REVIEW PAPER

Bile salt-mediated surface-engineered bilosome-nanocarriers for delivering therapeutics

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ABSTRACT

Several formulations have been developed in the current era using liposomes and niosomes as vesicular carriers, which have proven useful in oral drug delivery; nevertheless, their use is limited due to their gastrointestinal environment, including pH, enzymes, and bile salts. To overcome these difficulties, researchers are working on finding ways to improve the efficacy and stability of vesicles. Therefore bilosomes have been developed as promising vesicular carriers with the potential to deliver oral vaccines, parenteral and transdermal targeted drug delivery. In addition to incorporating hydrophilic as well as lipophilic drugs into vesicles, bilosomes are considered one of the most effective methods for enhancing bioavailability and efficacy. Bile acid-based bilosomes are rapidly growing in the current research areas and are expected to provide multiple applications in the pharmaceutical and biomedical fields that will occur in the future with bile salts. This paper briefly introduces the bilosomes of a new generation (structure), their mechanism of action, stability, physicochemical properties, and potential biomedical applications including in oral immunization. Furthermore, surface-engineered bilosomes are more effective than bare bilosomes in various animal models, but clinical trials are needed to assess their safety and efficacy. There is also a need for more research on scaling-up factors for commercializing bilosomal systems.

Keywords: *Bilosomes, Bile salts, Drug delivery, Nanoformulations, Vesicular carrier*

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INTRODUCTION

By employing various structural design techniques in nanotechnology over the past few years, it has been observed that functional carriers can be developed by embedding different molecules into their structure, for example, polymers, proteins, polysaccharides, or nucleic acids [1-4]. These structuring agents can modify main carrier properties, including helping carriers be more stable, making them biodegradable, and decreasing their toxicity. For the diagnosis or treatment of many diseases, functionalized carriers are used as an effective tool [5]. In the current era, liposomes (vesicular carriers) are the mostly used nanocarriers for active substance delivery (Table 1). They are concentric bleeder vesicles formed from phospholipids with hydrophilic (water-loving) heads and hydrophobic (water-fearing) tails, ranging from nanometers to several micrometers in size. Liposomes offer several advantages in drug delivery systems, like high biocompatibility, biodegradability, self-assembly capability, increased efficacy and bioavailability, lower toxicity, and easy removal from the body [6, 7]. Both hydrophilic and hydrophobic agents are encapsulated in this type of nanocarrier (aqueous core and phospholipid bilayers). The physiochemical properties like surface charge and size are modified by vesicular nanostructures [8]. In the agricultural field, cosmetics, food, and pharmaceutical industries, liposomes have been used a lot from the middle of the 20th century onwards. Liposomes are the first nanocarrier approved for clinical trials because of their promising and dynamic drug delivery systems [9].

Lack of physical and chemical stability limited the usage of liposomes in clinical practice. Innovative

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Table 1. Comparison between the various vesicular structures

S. No.	Characterization parameters	Bilosomes	Liposomes	Niosomes
1.	Composition	Charge inducers, lipids, bile salt, and Non-ionic surfactants	Charge inducers, natural phospholipids with cholesterol.	Charge inducers, non-ionic surfactants with cholesterol
2.	Oral bioavailability	High	Low	Low
3.	Chemical stability	Highly stable	Phospholipids undergo oxidative degradation	Stable
4.	Gastric fluid stability	Stable	Unstable	Unstable
5.	Intestinal fluid stability.	Stable	Unstable	Unstable
6.	Required protein and peptide dose	Low	Low	High
7.	Antigen dose	Relatively low	Relatively high	Relatively high
8.	Drug leakage in GIT	Negligible	High	High
9.	Storage condition	Does not require any particular storage condition	Requires particular storage condition (liquid nitrogen storage)	Does not require any particular storage condition
10.	Gastric irritation	Low	Low	High
11.	Entrapment efficiency	Higher than liposomes	Varies depending on the composition	Varies depending on the composition
12.	Size	The smaller size (less than 250 nm)	Size varies (50 nm to several µm)	The larger size (100 1000nm)

Note: These characteristics may vary depending on the composition, preparation method, and application of the bilosomes, liposomes, or niosomes.

structural liposomes incorporating cholesterol, non-ionic surfactants, or ethanol were developed to enhance the drug loading effectiveness and stability of conventional vesicular-type vehicles [10, 11]. Incorporating alcohol may make nanovesicles more pliable and soft because alcohol increases membrane permeability and lipid fluidity [12]. The entrapment efficacy of bioactive compound and drug will improve by 40-45% of ethanol concentration increment, and it also helps to reduce vesicle aggregation because of electrostatic repulsion by liposomal surface modification with a negative charge. Additionally, this alcohol plays a humectant function in increasing penetration capacity into the deep skin layer, and it increases the delivery efficiency of active substances [13]. Non-ionic surfactants with additional cholesterol formed niosomes, the additional single-chain surfactants with second group phospholipids (liposome) formed transferosomes, and the third group of phospholipids (vesicular structure) with high volume ethanol form ethosomes [14].

"Soft" lipid vesicular nanocarriers - bilosomes with bile salts acting as edge activators, such as sodium cholate, glycocholate, sorbitan tristearate, deoxycholate, taurocholate, or deoxycholic acid. When compared to traditional liposomes, colloidal stability was increased by these surfactants. The bioavailability of encapsulated cargo was consequently improved, and absorption of the drug was made more accessible by bilosomes [13]. It is advantageous to administer bilosomes orally since bile salts make the nanocarrier less susceptible to gastrointestinal tract degradation and increase mucous membrane permeability. A greater degree of flexibility of the structure also enhances their transdermal delivery, allowing for greater penetration into the outermost layer of the epidermis and deeper layers of the skin [15]. The present review discusses the structural importance and biomedical applications of bilosomes.

Bile salt integrated delivery system

Size tunable cholate nanocarriers

Nanotechnology based delivery systems are gaining intensive research interest to deliver the payload to the diseased tissues [16-19]. In gene delivery and cancer targeting, bile salt (BS) core copolymers were introduced as a new class of amphiphilic copolymers. Poly[lactic-co-glycolic acid] (PLGA), polyethylene glycol (PEG), dextran, chitosan, and pullulan are examples of hydrophilic linear biodegradable polymers, and hydrophobic core-forming block of polymer is cholic acids [20]. Amphiphilic structures (consists hydrophilic and hydrophobic properties) form nano aggregates in water via interactions between the hydrophobic and hydrophilic portion that decreases interfacial free energy that tunes the size of nanocarriers. Since bile acids are amphiphilic and possess steroidal polycyclic backbones, the resultant nanocarriers possess tunable properties [21].

Bile salt-phospholipid mixed micelles

Due to synergistic interaction, mixed micelles are formed by bile salt in the presence of amphiphilic drugs, conventional surfactants, or polar lipids, with lower critical micellar concentration (CMC) and a higher solubilization capacity than the individual compounds [22]. The poorly water-soluble drugs' bioavailability is improved by using mixed micellar systems, which can prepare without organic solvents. The phospholipids, which are present in the bile, help to stabilize the micelles and promote the formation of a stable emulsion of the lipids. This emulsion allows the lipids to be more easily digested and absorbed by the enterocytes lining the small intestine [23].

Bile acid-based polymer nanocarriers

In comparison to lipid-based carriers such as

liposomes or micelles, polymeric nanoparticles offer several advantages due to their higher cell penetration capacity and smaller size, allowing them to circulate for longer periods and accumulate preferentially at the target site due to their increased permeability and retention effect [24-27]. The bile acids are incorporated into the nanocarrier system and act as targeting moieties to deliver drugs to the liver and the gut specifically. Polymeric materials, such as polyethylene glycol, form the backbone of the nanocarrier and provide stability and biocompatibility [28]. PEGylated bile acids were found to have different solubilization efficiency depending on the nature, number, and length of the grafted PEG chains. Bile acid-polymer nanocarriers can improve the pharmacokinetics of drugs by increasing their solubility, reducing toxicity, and providing targeted delivery to specific organs or cell types [29]. These nanocarriers are being investigated for various therapeutic applications, including cancer therapy, gene therapy, and drug delivery to a gut-liver axis.

Architecture of Bilosomes

Bilosomes are a type of liposome (spherical lipid bilayer vesicles) that are formulated by a bilayer of phospholipids and can be used as drug delivery vehicles. These nanovesicles contain lipids enriched with bile salts and are capable of penetrating biological membranes far more effectively than other nanovesicles [30]. The inner layer of the bilayer acts as a compartment to encapsulate drugs, while the outer layer helps to protect the drug and control its release. These materials have promising applications in the fields of biomedicine and biotechnology, such as tissue engineering and drug delivery, due to their ability to protect and encapsulate therapeutic agents while also offering mechanical stability and biocompatibility, as shown in Table 2 [31].

Table 2. Different nanoformulations of bilosomes for the delivery of drug

S. No	Formulation	Preparation method	Size	Encapsulation efficacy	Applications	Ref.
1.	Lornoxicam (LOX) loaded bilosomes	Thin film hydration method	94.04 ± 6.5 to 292.5 ± 5.3 nm	56.01 ± 3.26 to 82.70 ± 1.80%	It shows a reduction in the amount of inflammatory cells infiltrating with average articular cartilage and average synovial lining. An effective delivery system for osteoarthritis treatment	[34]
2.	Dual laden bilosomes- doxylamine succinate (DAS) and pyridoxine hydrochloride (PDH)	Thin film hydration method	243.23 nm	59.18 and 41.63%,	DAS has a 1.64- and 2.3-fold increased bioavailability, and PDH has a 1.7- and 3.73-fold increase compared to free *in situ* gel and oral solution	[35]
3.	Acyclovir (ACV) loaded bilosomes	Thin film hydration method	121.2 ± 3.21 nm	83.32 ± 5.46%	The relative bioavailability of the bilosomes ($p<0.05$) was 4.36 and 2.5 fold higher than the commercial formulation and ACV suspension	[36]
4.	Eprosartan mesylate-loaded nano-bilosomes	Thin film hydration method	63.88 ± 3.46 nm	61.19 ± 0.88%	It is shown that oral administration reduces the increased expressions of Angiotensin II type 1 receptor, inducible transforming growth factor-β1 and nitric oxide synthase in Wistar rats	[37]
5.	SMV (Simvastatin)-loaded bilosomes	Thin film hydration method	172.1 ± 8.1 nm	89.2 ± 1.8%	Compared to SMV suspension and SMV gel, SMV-BS gel shows 2- and 3-fold greater relative bioavailability. (transdermal delivery)	[38]
6.	BUD (Budesonide)-loaded bilosomes	Thin film hydration method	215.9±5.06 nm	71.96±1.34%	Formulation reduces the levels of pro-inflammatory cytokines like TNF-α and TGF-β, as well as PKC in lung tissue	[39]
7.	Silymarin (SYL)-loaded Dextrose (DEX) modified bilosomes	Thin film hydration method	219.3 ± 2.99 nm	62.32 ± 4.23%	Comparing DEX-SYL-BL with pure SYL and pure SYL-BL in cytotoxicity studies, DEX-SYL-BL reduced the viability of Hep-G2 cells	[40]
8.	Bilosomes-entrapped antibiotics (levofloxacin & doxycycline)	Melt method	2700–3400 nm	>50%	In *B. Pseudomallei* infected mice found that bilosomal levofloxacin prevents weight loss. Levofloxacin treatment depleted mice's microbiomes of all phyla except Firmicutes, while doxycycline treatment had minimal effects	[41]
9.	Berberine (BER) loaded chitosan (CTS) coated bilosomes (BLS)	Thin film hydration method	202.3 nm	83.8%	The BER-CTS-BLS gel significantly reduced rat paw edema swelling to 24.4% after 12 hours compared to other groups	[42]
10.	D-alpha-tocopheryl polyethylene glycol succinate (TPGS) surface-modified bilosomes	Thin film hydration method	187.2 ± 2.2 nm	93.4 ± 5.1%	After 48 hours of incubation with doxorubicin resistant breast cancer (MCF-7/ADR) cell line, TPGS-CUR (curcumin)-BIL demonstrated excellent response in multidrug-resistant (MDR) tumors	[43]
11.	TCZ (terconazole) loaded UBs (ultradeformable bilosomes)	Ethanol injection method	273.15 ± 2.90 nm	95.47 ± 2.57%	The fabricated UBs were demonstrated to be safe *in vivo* ocular tolerance and histopathological studies in albino rabbits	[44]
12.	Piceatannol-loaded bilosomes-zein (PIC-BZ)	Thin film hydration method	157.45 ± 1.62 nm	93.14 ± 2.15%	Compared to pure PIC, PIC-BZ had a greater ability to induce cell death in A549 cells.	[45]
13.	Olmesartan medoxomil (OLM)-loaded PEGylated bilosomes	Thin film hydration method	559.30±10.70 nm	72.49±0.38%	The relative bioavailability of PB15 was found to be 235.04% when measured *in vivo* compared to transethosomes and OLM suspension.	[46]
14.	Tenoxical (TX) loaded bilosome	Thin film hydration method	242.5 ± 6.43 nm	68.33 ± 2.33%	Both *in vivo* and *ex vivo* skin deposition studies established that flourolabeled bilosomes are superior to drug solutions in delivering TX transdermally.	[47]
15.	Tizanidine hydrochloride (TZN)-loaded bilosomes	Thin film hydration method	165.8 nm	82.3%	This formulation showed the highest enhancement of TZN permeation compared to the unformulated drug, showing good stability after three months of storage at -4°C and 40°C.	[48]

The biopolymer matrix of bilosomes can be composed of various natural or synthetic materials, including proteins, polysaccharides, and synthetic polymers. The choice of matrix material can affect the mechanical stability, biodegradability, and release behavior of the bilosome, which can be tailored to meet specific needs. In developing and using bilosomes, there are still challenges to overcome. For example, improving the stability and shelf life of bilosomes, as well as ensuring consistent and controlled drug release, are important areas of ongoing research. Additionally, optimizing the targeting and delivery of bilosomes to specific tissues and cells, and reducing toxicity, remain significant challenges in the field [32]. Another area of active research is the development of new bilosome-based therapies for various diseases and conditions, such as cancer, inflammation, and infectious diseases [33]. It has the capability to encapsulate and deliver various therapeutic agents, such as proteins, small molecules, and nucleic acids, making bilosomes a versatile platform for developing new treatments.

Structural engineering of bilosomes

The structure of bilosomes typically consists of liposomes or niosomes embedded in a biopolymer matrix. The liposomes are spherical structures composed of a lipid bilayer that encapsulates an inner aqueous. The phospholipid bilayer is made up of two layers of phospholipid molecules with hydrophobic (water-repelling) tails facing each other and hydrophilic (water-attracting) heads facing outwards [49]. Among the major differences in bile acid structure are the stereochemistry, hydroxyl groups, number, and position in the cholesterol-derived steroid nucleus. Compared to conventional surfactants and lipids, bile salt possess unique characteristics. In contrast to typical hydrophilic heads and flexible hydrophobic tails (head-tail structure), BS has a planar polar structure instead. The hydrophobic and hydrophilic surfaces of BSs are weakly separated, making the molecules rigid and almost flat. Molecules containing multiple hydroxyl and carboxylic groups on the concave side (hydrophilic surfaces) and hydrophobic surfaces lie on the rigid steroid ring systems of the convex side, as shown in Fig. 1 [50]. The inner compartment is filled with an aqueous solution, typically containing the active ingredient of the bilosome. The phospholipid bilayer acts as a barrier, protecting the inner contents of the bilosome and controlling the release rate of the active ingredient. The lipid bilayer is made up of a double layer of phospholipid molecules, which form a stable and semi-permeable barrier around the inner compartment [51].

The unique structure of bilosomes can be attributed to several key features:

1. Hybrid nature: Bilosomes combine the benefits of both liposomes and biopolymers, resulting in a hybrid material with improved properties. The liposomes provide stability and protection for the encapsulated contents, while the biopolymer

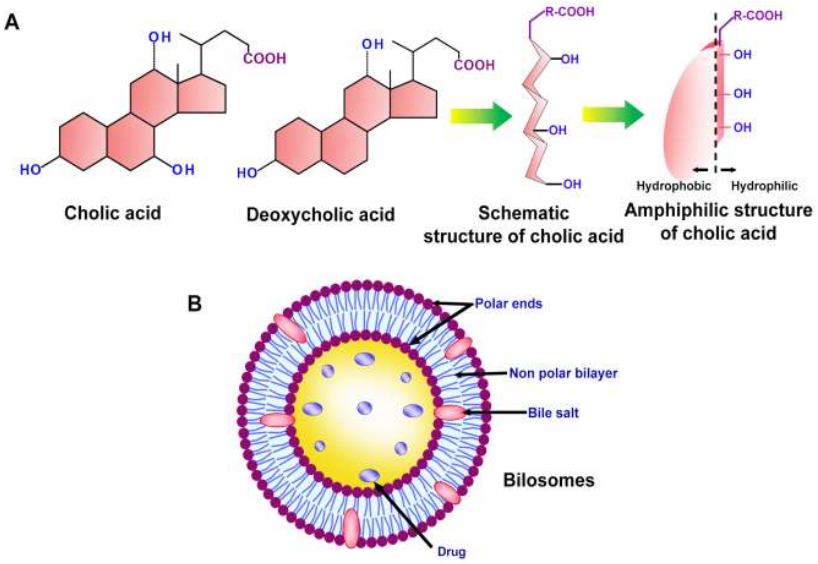

Fig. 1. Composition and the architectural fabrication of bilosomes: A. Structure and schematic representation of amphiphilicity of cholic acid derivatives, B. bilosomes formation

matrix enhances the mechanical stability and biodegradability of the bilosome.

2. Encapsulation ability: The liposomal component of bilosomes can encapsulate a broad range of therapeutic agents, including nucleic acids, proteins, and small molecules. This ability to encapsulate and protect these agents is critical for their effective delivery and use in various applications.

3. Targeting capabilities: The lipid bilayer surface is attached to functional molecules and can be used to target specific tissues or cell types, improving the delivery and efficacy of the bilosome -encapsulated contents [52].

4. Controlled release: The biopolymer matrix can be drafted to allow for controlled delivery of the encapsulated contents, which can be tailored to meet specific needs. This can be achieved through factors such as the composition of the matrix, the size and structure of the bilosome, and the presence of functional molecules.

5. Biocompatibility: The natural or biodegradable components of bilosomes make them biocompatible, reducing the risk of toxicity and improving their safety for use in various applications [53].

6. Multifunctionality: Bilosomes can be functionalized with various molecules, including therapeutic agents, targeting moieties, and other functional materials. This makes bilosomes a versatile platform that can be tailored to meet specific needs in different applications.

7. Improved stability: The biopolymer matrix of bilosomes can help to stabilize and protect the liposomes, which can otherwise be vulnerable to environmental factors such as temperature, pH, and mechanical stress.

8. Versatile synthesis: Bilosomes can be synthesized using various techniques, including physical entrapment, chemical cross-linking, and self-assembly. This versatility allows for the preparation of bilosomes with different structures and properties to meet specific needs.

Overall, the unique structure of bilosomes offers a range of benefits that make them a promising platform for developing new therapies and treatments in the fields of biomedicine and biotechnology. Further research and development in this area are expected to lead to new and innovative applications for bilosomes [52-54].

Physiochemical properties of bilosomes

In order to achieve high therapeutic efficacy, different varieties of phospholipids vesicles are used to deliver drugs for anticancer therapy. The physiochemical properties like surface charge and particle size distribution will help the nanostructures for the effective accumulation of target tissues. The developments of monodisperse colloidal systems with a particular size of nanocarriers are stable and safe [55]. The functional bilosomes has multiple stages of the design process, and their preparation technique influences the properties of phospholipid vesicle. At first, the pluronic/ cholesterol/ phosphatidylcholine mixture organic phase produces a dry lipid film by a solvent evaporation method [13]. Next, multilamellar vesicles are formed by the hydration of an already-formed lipid film using anionic biosurfactant sodium cholate with an aqueous solution [56]. This kind of nanocarrier size is around 500 nm and may be quickly removed or captured from blood circulation by MPS cells. After this process, due to the "stealth" effect of polymer shells, it reduces the immune response and extends the time those nanocarriers are in blood circulation. The poly (ethylene oxide) chains attached to PEO-based nanostructures form highly hydrated polymer brushes on the surface of vesicles [57]. The bilosomes are protected from the MPS cell uptake by the electrostatic and hydrophobic interaction between plasma proteins and vesicles. The targeted delivery efficiency was enhanced by attaching functional groups to PEO-PPO-PEO block copolymers (hydroxyl terminal group). This process reduces active substance adverse effects in the body because it prevents the interactions of normal cells and encapsulated drugs. In the presence of poor aqueous solubility or low membrane permeability, bile salts act as bio-surfactants to provide increased bioavailability of active cargo [58]. For anticancer therapy, sodium cholate-pluronic P123 hybrid nanocarriers are developed as a unique delivery system.

Bile salt had a consequential influence on the size of nanocarriers, with most of them showing lower hydrodynamic diameter values when stabilized with sodium cholate. The lipid bilayer affects the flexibility of the nanocarriers due to the addition of bile salt, and it also decreases the size of liposomes [59]. A higher sodium cholate weight ratio resulted in enlarged vesicles, possibly due to a higher bile salt concentration causing the medium viscosity to increase. The selection of preparation technique will affect the size, but it

does not affect the stability of liposomes.

Formulations using bilosomes

Bilosomes are small, spherical structures that are composed of lipids and proteins. They are formed by self-assembling these molecules and are often used to encapsulate and protect hydrophilic compounds, such as drugs or enzymes, within a hydrophobic environment. Bilosomes can be formed by a diversity of methods, including the use of liposomes or reverse micelles, and can be formulated with specific properties like size, stability, and drug-loading capacity [60]. Once formed, bilosomes can be administered to the body, where they can target specific cells or tissues and release their contents in a controlled manner.

Bilosomes are similar to liposomes but have a different composition. Bilosomes are composed of both lipids and proteins, whereas liposomes are composed of lipids alone. The inclusion of proteins in bilosomes can provide additional functionality to the bilosomes, such as targeted drug delivery, increased stability, and improved pharmacokinetics [61]. The bilosomes can also be formed by using a combination of lipids and proteins in a co-extrusion or co-hydration method [62].

The properties of bilosomes can be tailored to suit specific application by adjusting the composition of the bilosomes. For example, the size of the bilosomes can be adjusted by changing the composition of the lipids and proteins used to form the bilosomes. The stability of the bilosomes can be increased by incorporating certain lipids or proteins that provide additional structural support.

Bilosomes are a class of biomaterials that can be used for targeted drug delivery and are composed of lipids and proteins. They can be formed by various methods, have adjustable properties and have improved pharmacokinetics [63]. In addition to drug delivery, bilosomes have also been investigated for use in various applications, such as gene therapy, imaging, and vaccine delivery. For example, bilosomes have been used to deliver genes to target cells, allowing for the expression of therapeutic proteins within the cell. In imaging, bilosomes can be used to encapsulate contrast agents and target specific cells or tissues [64]. In vaccine delivery, bilosomes can be used to protect and deliver antigens to the immune system, leading to improved immunogenicity and protective response [65].

The use of bilosomes in these applications has shown promising results in preclinical studies, but more research is needed to fully understand their potential as a therapeutic and diagnostic tool. Moreover, the safety and toxicity of bilosomes also need to be evaluated in clinical trials before they can be used in humans.

Non-oral bilosomes

BS-modified liposomes have been found to enhance permeation via other routes due to their elasticity; however, BS content strengthens the wall of oral bilosomes against digestion. To enhance percutaneous drug absorption, multiple researchers investigated transdermal transferosomes [50]. The efficacy of transdermal permeation in pharmaceuticals molecules and cosmetics was improved by bile salt-containing transferosomes (ultra-deformable liposomes) concentrations under 0.2% [66]. In corneal penetration, bilosomes have novel applications that have not been investigated fully so far. According to Dai et al., bilosomes corneal permeation and elaboration have unique ocular delivery for tacrolimus, new immunosuppressive agents were used, and the potency of cycloporin exhibited up to 100 times [67]. Earlier research suggested liposomes containing tacrolimus can facilitate drug penetration through the corneal aqueous humor.

Probilosomes

Probilosomes are dry, free-flowing granules, ingestion of probilosomes results in an instantaneous dispersion of bilosomal components. It has very high permeability and stability because of its dry nature [50]. The drug encapsulation efficacy was increased because of the lipophilic ion pair formation between the drug and bile salt. Harita et al., developed probilosomes vesicles by thin-film hydration technique to improve the bioavailability by preventing the drug from acidic pH of the stomach. *In vitro* studies shows a sustained drug release for up to 8 hours in the pH 6.8 phosphate buffer, and *in vivo* studies show the probilosomal formulation has 2 folds increase in oral bioavailability compared to free drugs [68].

Surface-engineered bilosomes

The bilosomes surface was modified by anchoring suitable ligands for suitable receptors to improve the targeting efficacy and stability of the

drugs. Some examples of surface molecules that can be used to modify bilosomes include antibodies, peptides, and polymers. These molecules can be attached to the surface of bilosomes using multiple methods, such as covalent binding or adsorption [69]. Surface-engineered bilosomes have been shown to improve the delivery of multiple types of drugs, including anticancer agents, gene therapies and vaccines. One of the advantages of surface-engineered bilosomes is their ability to target tissues or specific cells. For example, antibodies can be used to target cancer cells, while peptides can be used to target specific organs or tissues. This allows for more efficient drug delivery and reduces side effects associated with non-specific drug distribution [70].

In addition to targeting, surface-engineered bilosomes can improve drug uptake and release. Polymers such as polyethylene glycol (PEG) can be used to shield the bilosomes from the immune system, which prolongs their circulation time in the body. This can lead to a higher drug concentration at the target site and increased therapeutic efficacy [71]. Silymari-loaded dextrose modified bilosomes show higher stability and perform better than the silymarin-loaded bilosomes and pure silymari [40]. Overall, surface-engineered bilosomes are a promising drug delivery system that has the potential to increase the efficacy and safety of various medical treatments. Ongoing research is focused on optimizing the design and functionality of these systems for various applications.

Stability of bilosomes

A primary goal of stability testing for bilosomes is to determine their stability during storage and in simulated fluids. Stability studies aim to ensure bilosome quality, efficacy, and safety. Testing parameters range from in-process stability tests to stability tests in simulated fluids to determine the stability of developed bilosome formulations in various storage conditions. The chemical stability of bilosomes entrapped with antigens is examined by SDS-PAGE [72]. All studies showed that bands were symmetrically positioned between pure and extracted antigens and additional bands did not appear, confirming that bile salts and the preparation method were sufficient to alter entrapped antigens irreversibly or denature them. The 20 mM concentration of bile salt resulted in niosomes losing all their BSA (Bovine serum albumin) in contrast to bilosomes retaining 85%, thereby confirming the stabilizing part of bile salts in vesicle structure [73].

The storage stability of immunological products at room temperature was also examined in order to investigate the protective effect of the nanobilosomes towards the encapsulated antigens. For 30 days, screw-capped glass bottles were kept at 5 ± 3C and 25 ± 28C at 70% relative humidity to determine their stability over time. When nanobilosomes were stored at room temperature for one month, nearly 94% of the antigen endure, whereas samples stored at refrigerator temperature showed more than 98% of the antigen retained [74]. A significant amount of stability was observed at both temperatures for the nanobilosomes. The stability could be explained by the negative charge induced on the nanobilosomes (charge inducer: DCP), which prevents aggregation and fusion during storage. Because of electrostatic repulsion, a negative charge retards fusion and aggregation [75].

Bilosomes are used to deliver the drug into GIT

Bilosomes are a favourable drug delivery system for delivering drugs to the gastrointestinal tract (GIT). The GIT is a challenging environment for drug delivery due to factors such as low pH, enzymes, and rapid transit times. Bilosomes can overcome these challenges by encapsulating drugs within a phospholipid and bile salt-based vesicle (Fig. 2). Niosomes have been incorporated with bile salts to create bilosomes, one of the most

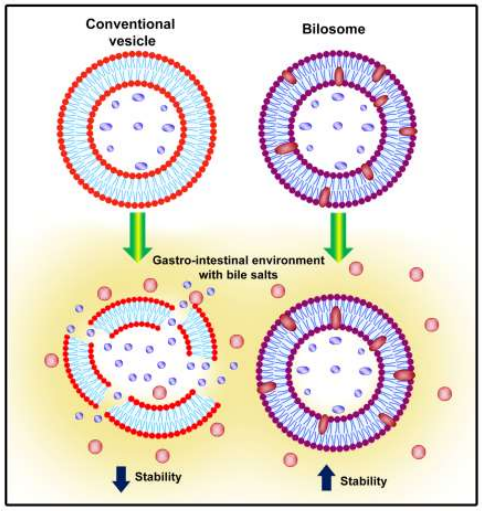

Fig. 2. Schematic representation of bilosomes stability compared to conventional vesicles carrying protein/peptide/drug in the gastrointestinal environment

novel nanocarriers [76]. However, bile salt present in the GIT inhibits the ability of conventional nano vesicular carriers. This leads to the vesicle membrane deforming and lysis, resulting in the initial release of the drug before reaching its target site. Compared to other nano-vesicles, bilosomes are more flexible, ultra-deformable, and elastic. It is possible to protect the drug in the gastrointestinal tract (GIT) from enzymatic degradation using conventional nano vesicular carriers. For intestinal penetration, mostly bile salts are used in pharmaceutical industries to increase the drugs oral bioavailability, like intestinal permeability and low aqueous solubility [36]. Sodium deoxycholate (SDC), sodium glycocholate (SGC), and sodium taurocholate (STC) are bile salts used to increase stability. Among these, bile salt SGC is widely used to improve the permeation effect, exhibits low toxicity, and protease enzyme obstructing potential enhances in the gastrointestinal system [77].

Against pH, gastric gastrointestinal enzymes, and bile contents, bilosomes have protective properties and higher stability when compared with traditional vesicles. When bilosomes are administered orally, they can protect the drugs in the stomach from degradation and enhance their absorption in the small intestine. Bilosomes can also increase the bioavailability of drugs and reduce their side effects by targeting specific sites in the GIT [69]. The uptake of drug by M cells in Peyer's patch was done by maintaining the vesicle in GIT for a long period. In most cases, the bilosomes positive effect on bioavailability is attributed to their gastroprotective properties [50]. In GIT, the oral stability of bilosomes is higher than the BS-deprive nanovesicles. A comparative study was done with niosomes (non-ionic vesicles) and bilosomes for oral immunization potential. The same amount of BSA was entrapped into both vesicles was incubated with 5mM BS solution. BS concentration was increased to 20mM and incubated in both vesicles, where a lot of BSA entrapped initially in niosomes was lost, and in bilosomes, 85% of BSA was retained still in initially loaded protein. This result shows that the impact of external bile content on bilosomes has a higher resistance to gastric degradation [15]. In addition to oral administration, bilosomes have also been investigated for other routes of administration, such as topical and intravenous routes. Bilosomes show an increased delivery of drugs to the skin in topical applications and enhance the circulation time and biodistribution of drugs in the body in intravenous applications [78].

Factors affecting the performance of bilosomes

There are still challenges in delivering therapeutic proteins and peptides orally due to enzymatic and gastric degradation and poor permeation across the intestinal epithelium. According to Niu et al., bile salt used to prepare bilosomes plays an important role in its performance *in vivo*. Bilosomes containing sodium glycholate have been shown to have a higher oral bioavailability of insulin than bilosomes containing sodium deoxycholate or sodium taurocholate bile salts [79]. The performance of any vesicular carrier is affected by its particle size because the size distribution of vesicles plays a major role in facilitated diffusion in the absorption of the particles. Neither very large size of the vesicles nor very small size of vesicles could be effectively absorbed [80]. There is no pharmacological effect and slight oral bioavailability of entrapped insulin when encapsulated in 2000 nm vesicles. Due to increased vesicle flexibility and decreased surface tension, bile salt concentration in the phospholipid bilayer reduces the particle size [81].

The performance of bilosomes can be influenced by a variety of factors. The type and proportion of lipids used in the bilosome formulation can significantly impact their performance. The choice of lipids can affect the size, stability, and surface charge of the bilosomes, which in turn can influence their drug loading capacity, release rate, and uptake by cells. Bilosome stability can be improved by the addition of a surfactant, which can also aid in drug solubilization and enhance drug delivery [82]. However, excessive surfactant concentrations can lead to bilosome destabilization, membrane disruption, and drug leakage. The optimum pH and temperature for bilosome stability depend on the lipid and surfactant composition, as well as the drug being delivered. The storage conditions of bilosomes, such as temperature, light exposure, and humidity, can also impact their stability and performance over time. Proper storage conditions should be maintained to ensure long-term stability and efficacy [83]. Due to the impact of pH and temperature*, in vitro* controlled release of phytochemical/dye from modified bilosomes was studied by Wanglewska and his Team. The stability and physical characteristics of the

double-layered bilosomes were increased by the phospholipid bilayers. The polymer chain present on the surface of the vesicle shows protection against aggregation, confirming the biological stability under different environmental conditions. The double-loaded bilosomes [methylene blue-aqueous core & curcumin-phospholipid bilayer] shows an initial burst release followed by a slower and controlled release and additionally exhibit excellent stability [84].

Conclusion and future prospective

For the last few years, proteins and peptides have been the choice of drug for various diseases because of their lower side effects, specific, and potent. The most preferred route of administration is oral route, but it is very challenging for these biopharmaceuticals due to its poor intestinal membrane penetration and pre-systemic enzyme degradation. There are growing applications for vesicular delivery systems in the pharmaceutical, cosmetic, and food industries. Bilosomes is a novel colloidal carrier, similar to niosomes, but their vesicular lipid bilayer membrane was encapsulated with bile salts. It is stable and more efficient in intestinal bile salt in the presence of pancreatic lipase and gastric acid. Bilosomes encapsulate peptides and proteins that enhance intestinal lymphatic circulation for improved delivery, which is currently being investigated for use as a method of giving antigens orally for immunization. Bilosomes may able to traverse tight junctions without the need for transport carriers or other auxiliary components to carry large molecular weight peptides and proteins. The unique structure of bilosomes, smaller size (100 – 200nm), and high encapsulation ensure high oral bioavailability for biologicals and do not pose any problem in transporting drugs across the bilayer of lipids.

The method of preparing and sterilizing bilosomes preparations remains a concern. The most common method of obtaining parenteral liposomes is aseptic manufacturing and filtration, but the procedures involve time-consuming procedures, and the equipment is extremely expensive and difficult to maintain. In addition to preventing the scaling-up of liposomal formulation production, this creates an additional cost barrier for such preparations. Therefore, there is still a need for an efficient and cost-effective sterilization technique for preparations, one that maintains physicochemical stability and encapsulated content.

Despite the fact that bilosomes increase the bioavailability of drugs, they also enhance their efficacy as well as their ability to encapsulate peptides, proteins, and antigens, according to the reviewed literature. Immunologists are facing a significant challenge in developing an impressive oral delivery system for mucosal vaccines. For this reason, various lipid-based delivery systems, including bilosomes, are developed and studied for oral vaccination.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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