilosomes-loaded with diacerein for transdermal delivery. Diacerein is a poorly water-soluble drug with an aqueous solubility of 3.197 mg/L (BCS class II drug). Bilosomes-loaded with diace^{r90n} exhibited excellent transdermal permeability due to the small PS that makes bilosomes permeate deeply through the skin layers instead of depositing on the outer surface of the skin. In addition, the elasticity of the formed bilosomes plays a major role in enhancing skin permeability by enabling the vesicles to squeeze through smaller pores and penetrate through the biological membrane without the risk of vesicle rupture. This is confirmed by the results of the deformability index obtained from the elasticity test for both the developed bilosomes and the conventional

niosomes that ensured the superiority of the developed bilosomes in the elasticity and consistency in the *ex vivo* skin permeability study. A list of other therapeutics applications is shown in **Table 2.**

Ocular Route

The penetration of drugs into the ocular compartments is influenced by both the physical and chemical properties of the drug and the nature of the nano-vesicular system.

Bilosomes extend the existence of the entrapped drug on the corneal surface due to the presence of bile salt in the bilayer membrane of the bilosomal formulations that improves the permeability of the eye for a fat-loving drug and prolongs the duration of action by inhibiting ocular digestion by enzymes in the lachrymal fluid and enhancing residence time. Also, the inclusion of bilosomes into in-situ gel systems increases the ocular residence time and transit time in the cul-de-sac[107]. For this reason, much drug delivery research has used bilosomes to deliver ophthalmic drugs. Specially to deal with diseases that affect the posterior parts of the eye.

The first report on the usage of the non-ionic surfactant nano-vesicular systems as an ocular carrier was reported by Saettone et al.[108] fabricated non-ionic surfactant-based vesicles (niosomes) encapsulated cyclopentolate for ocular delivery. Niosomal vesicles were formulated using equimolar mixtures of polysorbate20 (Tween20) and cholesterol and containing different concentrations of cyclopentolate (0.5 or 1 % w/v) at different pH values (5.5 or 7.4). The formed niosomes enhanced the transcorneal permeation of the entrapped cyclopentolate at pH 5.5, but the reverse effect happened at pH 7.4. In the *in vivo* pharmacodynamic study, the obtained niosomes enhanced the ocular bioavailability of cyclopentolate independent of their pH values. So, niosomes encouraged the transcorneal permeation of cyclopentolate by altering the permeability characteristics of the conjunctival and scleral membranes[108]. Examples of the therapeutic applications of bilosomes in the ocular route are expressed below:

Table 2: Therapeutic Applications of Bilosomes Through Transdermal Route.

Drug delivered via bilosomes	Therapeutics efficacy	Route of delivery	Conclusion	Ref
Olmesartan medoxomil	Anti-hypertension drug	Transdermal Delivery	Exhibited enhanced in skin permeation and higher skin deposition in comparison with the drug suspension.	102
Lornoxicam	Anti-inflammatory drug.	Transdermal Delivery	Superior *in vivo* permeation and showed that bilosomes could enhance the transdermal delivery of lornoxicam.	103
Tizanidine	Skeletal muscle relaxant agent.	Transdermal Delivery	Improved permeation through the skin barrier and thus improved the bioavailability of the drug.	104
Berberine	Rheumatoid arthritis treatment.	Transdermal Delivery	Showed a delayed-release effect and increased in skin permeability. Also, reduce inflammation and exhibited a dramatic reduction in edema swelling.	105
Metformin hydrochloride	Diabetes type II treatment.	Transdermal Delivery	Improved skin permeation and enhanced the bioavailability of the drug.	106

Terconazole-loaded bilosomes was fabricated by Abdelbary et al.[18] for ocular drug delivery that are prepared simply by the ethanol injection technique utilizing SP60 as a surfactant, STC as bile salts, cremophore EL as an edge activator, and cholesterol as a stabilizer[109]. Terconazole belongs to BCS class II drug with low solubility and high permeability. In this study, the bilosomes were constructed utilizing both bile salts and edge activators. So, the obtained bilosomes exhibited a maximum EE% of 95.47%, a small PS of 273.15nm, PDI equal to 0.24, and ZP of -52.75mV. *Ex vivo* corneal permeation study of the optimum bilosomes formula, conventional bilosomes, niosomes, and drug suspension (poorly water-soluble drug) showed that the cumulative amount of terconazole permeated per unit area at the end of the study from the optimum bilosomes formula relative to the conventional bilosomes, niosomes, and drug suspension was 97.49, 61.61, 45.96, and 29.52(μg/cm^2), respectively. This ensured the superiority of the optimum bilosomes to enhance the corneal drug permeation due to the role of bile salts that exert their fluidizing effect on both the bilayer membrane of the vesicles and the corneal lipid layers. Also, the enhancement ratio of the optimum bilosomes was more than 1.5 compared to conventional bilosomes. This could be explained by the presence of cremophore EL that increases the ability of the formed bilosomes to squeeze themselves through the cornea. Cremophore is an edge activator that has a higher tendency to withstand pressure and accumulate at the high-stress surface. This decreases the motion resistance of the formed bilosomes. Also, cremophore EL is hydrophilic, the same nature as the aqueous humor and vitreous of the eye, so it is expected that after the vesicles squeeze through the cornea, they will pass through the aqueous humor into the vitreous compartment in their intact form and exert their effects[110].

Nemr et al.[69] formulated agomelatine-loaded bilosomes (BCS class II drug) by a modified ethanol injection method to enhance the ocular permeability to the posterior segment of the eye and hence enhance the anti-glaucomic effect. The developed bilosomes showed a maximum EE% of 81.8 % and a slower drug release of about 75.14% after 24 hours (h). Also, the *in vivo* pharmacodynamic study showed a maximum reduction in the intraocular pressure

of the eye after 6 h by about 82.68% compared to 35.92% of the drug solution[69].

Acetazolamide-loaded bilosomes was fabricated by Mohsen et al.[111] for ocular drug delivery employing the thin film hydration technique. Acetazolamide (BCS class IV drug) has a slightly aqueous solubility of (0.7mg/ml) and a low permeability coefficient (4.1x10-6cm/s)[112]. The obtained bilosomes showed EE% of 74.24% with a better *in vitro* drug release profile of 97.71% at the end of 8h. Also, in the *in vivo* pharmacodynamic study, the developed bilosomes showed the highest ocular bioavailability, revealing an AUC$_{0-5h}$ value of 105.09% compared to niosomes, marketed dorzolamide eye drops, and marketed oral tablets, respectively. This can be attributed to the penetration-enhancing effects of both the non-ionic surfactant and the bile salts[113]. Bile salts can alter the rheological properties of the mucous membranes of the eye, leading to enhanced drug transport through ocular tissues[114,115].

Alsaidan et al.[116] developed ciprofloxacin-loaded bilosomes using the thin film hydration method for ocular drug delivery. Ciprofloxacin (BCS class IV drug) is a poorly water-soluble drug with 35mg/mL aqueous solubility and a low permeability with a log P value of 0.28[117]. The composition of the optimum bilosomal formula was 35mg of cholesterol, 65mg of SP60, and 20mg of SDC, respectively. It exhibited an EE% of 90.1% and a PS of 182.4nm. Then, it was incorporated into a mucoadhesive in-situ gel system composed of a mixture of carbopol 934P (pH-sensitive polymer) and hydroxyl propyl methyl cellulose (viscosity enhancer) to enhance the ocular residence time and hence boost ocular bioavailability. The formed gel was subjected to *in vitro* characterization studies. Based on *in vitro* characterization results, the in-situ gel system composition was selected. The *ex vivo* corneal permeation study showed that the permeation of ciprofloxacin from the in-situ gel system across the cornea was significantly higher than from the optimum bilosomal formula and pure ciprofloxacin (48.44, 39.24, and 15.69, respectively). Also, the enhancement ratio of the in-situ gel system was 3.08-fold higher than pure ciprofloxacin and 1.16-fold higher than the optimum bilosomal formula. This could be attributed to the bio-adhesive nature of the forming gel agents.

Future Prospections

Depend on the reviewed literature, bilosome vesicles can be utilized for oral vaccine delivery. Conacher et al.[5] demonstrated the effectiveness of oral bilosomes in entrapping antigens and alerting both mucosal and systemic immune responses. Furthermore, they showed the ability of the bilosomal vesicles to protect the entrapped peptides and proteins after oral administration. Although bilosomes as vaccine delivery systems have not been commercialized yet, there are two patents for technology concerning oral vaccines delivery (US 5,876,721 and EP 0722341B1), and patents relating to the delivery of small molecules and biologicals are also being developed[25,118].

So, Superficial modification of bilosomes by attaching ligands on the bilosomal surface reveals their ability to target specific immune cells. Directed research on the selective transfer of antigens to the intestinal lymphatic system, utilizing bilosomes, is the need of the clock. Also, the applicability of bilosomes in presenting a wide variety of antigens with different physical and chemical properties and instability in GIT should be studied. Thus, turning these carriers into attractive building blocks to target new drug carrier systems is a must.

Now, clinical researchers must leverage the knowledge of bilosomes to conduct safe and effective experiments in humans and to uncover the exact immune mechanism of oral administration of bilosomes.

Markets and Applications

Bilosomes have been reported as effective in vaccine delivery (currently under license) and can be applied to deliver biological therapeutics and traditional small-molecule drugs.

Conclusions

This review gives the most recent state of the art of bilosomes as a nano-vesicular drug delivery system. Drug delivery stand facing many obstacles such as: low solubility, poor absorption, poor penetration, hepatic first-pass effect, drug metabolism by metabolic enzymes, and low bioavailability. Bilosomes are becoming an efficient and effective move toward a recent drug delivery because they can be utilized effectively to enhance solubility, membrane permeability, and drug bioavailability. Moreover, bilosomes possess many superior advantages over conventional drug delivery systems as they provide controlled, targeted, and effective drug delivery with the capability to load both water-loving and fat-loving drugs.

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نشرة العلوم الصيدلية
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مراجعة شاملة على البيلوسومات: كنظام لتوصيل الدواء بواسطة الحويصلات النانومتريه لتعزيز التوافر البيولوجي للدواء من خلال طرق توصيل مختلفة

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في الآونة الأخيرة، تم تطوير العديد من أنظمة توصيل النانو الحويصلية لحل المشكلات المرتبطة بالعديد من الأدوية مثل الجسيمات الشحمية والجسيمات النانوية والنيوسومات. لسوء الحظ، يقتصر استخدامها عن طريق الفم بسبب العقبات التي تواجهها في الجهاز الهضمي (GIT) مثل درجة الحموضة والأملاح الصفراوية والإنزيمات الأيضية.

بسبب هذه الصعوبات مثلت البيلوسومات، وهي أنظمة توصيل حويصلية نانومتريه غير أيونية خافضة للتوتر السطحي تشمل الأملاح الصفراوية في غشائها نهجا حديثا لاحتواء الدواء بداخلها وحمايته من التكسير في المعدة وزيادة اتاحته البيولوجية. يؤثر وجود المواد الخافضة للتوتر السطحي غير الأيونية والأملاح الصفراوية في تركيباتها على خصائصها لأنها تجعلها أكثر استقرارا كيميائيا وتضيف مرونة لجدار الحويصلة التي تمكنها من المرور من خلال مسام أصغر من أقطارها والتغلغل بعمق من خلال طبقات الأنسجة، على التوالي. بهدف تقد يم اللقاح الفموي والعلاجات البيولوجية والأدوية التقليدية ذات الجزيئات الصغيرة، أظهرت البيلوسومات قدرتها العالية على حماية الببتيدات والبروتينات التي بداخلها بعد تناوله عن طريق الفم .

في هذه المراجعة، سنوضح الخصائص الفريدة للبيلوسومات التي تجعلها الخيار الأفضل كأنظمة توصيل الأدوية الحويصلية النانومترية، والتركيبات، وتقنيات التحضير، وطرق التوصيف التي تمثل العوامل التي تؤثر على معاييرها. علاوة على ذلك، تمثل هذه المراجعة التطبيقات العلاجية للبيلوسومات بطرق توصيل مختلفة وتأثير تغيير مكونات البيلوسومات إلى فعاليتها العلاجية. أخيرا، يتم تناول المعلومات المتعلقة بالتنقيب المستقبلي عن البيلوسومات من خلال طرق مختلفة ومناهجها التسويقية.