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Mini-Review

Bilosomes As Oral Drug Delivery Carrier

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ABSTRACT

Bilosomes are novel vesicular colloidal carriers that contain bile salts as one of the main components. Recently, bilosomes have gained significant attention as a drug delivery system due to the reported flexibility and stability, relative to other traditional vesicular carriers. As bile salts are natural endogenous surfactants, their presence in bilosomes imparts important characteristics to the vesicles such as biodegradability, compatibility, and minimal toxicity. Bilosomes were investigated for drug delivery through different routes of administration (such as transdermal, ocular, nose to brain) with improved therapeutic efficacy of the entrapped drug. This mini-review attempted to present a brief overview of the application of bilosomes in the oral delivery of drugs. When administered orally, the presence of bile salts in the main structure of bilosomes offers benefits over other vesicular systems. Bile salts provide protection to the vesicles against the effect of endogenous bile salts with improved therapeutic performance of the entrapped active pharmaceutical ingredient(s).

Keywords: Bile salts, vesicles, niosomes, liposomes, protein oral delivery

1. INTRODUCTION

Different vesicular drug delivery systems have been successively evolved to improve the therapeutic performance and stability of many drugs. These systems include liposomes, transferosomes, niosomes ethosomes, cubosomes, and bilosome[1,2]. To justify bilosomal development, knowledge about the main vesicular systems should be briefly discussed. The first vesicular system that was reported to be of great potential for drug delivery was liposomes. Lipoosmes are spherical nano-sized vesicles formed of one or more layers of phospholipids bilayer (also known as lamella) enclosing hydrophilic aqueous core **(Figure 1)**. Liposomes may be composed of one lipid bilayer shell (i.e. unilamella) or many bilayers (i.e. multilamellar) Therefore, they are characterized by the capability of entrapping both lipophilic drugs (in the phospholipid bilayer shell(s)) and hydrophilic drugs (in the aqueous core). Liposomes reflected many advantages such as increased efficiency and therapeutic index of many drugs, and reduced toxicity of the entrapped drug via site-specific active targeting. However, they suffer from many limitations such as, for example, instability problems and high production costs. Additionally, it was found that orally administered liposomes can form aggregates and flocs resulting in a change in size and lysis for the ester linkage under the influence of the intestine bile salts, resulting in chemical instability[3]. Consequently, modification in the liposomal bilayer composition was necessary to minimize these drawbacks and to improve their *in vivo* durability. In the meantime, novel modulated liposomes were evolved containing supporting additives such as, for example, edge activators (i.e. transfersome), ethanol (i.e. ethosomes), or nonionic

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surfactants to improve the loading efficiency and shelf life of the traditional vesicles.

Transferosomes (also called ultra-deformable liposomes) are the modified form of liposomes where the so-called edge activator (such as sodium cholate) is incorporated into the vesicular bilayers. The role of the edge activator was to impart more flexibility to the vesicle rendering the vesicle elastic enough so that it can squeeze itself through the skin layers when applied non-occlusively to the skin. This specific property of high penetration is reported to be due to the influence of hydration gradient[4]. Therefore, their application is restricted to the transdermal route of drug delivery.

Niosomes are spherical vesicles that appeared shortly after liposomes to overcome some of the drawbacks of liposomes such as limited stability and high production cost[5]. The building blocks of the bilayers are nonionic surfactants and cholesterol, instead of phospholipids (Figure 1). Niosomes reserve the capability of encapsulating lipophilic and lipophobic drugs with high efficacy and improved stability. Niosomes became a reliable alternative to liposomes due to the more convenient method of preparation with cheaper raw materials[6], higher chemical stability, lower toxicity, and higher biocompatibility[7].

The recent member of the vesicular nano-system was bile salt-containing liposomes/niosomes. Bilosomes can be considered as the modified form of liposomes/niosomes, as they are prepared to contain bile salts within the lipophilic/nonionic surfactant bilayers[8]. In the bilosomal structure, the bile salts are inserted within the lipid layers forming a unique closed shape. Compared to niosomes and liposomes, bilosomes have been reported to improve the oral bioavailability of many drugs such as acyclovir [9], carvedilol[10], and doxorubicin[11]. When compared to niosomes, the composition of the vesicular wall is the key difference between the two systems **(Figure 1).**

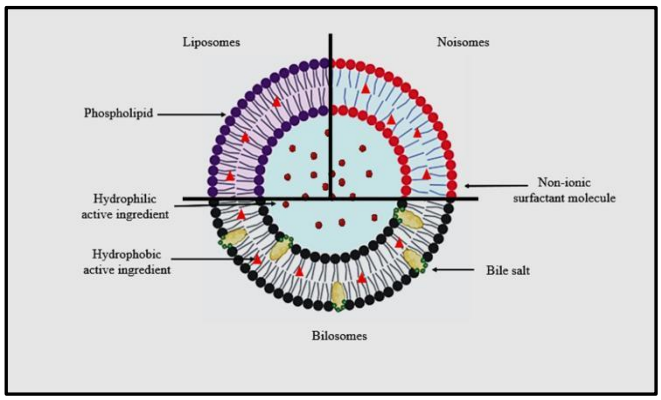

Figure 1. Schematic representation of some common nano-vesicular drug delivery systems namely liposomes, niosomes, and bilososmes.

Niosomes lack the presence of an edge activator (i.e. bile salts) which results in membrane rigidity and destabilization of niosomes that would hinder their tissue penetration[12]. As bilosomes are developed from niosomes, they share the advantages of stability with normal shelf storage conditions and low cost of production. However, as they vary in their structural composition, they are dissimilar regarding gastrointestinal tract stability, chemical durability, and life prospects. These advantages arise from the ability of bilosomes to withstand disruption by physiological bile salts present in the gastrointestinal tract. The main differences between bilosomes and niosomes are collected in **Table 1**[12]

Table 1: Comparative assessment between niosomes and bilosomes[12].

Comparison criteria	Niosomes	Bilosomes
Durability against GIT enzymes	Nondurable	Durable
Durability against pH change	Nondurable	Durable
Gastric irritation	High	Low
Oral absorption	little	High
Drug leakage in GIT	High	Very low
Lymphatic drainage	weak	strong

Bilosomes overcome many of the burdens confronted by other nano-dispersions. **Table 2** illustrates some of the advantages and limitations of bilosomes[13].

Table 2. Advantages and disadvantages of bilosomes[13].

Advantages	Disadvantages
Bilosomes display efficient emulsifying and solubilizing effects and increase the encapsulation efficiency.	Poor correlation between in-vivo and in-vitro profiles and drawbacks in ex-vivo permeation
They are non-invasive drug delivery systems with a wide range of therapeutic activity.	They show poor entrapment for anionic active agent due to the negative charge of bile salts.
Bilosomes show minimal toxicity and high stability.	Some types of bile salts exhibit cytotoxic side effects or cause minor irritation, which makes the choice of integrated bile salts essential.
Bilosomes enhance the bioavailability by enhancing the permeability.	
They can prolong the drug release and extend the duration of action	

2. ROLE OF BILE SALTS IN BILOSOMES PERFORMANCE

The presence of bile salts within the vesicular shell provides bilosomes many benefits over other vesicular nano-systems with especially unique gastrointestinal stability as well as high transdermal permeability. As bile salts are natural endogenous surfactants, they show specific biological compatibility, minimal toxicity, and biodegradability. Bile salts can also enhance transport through biological

membranes by improving drug dissolution and/or altering the permeability through cell membranes[12].

When administered orally, bilosomes provide protection from harsh conditions, such as the acidic environment of the stomach and enzymes, owing to the incorporation of bile salts as the main component in their structures[14]. Furthermore, they offer high stability towards the hostile gastrointestinal tract environment for the entrapped therapeutic agent compared to standard niosomes and liposomes which rapidly break down and release the entrapped drugs before reaching the target cells **(Figure 2).**

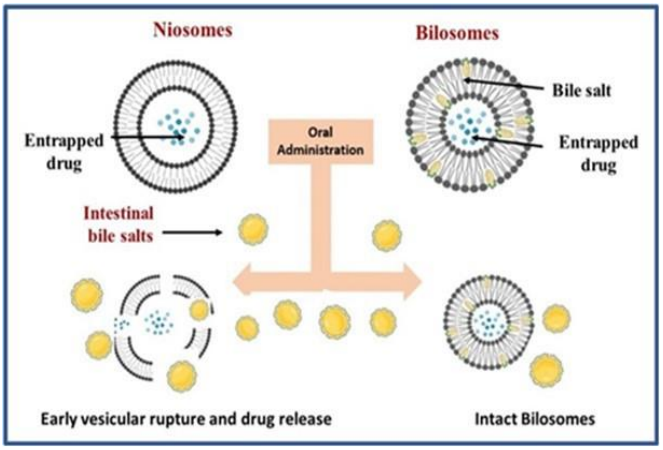

Figure. 2. Illustration of bilosomes strength against intestinal bile salt compared to niosomes in the gastrointestinal tract; rupture and erosion of niosomes contrary to more stability of bilosomes.

It was also claimed that bilosomes can achieve improved absorption via intestinal cells owing to their flexibility, so they are more efficient for the oral delivery of biological macromolecules, vaccines, and antigens than conventional delivery systems[15,16]. In addition, bilosomes show many benefits to be a suitable candidate for application to deliver therapeutic agents through different administration routes, beside the oral one[17,18]. Their efficient solubilizing and emulsifying characteristics enabled them to be suitable permeation enhancers for trans-mucosal delivery.

Regarding stability, bilosomes show good stability at room temperature as well as in a refrigerator mostly due to the relatively high negative charge imparted by bile salts[18,19]]. From the commercial point of view, bilosomes may be preferred over other nanocarrier systems due to accessibility, with easy and economical manufacturing techniques. In addition to high patient compliance. Previous works revealed that bilosomes reflect higher deformability with increased intestinal stability and absorption[11,20].

Based on these properties, bilosomes were exploited in delivering vaccines, drug polysaccharides, antigens, anticancer, and other macro-molecules[9,11,20,22]. Additionally, bilosomes were successively used to encapsulate risedronate with an increased bioavailability proofing the potential of bilosomes to deliver hydrophilic molecules[23].

3. COMPOSITION OF BILOSOMES

The main compositions of bilosomes are phospholipids (in the case of liposomes) or nonionic surfactants (in the case of niosomes), cholesterol, and bile salts. These ingredients are used at different ratios based on the nature of the entrapped drug and the intended route of administration. The structural components are very crucial for the good manufacture of bilosomes with the desired characteristics.

The following section discusses the major components of bilosomes and reflects the significant effect of composition on the properties of the obtained vesicles. The ingredients used in the preparation have GRAS (generally recognized as safe) status and are commercially available.

3.1. Lipids

Lipids are mainly cholesterol or phospholipids, which are the main ingredients for the preparation of vesicular lipid bilayers. Cholesterol and phospholipids are amphiphilic in nature; therefore, they show significant biocompatibility with the cell membrane and good emulsification properties[19]. It is possible to keep vesicular integrity and stability by selecting lipids with the required phase transition temperatures. The cholesterol content controls membrane permeability, aggregation, and fusion processes due to increased membrane rigidity[24]. Additionally, it stabilizes the vesicular membrane against elevated temperature[25].

On the other hand, lecithin (a mixture of phospholipids with hydrophilic-lipophilic balance (HLB) value of about 8.0 in which phosphatidylcholine and phosphatidylethanolamine dominate) may be incorporated in bilosomes to modulate the solubility of the encapsulated drug with other lipidic components to support nanovesicles stabilization[16]. It is important to ensure that the used lipid mix is easily blended at the used concentration range to be used in the formulation. The oil, if present, should be more resistant to chemical degradation (e.g. lipolysis and oxidation). The lipids should be of high purity and free from any toxic residues to achieve an acceptable toxicological profile.

3.2 Bile salts

Physiologically, surface active agents are created in the liver from cholesterol as conjugated bile acids. Their structure improves the aqueous solubility of many compounds[26]. The non-lipoidal characters of bile salts dictate their use in the preparation of traditional dosage forms (e.g. tablets) as well as micellar drug delivery systems. Moreover, bile salts are considered elegant building blocks employed in the preparation of many novel pharmaceutical techniques for the delivery of vaccines and biomolecules cause of simple derivatization procedures[26]. Although bile salts have many benefits, some hydrophobic bile acids, such as glycocholic acid, have adverse effects when produced in high concentrations, leading to hepatotoxicity. On the other hand, it was documented that the effects of blending hydrophobic

and hydrophilic bile salts may display cytoprotective activity (i.e. protecting the cells from harmful agents). Therefore, small structural alterations were carried out to decrease toxicity and maintain absorption-enhancing activity[27].

Importantly, bile salts cause repulsion to gastrointestinal bile thus protecting vaccines or biomolecules against degradation with improved intestinal stability[16]. Moreover, bile salts increase drug transport through biological membranes either by increasing drug dissolution or by increasing penetrability through phospholipid cell membranes. They also destabilize the intestinal membrane, thus improving the absorption of hydrophilic drugs that have poor intestinal permeability[9]. When present at concentrations above their critical micelle concentration in the gastrointestinal tract, bile salts form micelles that can accelerate transcellular absorption[16].

The most employed bile salts in bilosomes preparation are cholates; sodium deoxycholate; sodium glycolate, and sodium taurodeoxycholate[15,28]. Among them, sodium deoxycholate is the most widely used in pharmaceutical research due to its non-toxic effect. It is worth noting that the amount of bile salt used in preparing bilosomes has a significant impact on the amount of entrapped drug. Meaning that high bile salts concentration leads to higher drug solubility in the medium, because of increasing mixed micelles formation, thus decreasing the encapsulation efficiency[29]. Additionally, high bile salt concentration may lead to increased leakage of the drug because of the fluidization of the lipid bilayer of the vesicles[28,30].

3.3. Surfactants

Non-ionic surfactants are widely used in bilosomes preparation due to their reported compatibility with other bilosomal components. Non-ionic surfactants such as Sorbitan fatty acid esters (Span 40, Span 60, and Span 80), Sorbitan mono-oleate, and Sorbitan triestearate are examples of the used surfactants. It was reported that neutral surfactants can improve targeting to a specific tissue with little irritation effect on the cellular surfaces keeping the pH value nearest to the physiological one[31]. The HLB values of the used surfactants must be suitable for bilosomes fabrication to obtain high entrapment efficiency. The higher the HLB value of the surfactant, the higher the entrapment efficiency [12].

4. PREPARATION OF BILOSOMES

The published articles proposed different techniques for the fabrication of bilosomes. The most important techniques are thin-film hydrating, hot homogenization, and reverse-phase evaporation.

In the thin film hydration technique, the lipid ingredients and the drug (if lipophilic) are solubilized in a suitable organic solvent or combination of solvents, which are then vaporized under a vacuum (negative pressure) using a rotary evaporator. The obtained thin film is then hydrated using an aqueous solution of the bile salts and the hydrophilic drug(s), if present. This hydration step is usually aided by sonication and followed by extrusion. In the reverse-phase evaporation process, a W/O emulsion is prepared. The oily phase comprises the lipids, bile salts, and drug (if lipophilic) dissolved in a suitable organic solvent(s). The aqueous phase is then mixed with the oily phase to form a W/O emulsion that is then roto-evaporated to produce a gel-like thin layer. This layer is then hydrated with an aqueous phase to form the final colloidal dispersion. Regarding the hot homogenization technique, the lipid components are usually heated at an elevated temperature (about 140 °C based on the type of lipid used) till complete melting and then moistened with an aqueous phase. The obtained lipid dispersion is homogenized, and bile salt solution is then added while mixing to form the vesicles which are then downsized by homogenization[31,33].

Strathclyde University published a patent about the possibility of using microwave irradiation technique for the mass production of bilosomes. This process is a time-saving technique allowing rapid manufacturing with the preparation of elegant bilosomes with no deterioration in their physicochemical properties. This method alleviates the use of organic solvents avoiding, therefore, its harmful effect on vaccines (WO 2018/011553 A2)[34]. Recently, bilosomes were prepared from their pro-concentrates with promising results[11].

5. APPLICATIONS OF BILOSOMES IN ORAL DRUG DELIVERY

Bilosomes are known to be flexible for improving the oral bioavailability of both hydrophilic drugs (entrapped in the aqueous core) or hydrophobic drugs (impeded in a lipid double layer) or both. Bilosomes were investigated for drug delivery via transdermal, ocular, and nose-to-brain routes of administration with improved therapeutic efficacy of the entrapped drug. However, this review attempted to present a brief overview of the application of bilosomes in the oral delivery of thedrug.

5.1 Oral immunization

Scientists have reported the effectiveness of bilosomes in improving the oral bioavailability and efficiency of vaccines over traditional liposomes and niosomes. They showed promising in-vivo performance owing to the protective effect of bilosomes against gastro-intestinal conditions as illustrated in **Figure 2**[27,35]. Bilosomes were also stated to raise the numbers of Immunoglobulin A plasma cells located in the small intestine increasing, therefore, mucosal and systemic immunity towards the bacterial antigen[18]. Compared with the parenteral form, oral delivery of tetanus toxoid in bilosomes was improved[19]. Bilosomes were used for oral immunization of other types of vaccines such as Diphtheria toxoid that induced higher efficiency for anti-Diphtheria toxoid in the mucosal exudations relative to its intramuscular injection form. Furthermore, the generated serum antibody titers

following oral administration of bilosomal Diphtheria toxoid were 4-fold higher when compared to intramuscular administration, thus avoiding tolerance stimulation[35].

5.2. Improving oral bioavailability of hydrophobic and hydrophilic drugs

In addition to the effect of bilosomes in enhancing oral immunization of different vaccines, many studies reported their enhancing ability to oral bioavailability of water-soluble drugs with improved stability in gastrointestinal conditions. The fluidizing effect, the nano-size range of bilosomes, and their distinctive structure in relation to the presence of bile salts would enhance systemic bioavailability following oral administration and assist in penetration through biological barriers. As mentioned before, bilosomes withstand the harsh effects of physiological acids in the stomach because of the stabilizing action of bile acids. Additionally, they improve absorption by the intestinal epithelial cells and inhibit enzyme activity at absorption sites[37].

The oral delivery of bilosomal carvedilol was compared with plain niosomes. Bilosomes increased drug stability in simulated gastrointestinal conditions, with sustained drug release and enhanced intestinal absorption[10]. The oral bioavailability of bilosomal fenofibrate was significantly higher than that obtained from traditional liposomes and micronized pure drugs [38].

Recently, a combination between bilosomes and penetration/permeation enhancers was investigated for enhancing the oral bioavailability of doxorubicin. Bilosomes encapsulating penetration enhancers enhanced the cytotoxicity and oral absorption of doxorubicin[11].

5.2.1. Delivery of peptides and proteins

Oral delivery of therapeutic proteins and peptides are very attractive means of administration. However, they require great efforts to overcome chemical and physical barriers of the gastrointestinal tract that are responsible for the very low oral bioavailability (about 10%) of these therapeutic agents. Such low oral bioavailability is because of their rapid degradation by the proteolytic enzymes found in the gastrointestinal tract. Another contributing factor for such low bioavailability is that most of these therapeutic proteins and peptides are hydrophilic in nature with very large molecular weights leading to poor permeation through the gastrointestinal membrane[39,40].

Bilosomes were investigated for the oral delivery of peptides and protein for improving therapeutic performance and were compared to the traditional vesicular carrier with promising results. For example, cyclosporine-A-loaded bilosomes were compared with that loaded in conventional liposomes and commercial products (Sandimmune Neoral®) in male Wistar rats. The relative bioavailability of Cyclosporine from bilosomes was higher than liposomes compared with Neoral, most probably due to the facilitated absorption of bilosomes[41].

Oral delivery of insulin in treating Type I diabetes offers many advantages such as more patient compliance, rapid hepatic insulinization, reduced possible hypoglycemia, and weight gain. However, oral delivery of insulin is challenging because of poor oral bioavailability. This is mainly due to enzymatic degradation and lack of transport through the intestinal epithelial wall[42]. Bilosomes were also investigated for the delivery of insulin. Bilosome containing sodium glycocholate was superior to conventional liposomes in rats with more hypoglycemic effect [43].

6. CONCLUSION

The vesicular nanosystem stabilized by bile salts (bilosomes) is a novel carrier in which bile salts are embedded within the lipidic bilayer(s) of niosomes. Bilosomes have many extra advantages over other lipid nanocarriers namely inherent gastrointestinal stability, higher solubility, and permeability. Therefore. enhanced drug bioavailability with improved therapeutic performance is expected. Bilosomes can be prepared to employ different reproducible and applicable methods that can make them suitable for commercialization.

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