BILOSOMES: NEXT GENERATION VESICULAR NANOCARRIERS FOR TARGETED AND ENHANCED DRUG DELIVERY

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ABSTRCT

Bilosomes are novel vesicular nanocarriers engineered by incorporating bile salts into phospholipid-based bilayers, offering enhanced drug delivery especially for orally and topically administered therapeutics. These nanocarriers exhibit superior stability, improved drug permeability, and increased systemic bioavailability compared to conventional systems such as liposomes. Their resistance to enzymatic degradation and capability to traverse biological barriers make bilosomes highly promising for the delivery of peptides, proteins, vaccines, and poorly water-soluble drugs. This systematic review summarizes bilosome composition, preparation techniques, benefits, challenges, and therapeutic applications with critical insights on future potential. Bilosomes represent a robust strategy to overcome conventional delivery limitations, particularly for mucosal and oral administration, and are poised for broader clinical translation with ongoing technological improvements.

KEYWORDS

Bilosomes, Vesicular Nanocarrier, Bile Salts, Oral Drug Delivery, Nanotechnology, Vaccine Delivery, Bioavailability

BACKGROUND

Conventional drug delivery systems frequently face critical limitations such as poor aqueous solubility, limited permeability across biological barriers, rapid enzymatic degradation, and low bioavailability—especially for macromolecules, peptides, and poorly water-soluble drugs.

These barriers often necessitate high dosing frequencies or invasive administration routes, which may lead to poor patient compliance and suboptimal therapeutic outcomes.[1]

In recent years, nanotechnology-based delivery systems have garnered significant attention for their ability to overcome these challenges. Among them, bilosomes have emerged as a promising class of non-invasive, lipid-based vesicular carriers designed to enhance drug stability, protect sensitive therapeutics, and improve absorption, particularly via oral and mucosal routes. [2]

Bilosomes are structurally similar to liposomes but are uniquely characterized by the incorporation of bile salts—amphiphilic molecules naturally present in the gastrointestinal tract—into the phospholipid bilayer.[3] The presence of bile salts not only enhances the structural stability of the vesicles but also confers resistance to bile-mediated and enzymatic degradation, thereby improving the survival of the vesicles in hostile biological environments such as the gastrointestinal (GI) tract. Furthermore, bile salts facilitate paracellular and transcellular absorption by modulating tight junctions and interacting with membrane transporters, making bilosomes especially suitable for the delivery of peptides, proteins, vaccines, and poorly soluble drugs. [4]

The existing literature highlights the successful application of bilosomes in various delivery routes, including oral, transdermal, intranasal, and ocular. Numerous in vitro and in vivo studies have demonstrated improved pharmacokinetics, enhanced mucosal immunity (in the case of vaccines), and better therapeutic efficacy compared to conventional delivery systems.[5] Bilosomes have also been adapted for ligand-targeted drug delivery, stimuli-responsive release, and theranostic applications, reflecting their versatility and potential for clinical translation.

However, despite their advantages, challenges remain in terms of long-term stability, cytotoxicity at higher bile salt concentrations, standardization of manufacturing processes, and

limited clinical validation. Regulatory hurdles and a lack of standardized characterization protocols also hinder their widespread adoption in pharmaceutical product development.[6]

This review aims to provide a comprehensive and critical overview of the recent advances in bilosomal drug delivery systems. It discusses their structural design, formulation strategies, pharmaceutical advantages, and diverse applications, while also addressing current limitations, regulatory considerations, and future research directions. This review compiles evidence from current research to emphasize the potential of bilosomes as advanced nanocarriers for both treatment and preventive healthcare applications.

1. STRUCTURE AND COMPOSITION

Bilosomes are advanced lipid-based vesicular systems specifically engineered to improve drug stability and bioavailability, particularly for oral and mucosal routes of administration. Structurally, bilosomes are similar to traditional liposomes but are uniquely distinguished by the incorporation of bile salts, which play a pivotal role in enhancing vesicular integrity and membrane permeability.

1.1 Phospholipid Bilayer

The foundational framework of bilosomes is composed of phospholipid bilayers, typically using natural or synthetic phospholipids such as phosphatidylcholine or phosphatidylethanola mine. These lipids self-assemble in aqueous environments to form bilayered vesicles, encapsulating an aqueous core. The amphiphilic nature of phospholipids enables them to entrap hydrophilic drugs in the inner aqueous compartment, while lipophilic drugs are solubilized within the hydrophobic regions of the bilayer [7].

1.2 Bile Salts

A defining feature of bilosomes is the incorporation of bile salts, such as sodium deoxycholate, sodium cholate, or taurocholate. These amphiphilic compounds embed within the lipid bilayer, enhancing its flexibility, elasticity, and resistance to enzymatic degradation. Bile salts stabilize the vesicle against the harsh conditions of the gastrointestinal tract and facilitate paracellular and transcellular absorption across the intestinal mucosa by modulating tight junctions and interacting with membrane transporters [8,9].

1.3 Cholesterol

Cholesterol is commonly included in bilosomal formulations to modulate membrane fluidity and mechanical strength. It intercalates between phospholipid molecules, reducing membrane permeability and enhancing vesicle rigidity, which in turn improves entrapment efficiency, drug retention, and long-term physical stability. The cholesterol-to-phospholipid ratio is critical and can influence vesicle size, polydispersity, and drug release kinetics [10].

1.4 Aqueous Core and Drug Localization

Bilosomes possess a central aqueous core, which allows for the encapsulation of water-soluble drugs, peptides, proteins, or vaccines. Conversely, lipophilic drugs are embedded within the phospholipid bilayer. The presence of both aqueous and lipid regions allows bilosomes to efficiently encapsulate diverse pharmaceutical compounds, enhancing their suitability for various drug delivery applications. The drug’s physicochemical properties largely determine its partitioning and localization within the vesicle [11].

1.5 Optional Components and Surface Modifiers

To further enhance bilosome performance, additional components such as surface stabilizers (e.g., Tween 80 or Span 60), polymeric coatings (e.g., chitosan for mucoadhesion), or targeting ligands (e.g., folic acid, transferrin) may be included. These modifications can improve vesicle stability, circulation time, site-specificity, and mucosal adherence, thereby broadening the application of bilosomes across multiple routes and disease targets [12].

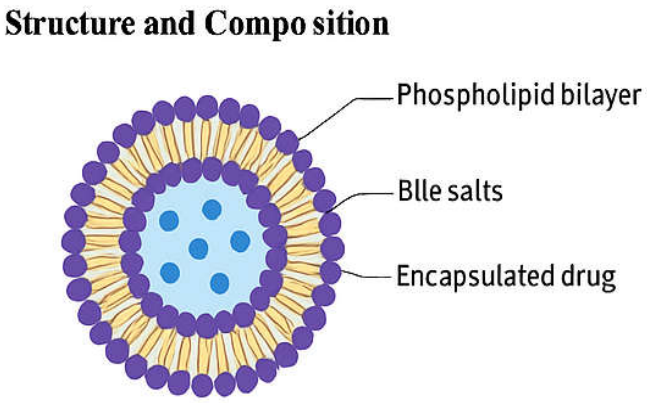

Fig 1: Structure and composition of Bilosomes

2. FORMULATION STRATEGIES

Bilosomes are versatile vesicular systems that can be formulated through various techniques, each offering specific advantages depending on the physicochemical properties of the drug and the intended application. The selection of the preparation method is crucial, as it influences vesicle size, entrapment efficiency, stability, and drug release kinetics.

2.1 Thin-Film Hydration Method: This conventional and widely adopted approach involves dissolving lipids and bile salts in an organic solvent, typically a chloroform-methanol mixture. The solvent is evaporated under reduced pressure to form a thin lipid film on the wall of a round-bottom flask. The film is then hydrated with an aqueous phase containing the drug under controlled temperature and agitation. The resulting bilosomes are often further sonicated or extruded to achieve uniform vesicle size distribution. This method is particularly suitable for both hydrophilic and lipophilic drugs and offers high reproducibility.[13,14]

2.2 Reverse Phase Evaporation Technique (REV): In this method, an emulsion is formed by sonication of a mixture of organic and aqueous phases, where the drug is usually dissolved in the aqueous phase. Upon evaporation of the organic solvent, bilayered vesicles are formed. This method is advantageous for encapsulating large volumes of aqueous drug solutions, thereby improving the loading efficiency of

hydrophilic drugs. The use of bile salts in this method helps stabilize the vesicles and enhances membrane fluidity.[15]

2.3 Ethanol Injection Method: This relatively simple technique involves the rapid injection of an ethanolic solution of lipids and bile salts into an aqueous phase under constant stirring. The immediate diffusion of ethanol into water leads to the spontaneous formation of nanosized bilosomes. This method does not require high energy input and is effective in reducing vesicle size without the need for further size reduction steps, making it suitable for thermolabile drugs.[16]

2.4 Microfluidization and High-Pressure Homogenization: These advanced techniques are suitable for scale-up and industrial production. In microfluidization, two fluid streams are forced through microchannels at high velocity, resulting in particle size reduction through shear forces and cavitation. High-pressure homogenization, on the other hand, passes the bilosomal dispersion through a narrow valve under high pressure, achieving uniform vesicle size. Both methods produce bilosomes with improved homogeneity, enhanced stability, and better scalability for commercial manufacturing.[17]

Several **critical formulation parameters** influence the physicochemical characteristics and therapeutic performance of bilosomes:

- ***Bile Salt Concentration:*** Bile salts such as sodium deoxycholate and sodium taurocholate play a pivotal role in stabilizing the vesicular membrane, enhancing permeability, and protecting vesicles from enzymatic degradation. However, excessive bile salt content may lead to vesicle disruption or drug leakage.[18]
- ***Type and Ratio of Lipids:*** The choice of phospholipids (e.g., phosphatidylcholine, cholesterol) and their ratios significantly affects the vesicle's rigidity, drug

encapsulation, and release profile. Cholesterol is often included to modulate membrane fluidity and prevent leakage.[19]

- ***Hydration Conditions:*** Factors such as hydration temperature, duration, and the ionic strength of the hydration medium influence vesicle formation. Proper hydration ensures complete dispersion of the lipid film and uniform vesicle formation.[20]
- ***Drug Properties:*** The solubility, polarity, and molecular weight of the drug influence its localization within the vesicle (aqueous core vs. lipid bilayer) and affect entrapment efficiency. Drug-lipid interactions can also alter the morphology and stability of the bilosomes.[21]

By optimizing these formulation variables and preparation methods, bilosomes can be tailored to enhance drug delivery through oral, topical, or parenteral routes.

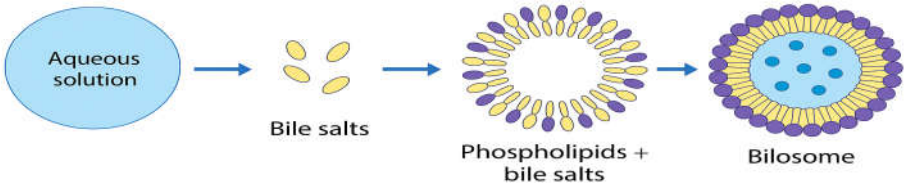

Fig 2: Formation of Bilosomes

3. ADVANTAGES

Bilosomes represent a next-generation vesicular delivery system with several pharmaceutical and therapeutic advantages over conventional liposomes and other nanocarriers. Their unique composition—phospholipids integrated with bile salts—confers distinctive properties that improve drug stability, absorption, and therapeutic effectiveness across multiple routes of administration.

3.1 Enzymatic and Gastrointestinal Stability

One of the most significant advantages of bilosomes is their ability to withstand enzymatic degradation in the harsh environment of the gastrointestinal (GI) tract. The incorporation of

bile salts such as sodium deoxycholate or sodium taurocholate enhances the stability of the lipid bilayer, thereby protecting encapsulated drugs from hydrolytic enzymes, bile acids, and pH fluctuations in the stomach and intestines [22]. This property is especially beneficial for the oral delivery of proteins, peptides, and vaccines that are otherwise rapidly degraded in the GI tract.

3.2 Enhanced Oral Bioavailability

Bilosomes significantly improve the oral bioavailability of both hydrophilic and lipophilic drugs by promoting absorption through the intestinal mucosa. Bile salts act as permeation enhancers, facilitating transcellular and paracellular transport. Moreover, they may modulate P-glycoprotein and efflux transporter activity, leading to increased drug retention and absorption [23]. Additionally, bilosomes can enable partial circumvention of hepatic first-pass metabolism, which is a major barrier to the bioavailability of many orally administered drugs.

3.3 Versatile Drug Encapsulation Capability

Due to their amphiphilic nature, bilosomes can efficiently encapsulate a broad range of therapeutic agents, including hydrophilic drugs in their aqueous core and lipophilic drugs within the lipid bilayer. This dual capability allows bilosomes to be employed for the delivery of small molecules, macromolecules (like peptides and proteins), genetic material (DNA/RNA), and vaccines [24]. Furthermore, their composition can be tailored to achieve controlled or targeted drug release.

3.4 Multimodal Routes of Administration

Bilosomes are highly versatile in terms of administration routes. In addition to oral delivery, they have demonstrated efficacy in transdermal, intranasal, ocular, pulmonary, and parenteral delivery systems. Their deformability and bile salt-induced membrane fluidity facilitate mucosal penetration and transdermal absorption [25]. For ocular and nasal routes, their

mucoadhesive nature and small size support prolonged retention and improved bioavailability at the target site.

3.5 Biocompatibility and Safety Profile

Bilosomes are composed of naturally occurring, non-toxic, and biodegradable materials such as phospholipids and bile salts. These components are generally recognized as safe (GRAS) and compatible with physiological environments, reducing the risk of irritation or toxicity upon administration. Their well-tolerated nature makes them suitable for chronic administration or use in paediatric and geriatric populations. [26]

3.6 Immunological Adjuvanticity

Bilosomes have shown promise in vaccine delivery due to their intrinsic adjuvant properties. They can stimulate both systemic and mucosal immune responses by promoting the uptake of antigens across Peyer's patches in the intestinal lining. The bile salt component enhances mucosal transport and acts as an immune stimulant, making bilosomes a valuable tool for oral and intranasal vaccine delivery platforms [27].

4. APPLICATIONS

Bilosomes have gained significant attention across various drug delivery domains owing to their ability to encapsulate a wide range of therapeutic agents, improve bioavailability, and facilitate mucosal and systemic absorption. Their structural adaptability and stability in hostile biological environments enable their use in diverse administration routes and therapeutic areas.

4.1 Oral Drug Delivery

Oral drug delivery represents one of the most significant uses of bilosomes, especially for unstable macromolecules like proteins, peptides, and vaccines. The incorporation of bile salts within the vesicular membrane provides resistance against digestive enzymes and bile-induced lysis, allowing for improved protection and prolonged residence in the gastrointestinal (GI) tract. Bilosomes have been effectively used to encapsulate drugs like

insulin, cyclosporine A, and desmopressin, resulting in markedly improved oral bioavailability over traditional formulations. [28]. Bilosomes also improve drug permeation by loosening tight junctions and enhancing transcellular transport across the intestinal epithelium.

4.2 Mucosal and Vaccine Delivery

Bilosomes are emerging as potent carriers for mucosal immunization. Their ability to be absorbed via Peyer's patches in the gut-associated lymphoid tissue (GALT) makes them effective for oral vaccine administration. Studies have demonstrated the successful delivery of antigens such as hepatitis B surface antigen (HBsAg), tetanus toxoid, and cholera toxin subunits via bilosomes, which led to both mucosal (IgA) and systemic (IgG) immune responses . Intranasal bilosomal formulations have also been explored for their potential to induce rapid immune activation and bypass the first-pass effect [29]. The combination of immune-stimulatory effects and bile salt-mediated mucosal permeability makes bilosomes ideal for non-invasive vaccine strategies.

4.3 Topical and Transdermal Delivery

Topical and transdermal drug delivery using bilosomes has shown enhanced skin permeation and dermal drug deposition. The deformable nature of bilosomes, aided by bile salts, enables them to squeeze through the intercellular lipids of the stratum corneum. This mechanism significantly enhances the penetration of drugs such as azithromycin, diclofenac sodium, ketoconazole, and clotrimazole for the treatment of localized infections and inflammatory conditions [30]. Moreover, bilosomes reduce systemic side effects by enabling site-specific delivery and maintaining therapeutic concentrations at the target site for longer durations.

4.4 Targeted and Site-Specific Drug Delivery

With advancements in nanotechnology, ligand-conjugated bilosomes have been developed for active targeting. Surface modification with ligands such as folic acid, transferrin, aptamers, and monoclonal antibodies allows bilosomes to selectively bind to receptors overexpressed in

pathological tissues. This approach has been particularly useful in cancer therapy for targeted drug delivery to tumor cells, brain targeting via receptor-mediated transcytosis across the blood-brain barrier (BBB), and inflammatory disease treatment such as ulcerative colitis and Crohn's disease where site-specific drug accumulation is desired [31]. Bilosomes have also been employed for colon-targeted delivery by incorporating pH-sensitive polymers or time-dependent release coatings**.**

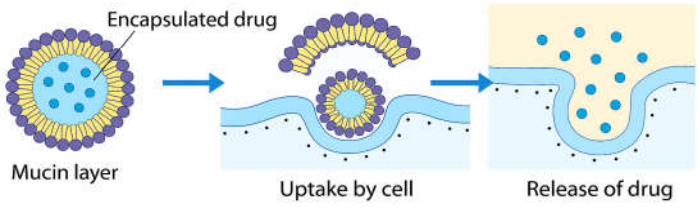

Fig 3: Mechanism of Bilosomes

5. LIMITATIONS AND CHALLENGES

While bilosomes present numerous advantages in drug delivery, their translation into clinically approved and commercially viable formulations is still constrained by several limitations. These challenges span physicochemical stability, toxicity concerns, scale-up difficulties, regulatory uncertainties, and analytical complexities.

5.1 Stability Concerns

One of the primary limitations of bilosomal systems is their susceptibility to physical and chemical instability during storage. Vesicles may undergo fusion, aggregation, or leakage of the encapsulated drug over time, particularly under fluctuating temperature or humidity conditions. Bile salts, though essential for vesicle flexibility and permeability, may also destabilize the lipid bilayer at higher concentrations or upon prolonged storage. Oxidative degradation of phospholipids and hydrolytic cleavage can further compromise vesicle integrity, especially in aqueous dispersions without proper cryoprotectants or stabilizers [32].

5.2 Potential Cytotoxicity and Irritation

Although bile salts enhance permeability and vesicle deformability, they may also exhibit membrane-disruptive behaviour at higher concentrations, posing risks of cytotoxicity or irritation, especially when administered via mucosal or topical routes. Sensitive tissues such as ocular, nasal, and intestinal mucosa may exhibit adverse responses if bile salt concentrations are not carefully optimized. In vitro cytotoxicity studies have indicated concentration-dependent effects of bile salts on cell viability and membrane integrity [33]. Therefore, it is crucial to optimize the formulation components to ensure safety is not compromised

5.3 Challenges in Scale-Up and Manufacturing

The scale-up of bilosomal formulations remains a critical hurdle due to the complexity of their preparation processes, which often involve multiple steps such as thin-film hydration, sonication, and size homogenization. Maintaining batch-to-batch consistency, vesicle size distribution, and drug entrapment efficiency during industrial production is difficult. Additionally, the requirement for sterile processing, lyophilization, and specialized equipment increases manufacturing costs and limits commercial scalability [34].

5.4 Regulatory and Clinical Barriers

Despite encouraging preclinical data, the lack of comprehensive clinical trials for bilosome-based products is a major barrier to regulatory approval. Regulatory agencies currently lack specific guidelines for evaluating the safety, efficacy, and quality control of bilosomal drug delivery systems. Moreover, the absence of standardized protocols for formulation, characterization, and toxicity testing creates uncertainty in the regulatory landscape. Long-term safety data, immunogenicity profiles, and human pharmacokinetic studies are urgently needed to support clinical translation [35].

5.5 Analytical and Characterization Complexities

Bilosomes pose significant challenges in analytical characterization due to their dynamic and complex structure. Parameters such as vesicle size, polydispersity index, zeta potential,

bilayer deformability, encapsulation efficiency, and in vitro drug release must be precisely measured using advanced techniques like dynamic light scattering (DLS), electron microscopy, differential scanning calorimetry (DSC), and Franz diffusion cells. Furthermore, reproducibility of these measurements across laboratories remains limited, complicating formulation optimization and quality assurance [36].

6. FUTURE DIRECTIONS

The field of bilosomal nanotechnology is evolving rapidly, and emerging innovations are aimed at overcoming current limitations and expanding the therapeutic utility of bilosomes. Future research is expected to focus on enhancing targeting precision, smart release mechanisms, clinical validation, and commercial scalability.

6.1 Ligand-Directed Targeting

One of the most promising areas of bilosome development is active targeting using ligands such as monoclonal antibodies, peptides, aptamers, folate, or transferrin. These ligands can be conjugated to the surface of bilosomes to achieve site-specific delivery by binding to overexpressed receptors on diseased cells, such as tumor tissues or inflamed sites. For example, folate-decorated bilosomes have shown enhanced intracellular uptake in folate receptor-positive cancer cells, offering potential for cancer chemotherapeutics with reduced off-target toxicity [37]. Targeted bilosomes also hold promise in brain delivery, colon-targeting, and autoimmune diseases.

6.2 Stimuli-Responsive Bilosomes

The development of stimuli-responsive bilosomes represents an innovative strategy for on-demand and site-specific drug release. These bilosomes can be engineered to respond to internal stimuli such as pH variations, enzymatic activity, or redox potential, or external triggers such as temperature or ultrasound. For instance, pH-sensitive bilosomes can release their payload selectively in acidic tumor microenvironments or the colon (pH ~7.5), improving

therapeutic precision and reducing systemic side effects [38]. Enzyme-responsive bilosomes may also be used for localized drug release in inflammatory tissues where enzymes such as matrix metalloproteinases (MMPs) are overexpressed.

6.3 Theragnostic Applications

Bilosomes offer potential in theragnostic, where a single platform is used for both therapy and diagnostics. This dual-functionality can be achieved by co-encapsulating imaging agents (such as fluorescent dyes, MRI contrast agents, or quantum dots) along with therapeutic drugs. Such theragnostic bilosomes can enable real-time tracking of drug distribution and accumulation, assisting in image-guided drug delivery and personalized treatment regimens [39]. This approach may be particularly useful in oncology, where monitoring treatment response is critical.

6.4 Clinical Translation and Human Trials

Despite promising preclinical data, clinical studies on bilosome-based formulations are limited. Future research must emphasize pharmacokinetic and pharmacodynamic profiling, long-term safety, immunogenicity assessment, and dose optimization in humans. Randomized clinical trials are essential to establish therapeutic efficacy and support regulatory approvals. The lack of standardized protocols and limited human data currently hampers the transition of bilosomes from laboratory to clinic [40].

6.5 Industrial Scale-Up and Commercialization

To enable commercial success, bilosome production processes should be optimized for scalability, consistency, and economic feasibility. This includes adopting continuous manufacturing processes, automated microfluidic systems, and lyophilization technologies for enhanced shelf-life and stability. Addressing Good Manufacturing Practice (GMP) requirements and developing scalable purification and sterilization techniques will be critical to facilitate regulatory acceptance and industry adoption [41].

CONCLUSIONS

Bilosomes have emerged as a novel and versatile nanocarrier system capable of addressing many of the limitations associated with conventional drug delivery platforms. By incorporating bile salts into their lipid bilayers, bilosomes offer enhanced membrane stability, improved permeability across biological barriers, and protection of encapsulated drugs from enzymatic degradation—making them especially advantageous for oral, transdermal, and mucosal administration routes.

Their ability to encapsulate both hydrophilic and lipophilic molecules, combined with their biocompatibility and capacity to induce mucosal immunity, further expands their potential in pharmaceutical and vaccine delivery. Moreover, ongoing innovations in ligand-mediated targeting, stimuli-responsive release, and theranostic applications continue to drive their relevance in modern drug delivery research.

However, challenges such as physical stability during storage, cytotoxicity at high bile salt concentrations, and limited clinical data must be addressed to ensure successful clinical translation. Future efforts should focus on standardized regulatory pathways, scalable manufacturing technologies, and comprehensive human trials to unlock the full potential of bilosomes as a next-generation delivery system.

LIST OF ABBREVATIONS

GI: Gastrointestinal

EPR: Enhanced Permeability and Retention

PE: Phosphatidylethanolamine

PBS: Phosphate Buffered Saline

PEG: Polyethylene Glycol

DECLARATIONS

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Consent for publication: Not applicable.

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