Scholars Academic Journal of Pharmacy
Abbreviated Key Title: Sch Acad J Pharm
ISSN 2347-9531 (Print) | ISSN 2320-4206 (Online)
Journal homepage: http://saspublishers.com

OPEN ACCESS

Pharmaceutical Science

Bilosomes as Novel Nanocarrier: A Review

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DOI: https://doi.org/10.36347/sajp.2026.v15i02.001 | **Received:** 26.12.2025 | **Accepted:** 05.02.2026 | **Published:** 09.02.2026

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Abstract **Review Article**

Bilosomes are emerging as a highly versatile and effective nanocarrier system in modern drug delivery. It offers improved stability, enhanced permeation, and targeted delivery of therapeutic moieties. Structurally, bilosomes consist of bile salts integrated into the lipid bilayers, which enhances vesicles stability and promote transit across biological barriers like skin and mucous membrane. This review highlights the fundamental components of bilosomes, various formulation techniques such as thin-film hydration, ethanol injection, and microfluidization, and physicochemical and biological evaluation parameters critical for their characterization. Furthermore, it explores the broad spectrum of bilosomal applications, including oral, transdermal, ocular, and intranasal routes for the delivery of diverse therapeutic agents such as antidiabetics, antidepressants, antibiotics, and anticancer drugs. The authors also made an attempt to include various release kinetics along with mechanisms. The review discusses the incorporation of bilosomes in innovative areas like vaccine delivery and gene therapy. Finally, future perspectives emphasize the need for advanced formulation strategies, scale-up technologies, and clinical validation to realize the full potential of bilosomes in precision medicine and commercial pharmaceutical applications are tried for incorporation.

Keywords: Bilosomes, Nanocarriers, Novel Delivery.

INTRODUCTION

Bilosomes are nano-vesicular drug delivery systems incorporating bile salts into its structure. These nano-vesicles are formulated using a blend of non-ionic surfactant; lipid like phosphatidyl choline; bile salts like sodium deoxycholate, sodium taurocholate, sodium glycocholate; and often cholesterol [1]. These vesicles being flexible and ultra-deformable are capable of passing through biological barriers like skin, intestines, and even cornea with ease [2]. These delivery devices are further capable of taking up issues like instability in the gut area and difficulty of retaining drugs till its spatial disposal. These nano-carriers were first introduced in the year 2001 by Conacher and team for the purpose of oral immunization using protein-based antigens. The surface modification of such 'bile salt stabilized vesicles' translating them into more stable, penetrable and efficient carriers when administered through oral, transdermal and ocular routes. Further, they have transformed into robust system capable of targeting cancer therapeutics and vaccine delivery [3]. Their improved stability and permeability quotient enhancing drugs absorption and therefore quickly changing the drug delivery scenario.

Structural components of bilosome:

Bilosomes are advanced nano-vesicular systems designed to improve drug delivery and bioavailability by enhancing the stability and permeability of the carrier. The key formulation components of bilosomes include bile salts like sodium deoxycholate, sodium taurocholate, sodium glycocholate; non-ionic surfactant; phospholipids like phosphatidyl choline; and cholesterol. Each constituent has very specific role in the performance of these nano-carriers.

1. **Bile Salts:** Bile salts are amphipathic molecules possessing both the hydrophilic and hydrophobic properties. They are crucial for bilosomes functionality. These bile salts make such nano-vesicles stable in the harsh environment of gastrointestinal tract. Bile salts like sodium deoxycholate, sodium taurocholate, and sodium glycocholate enhance the solubility and permeability of lipophilic drugs across biological membranes. Bile salts facilitate the penetration of the vesicle through the skin and intestinal walls for enhanced drug delivery [4].

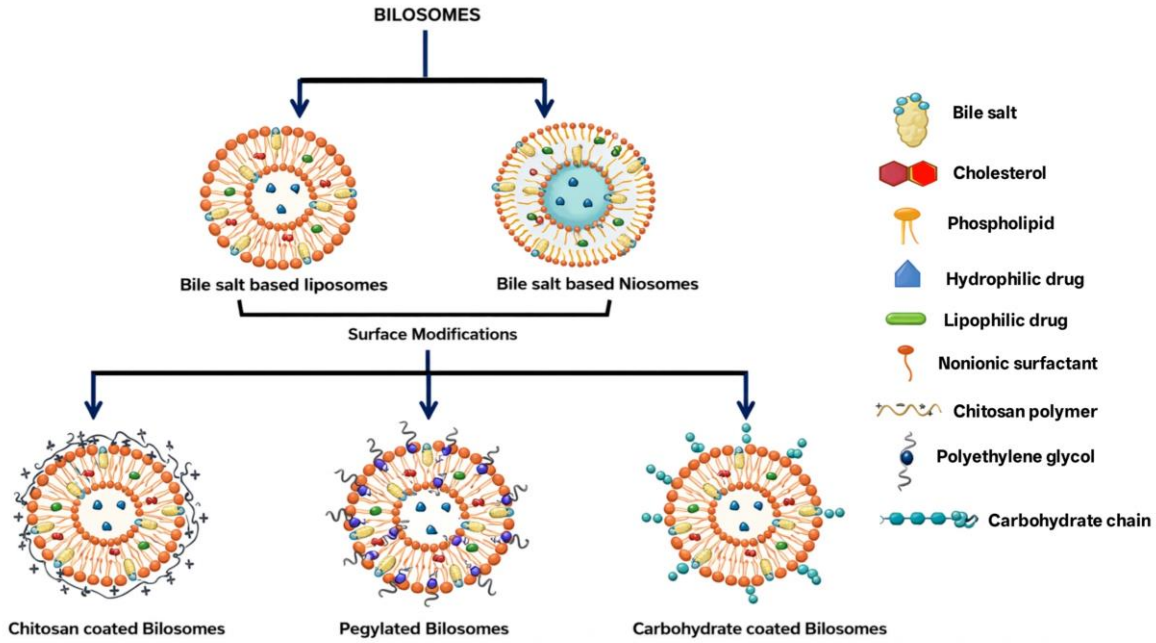
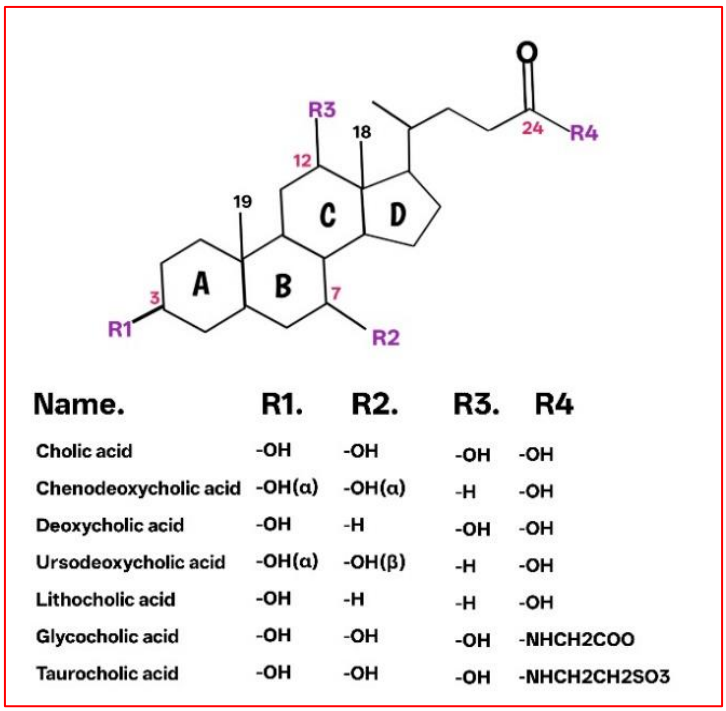


Fig 1: A schematic representation of bilosomes along with surface modifications.



Name.	R1.	R2.	R3.	R4
Cholic acid	-OH	-OH	-OH	-OH
Chenodeoxycholic acid	-OH(α)	-OH(α)	-H	-OH
Deoxycholic acid	-OH	-H	-OH	-OH
Ursodeoxycholic acid	-OH(α)	-OH(β)	-H	-OH
Lithocholic acid	-OH	-H	-H	-OH
Glycocholic acid	-OH	-OH	-OH	-NHCH2COO
Taurocholic acid	-OH	-OH	-OH	-NHCH2CH2SO3

Fig 2: Bile salt derivatives.

2. **Non-ionic Surfactants:** Non-ionic surfactants are key to bilosome formation due to their excellent stability, compatibility and reduced toxicity. These surfactants, such as Span 60 (sorbitan monostearate) and Tween 80 (polysorbate 80) help in solubilizing and emulsifying drugs. They are less irritating compared to anionic or cationic surfactants and are ideal for creating stable vesicular drug delivery system. Non-ionic surfactants promote drug permeation across bio-membranes, thus increasing drug's bioavailability. They are also used to control the size and surface properties of the bilosomes and therefore improves drug's entrapment and facilitates targeted delivery [5].

3. **Lipids**

a. **Cholesterol:** Cholesterol is an essential amphiphilic component in bilosomes that enhances membrane

stability and rigidity. By incorporating cholesterol into the bilosome structure, the vesicle's membrane becomes more rigid, reducing the leakage of water-soluble substances. Cholesterol determines the fluidity of bilosomes which in turn is crucial for its structural integrity under varying conditions.

b. **Phospholipids:** Phospholipid is a type of lipid with hydrophilic head and two hydrophobic tails making it amphipathic in nature. Phospholipids such as soybean phosphatidylcholine (Lipoid S 100) and phosphatidylcholine (PC) are the primary building blocks of bilosomes. These amphiphilic molecules form the bilayer structure that encapsulates the drug, facilitating self-assembly and emulsification. Phospholipids help to stabilize the vesicle, ensuring proper formation and functionality of the bilosomes in aqueous environments. They are biocompatible in nature which ensures minimal toxicity and irritation, making them ideal for drug delivery particularly in oral drug delivery and ocular formulations [6].

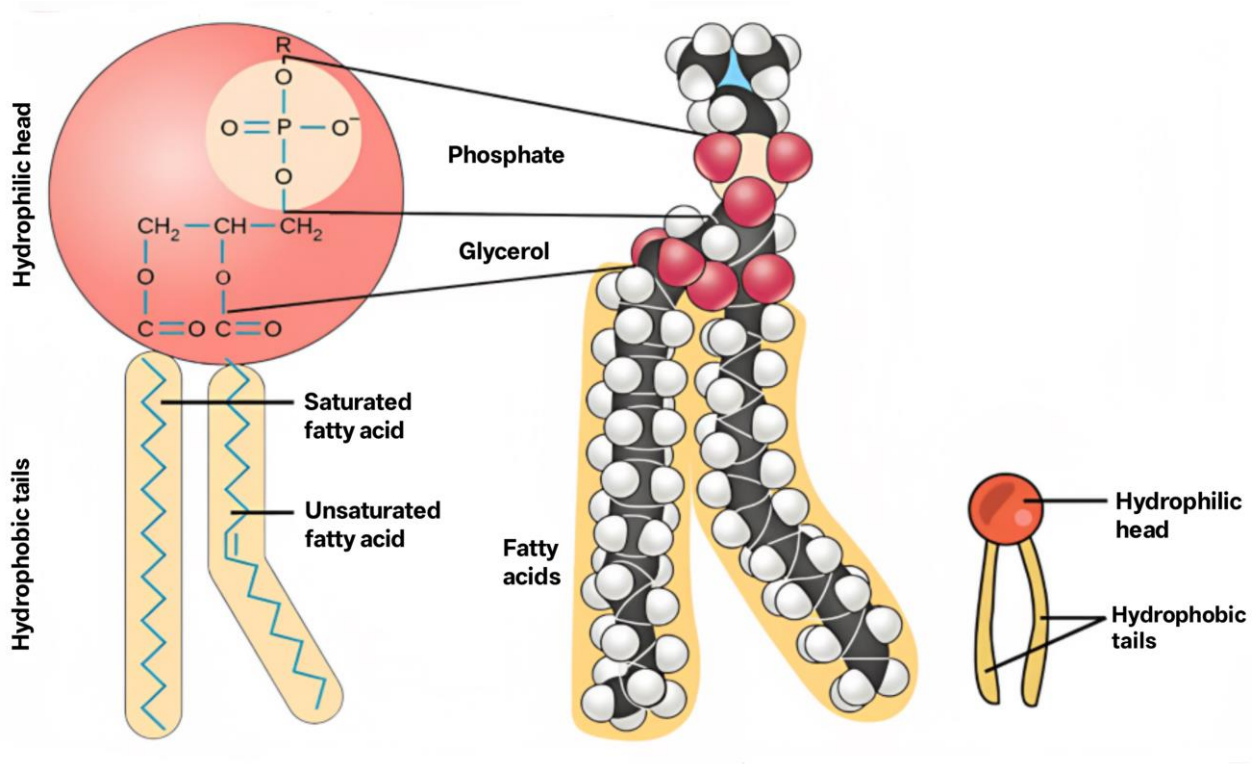


Fig 3: A schematic diagram of Phospholipid.

Table 1: Components of Bilosomes.

Main Components	Sub-category	Examples Reported in Bilosome Formulations
Bile Salts [4]	Primary bile salts	Sodium cholate, Sodium glycocholate, Sodium taurocholate
	Secondary bile salts	Sodium deoxycholate, Sodium chenodeoxycholate
	Semi-synthetic bile salts	Sodium taurodeoxycholate
Non-ionic Surfactants [5]	Sorbitan-esters (Spans)	Span 20, Span 40, Span 60, Span 65, Span 80
	Polysorbates (Tweens)	Tween 20, Tween 40, Tween 60, Tween 80
	Polyoxyethylene ethers	Brij 30, Brij 35, Brij 58
Lipids [6]	Sterol	Cholesterol (most widely used bilosome stabilizer)
a. Cholesterol	Natural phospholipids	Soy lecithin, Egg lecithin, Egg phosphatidylcholine (EPC)
b. Phospholipid	Hydrogenated phospholipids	Hydrogenated soy phosphatidylcholine (HSPC)
	Synthetic phospholipids	Dipalmitoyl phosphatidylcholine (DPPC), Distearoyl phosphatidylcholine (DSPC)

Novelty quotient of bilosomal drug delivery system: Bilosomes are novel drug carriers with novel features. The integration of bile salts into its lipid bilayer makes it more advanced in comparison to liposome or noisome. Few of the novel features of bilosome are narrated below.

i) Resistance towards gastrointestinal bile salts: Bile salts are the building block component of bilosomes. The presence of such salt in the bilosomal layers makes it stable in comparison to liposome & noisome when exposed to gastrointestinal bile salts [7].

ii) Oral delivery of macromolecules: Bilosomes enable oral administration of peptides, proteins, and vaccines by protecting them from enzymatic degradation and acidic pH. The bile salts integrated in the vesicular layers provide stability against the acidic environment and digestive enzymes of the GIT [8].

iii) Membrane flexibility and deformability: Bile salts impart elasticity to the bilosome nanovesicles, facilitating paracellular transport across the skin or mucous bio-membrane [7].

iv) Targeted delivery: Bilosomes facilitate targeted uptake via Peyer's patches and associated Microfold (M) cells in the intestine due to bile salt integrated in its structure. The bile salts protect the bilosome from harsh GI environment of acidity and enzyme and allow it to reach the Peyer's patch of the intestine [9].

v) Sustained and Controlled release: Bilosome nanocarriers provide sustained and controlled release of drugs owing to the presence of bile salts into its vesicular structure. Such bile salts contribute tremendously in the stability of the lipid vesicles. The bile salt-stabilized membrane shows slower release of the drug and prevents early leakage in the gastrointestinal tract. This allows the drug to be released gradually over a longer time, helps maintain effective drug levels, reduces dosing frequency and improves patient compliance [8].

vi) Avoid hepatic first-pass metabolism: Bilosomes are capable of avoiding hepatic first-pass metabolism. They are primarily absorbed through intestinal lymphatic system owing to their lipid and bile salt rich membrane. The negative charge due to bile salts encourages its uptake by M-cells in the Peyer's patch of intestine, which ultimately transport the drug through lymphoid tissues [10].

vii) Dual immunological response: Bilosomes are bile salt-stabilized vesicular systems that are capable to trigger dual immunological responses. They stimulate both mucosal (IgA) and systemic (IgG) immunity. They offer a needle-free, painless alternative to injections, thus increasing patient compliance [9].

viii) High entrapment efficiency: Bilosomes are reported to show higher entrapment efficiency of both hydrophilic and lipophilic drugs. Such payloaded nanovesicles have been shown to deliver drugs through oral, ocular or transdermal routes [11].

ix) Improved patient compliance: Oral, needle-free administration improves acceptability especially in pediatric and geriatric populations [11].

x) Translational potential and scalability: Simple preparation methods and use of biocompatible excipients favor industrial scale-up [8].

METHODS OF PREPARATION:

1. Thin-Film Hydration Method:

This is the most conventional technique used to produce bilosomes. In this method, first of all, lipids like cholesterol/phosphatidylcholine and surfactants like span 60, Span 80, or Pluronic P123 & bile salts are dissolved in a volatile organic solvent (chloroform/methanol). Then the solvent was evaporated using a rotary evaporator under reduced pressure. This creates a thin, dry lipid film on the inner wall of a round-bottom flask. The film is then hydrated using aqueous buffer, drug solution or bile salt solution to form multilamellar vesicles (MLVs) of bilosomes. The hydration is performed at a critical temperature above the lipid's phase transition temperature (e.g., 37°C) for several hours with agitation by magnetic stirring or sonication to form large multilamellar vesicles (MLVs). To obtain smaller uniform vesicles (SUVs), the MLV suspension is subjected to further processing. The methods like extrusion (forcing through polycarbonate membranes of specific pore sizes) or probe sonication to break down the MLVs are also widely practiced. This method is capable of creating stable, drug-loaded bilosomes with enhanced bioavailability. This method is also known as the 'solvent evaporation' method [3].

2. Ethanol Injection Method:

The ethanol injection method is used to prepare bilosomes. Such lipid-based nano-vesicles are prepared by three-step process. In the first step, an organic phase is prepared by dissolving the drug, phospholipids, and cholesterol in ethanol. In the second step, an aqueous buffer is prepared containing bile salts and other water-soluble components. In the third step, the ethanolic (organic) phase is rapidly injected into the aqueous phase with stirring. During stirring, the temperature was maintained between 40 and 60°C for hours to evaporate the ethanol completely. This results in the self-assembly of lipids into bilosomes. The dispersion was cooled down, and sonication was employed to reduce vesicle size. Dialysis may also be followed to remove any residual ethanol [5].

3. Hot Homogenization Method:

This method involves the melting of lipid components (like cholesterol, monopropylene glycol, or diacetyl phosphate) at high temperature (120-140°C) in a beaker in a hot oil bath, followed by rapid hydration with an aqueous buffer of pH 7.6, followed by high-speed homogenization (8000 rpm) for a short time to form an emulsion. In the final step, the preheated bile salt solution was added and homogenized again to form empty bilosomes. Thereafter, the drug was added to produce payloaded nanovesicles, which were then allowed to cool at room temperature, followed by incubation at 30°C for 2 hours at 220 rpm to stabilize the bilosomes [9].

4. Microfluidization Method:

It is a continuous, high-energy process that uses precisely controlled fluid dynamics to create uniform, small-sized vesicles with a low polydispersity index. This method is reported to be highly scalable in industry. In this method, two fluid streams, an *organic phase* (lipids, surfactants, cholesterol, and bile salts dissolved in a water-miscible solvent like ethanol or isopropyl alcohol) and an *aqueous phase* (containing the drug) are forced together through specialized microchannels at high pressure. The rapid diffusion of the organic solvent into the aqueous phase, combined with high shear rates & pressure drop, causes the dissolved components to quickly self-assemble into nanosized bilosomes [12]. Various steps of the method is presented below:

i. **Preparation of lipid phase:** Lipid phosphatidylcholine, non-ionic surfactant like Span 60, cholesterol, and bile salt like sodium deoxycholate are weighed accurately and dissolved in a water-miscible organic solvent, such as ethanol.
ii. **Preparation of aqueous phase:** The drug to be encapsulated is dissolved in a suitable aqueous phosphate buffer solution.
iii. **Microfluidization:** Then, the organic and aqueous phases are introduced into separate inlets of the microfluidizer and forced through the microchannels at specific, controlled flow rates using syringe pumps.
iv. **Vesicle formation:** The rapid mixing and solvent exchange at the microchannel junction leads to the instantaneous self-assembly and precipitation of the components into stable bilosomal vesicles typically less than 200 nm without sonication or extrusion.
v. **Collection and post-processing:** The resulting bilosome dispersion is collected from the outlet. Depending on the specific protocol and desired properties, post-processing steps like dialysis can be used to remove the organic solvent and any unentrapped material.

5. Probilosomal Method:

It is a technique for preparing bilosomes by first creating a dry, free-flowing powder, which is rehydrated later on to form the vesicular suspension. This approach produces a highly stable solid-state powder formulation capable of avoiding physical incompatibilities like aggregation, fusion, or leakage. This dry powder can be easily stored, distributed, and transformed into an active bilosome suspension just before consumption. The process involves two main steps: i) preparation of the probilosomal powder and ii) rehydration into a bilosomal dispersion [10].

i) Preparation of Probilosomal Powder:

Water-soluble compounds like sorbitol, mannitol, or microcrystalline cellulose are placed in a round-bottomed flask and vacuum-dried using a rotary evaporator. Then a solution containing the drug, phosphatidylcholine (or another non-ionic surfactant/lipid), and bile salt (e.g., sodium deoxycholate) dissolved in an organic solvent (e.g., a chloroform: methanol mixture) is added dropwise onto the said dried compound. The loaded particles are then subjected to freeze-drying (lyophilization) process to remove the solvent, and a dry, free-flowing probilosomal powder were produced.

ii) Conversion to Bilosomes:

The resulting probilosomal powder is converted into bilosomes by manually agitating or stirring it in a specific amount of an aqueous medium, such as distilled water or a buffer solution. The powder thus transforms into multilamellar vesicles (MLVs) upon contact with the aqueous phase.

Drug Release Mechanism:

The drug release from bilosomes involves a combination of Fickian and non-Fickian diffusion mechanisms. The pH of the surrounding environment, swelling of the 3D matrix, and spatial temperature all have profound contributions in exhibiting various release mechanisms.

Release Mechanisms:

Diffusion:

The non-Fickian or anomalous diffusion is the primary mechanism involved in the drug release from bilosomes. This mechanism deviates from that described by Fick's law. Here, concentration gradient-driven drug release is not followed; rather, the drug diffuses out from the nano-vesicles over time depending on the factors like polymer relaxation & swelling; chemical reactions, or structure of bilosomes [10].

pH-Dependent Release:

Bilosomes can be engineered for pH-dependent drug release. This facilitates the targeted delivery of drugs at various segments of the gastrointestinal tract. The selection of pH-sensitive bile salts or polymers endows stability to the bilosomes and improves drug solubility. At a low pH of 3.5, the bile salts incorporated in the bilosome membrane are protonated and resist the premature degradation of the vesicles, thus preventing drug release, which, however, is released in slightly alkaline conditions of pH 6.8 in the small intestine [14].

Swelling Dependent Release:

Bilosomes incorporated into the hydrogel matrix absorb moisture from the surrounding environment and swell. The swelling of the hydrogel alters the path length for drug diffusion. The swelling rate thus directs how rapidly a drug can diffuse out of the bilosomal/gel matrix into the surrounding tissue or bloodstream. An initial burst release of the drug can be seen to provide an immediate effect. Thereafter, a slower, sustained release can be observed for an extended period of time due to gradual diffusion of the drug from the inner core of the vesicles and the surrounding swollen gel matrix. Hence, an overall

biphasic release pattern can be observed from such composite delivery device [15].

Temperature Dependent Release:

In this mechanism, vesicles releasing the pay load at higher temperatures due to increased bilayer fluidity and permeability across the skin or mucous membrane. Such release patterns are supported by Higuchi or Korsmeyer-Peppas model indicating diffusion-controlled release facilitated by thermal stimuli [13].

'Higuchi' model describes diffusion-controlled drug release from matrix pores following Fick's law.

$$Q_t = k.\ t^{1/2}$$

Here,

Q_t represents Cumulative drug release.
k represents Higuchi Constant.
t represents time.

A linear relationship between Q_t and $t^{1/2}$ indicates Higuchi kinetics.

'Korsmeyer-Peppas' model on the other hand describes drug release from various geometries like spheres, cylinders etc. by incorporating release exponent (n) that specify the mechanism involve in practice [16].

$$M_t/M_\infty = k.\ t^n$$

Here, M_t/M_∞ represents fraction of drug released.
k represents release rate constant.
t represents time.
n represents release exponent.

Table 2: Various drug release mechanisms based on 'n' exponent value.

Drug Transport	Drug Release Mechanism	Value of 'n' exponent
Non-Fickian diffusion	-	$n > 0.45$
Fickian diffusion	Diffusion-controlled	$n \leq 0.45$
Non-Fickian/ Anomalous Transport	Combination of Diffusion & Polymer Swelling	$0.45 < n < 0.89$
Case II Transport	Swelling-controlled	$n \approx 0.89$
Super Case II Transport	Further Swelling/Relaxation- controlled	$n > 0.89$

The 'Hixson-Crowell' model is also used to describe drug release kinetics and mechanism from bilosome. In this model, drug dissolution rate is related to the change in surface area and diameter of the drug carrier where the matrix erodes or dissolves over time [13]. The Hixson-Crowell model is described by the cube root law equation and presented below:

$$W_0^{1/3} - W_t^{1/3} = k \cdot t$$

Here,
W_0 represents initial amount (or weight) of the drug in the bilosome.
W_t represents remaining amount of the drug in the bilosome at time t.
K represents a constant that incorporates surface-volume relation.
t represents time.

The data's obtained from *in-vitro* drug release studies are fitted into various kinetic models including zero-order, first-order, Higuchi, and Korsmeyer-Peppas etc. to determine which model best describes the release behaviour. The model with highest regression coefficient (R^2 value closer to 1) is consiered the 'best-fit' model.

Physical characters of bilosomes and its impact on therapy:

The physical state of bilosomes have profound impact on its permeation through the biological barriers like skin or mucous membrane. Such permeation translates into improved drug absorption reflecting better bioavailability and enhanced therapeutic efficacy. The important physical characters of bilosomes are outlined below:

i) Membrane Permeability:

The bile salts incorporated in the bilosome structure are capable of opening the tight junctions between epithelial cells (intestinal or ocular) temporarily. This facilitates the permeation of bilosomes across the bio-membrane through paracellular transport. As the permeation of bilosomes are facilitated automatically drugs absorption and its bioavailability was also improved. This results into improved therapeutic efficacy with reduced side effects [2].

ii) Bilayer disruption and increased fluidity:

The amphiphilic bile salt molecules incorporated in bilosome bilayer interact with lipids like cholesterol and phospholipids. The presence of bile salt amid phospholipids disrupts its tight packing. This results into reduction in *van der waals* force of attraction between phospholipid molecules. This results into less ordered, more flexible and ultra-deformable structure of bilosome. A bilosome with such unique physical character can easily pass through the tight junctions of bio-membrane. This results into increased permeability of the bilosomes translating into improved bioavailability and better therapeutic efficacy. The concentration of bile salts plays a significant role in

achieving the ultra-deformable structure of bilosomes [3].

iii) Enhanced Absorption:

Bile salts being amphiphilic in nature are capable of altering the permeability of the biological barriers like skin and mucous membrane. This action in turn promotes the permeability of bilosomes across bio-membrane translating into higher absorption and increased bioavailability. This results into improved therapeutic efficacy with reduced side effects [4].

Factors Influencing Drug Release:

iv) Bile salt type: Different bile salts (e.g., sodium deoxycholate and sodium glycocholate) affect the rate and extent of drug release.

v) Cholesterol content: The cholesterol content is responsible for maintaining vesicles rigidity. Increasing cholesterol typically reduces drug release, while excessive amounts can disrupt the structure.

vi) Surfactant-to-bile salt ratio: This ratio affects vesicle size, its stability and drug pay-loading, thereby influencing release kinetics [17].

Evaluation Parameters of Bilosomes:

To ensure successful development and therapeutic efficacy, bilosome-based formulations need to undergo comprehensive evaluation process considering multiple parameters as benchmark. These parameters cover physicochemical characterization, drug loading and release behavior, structural integrity, stability under various conditions, permeability performance and safety profiling. The **table 3** below summarizes the critical evaluation criteria along with literature references.

Table 3: Critical evaluation parameters of bilosomes.

Sl. No.	Category	Parameter	Description
1.	Physicochemical Evaluation	Vesicle Size & Distribution	Measured using Dynamic Light Scattering (DLS) or laser diffraction (e.g., Master sizer 3000E), indicating size homogeneity and efficiency of drug delivery.[18]
		Zeta Potential	Assesses surface charge; higher absolute values indicate more stable vesicles due to repulsion between similarly charged particles.[19]
		Polydispersity Index (PDI)	Indicates uniformity of vesicle size; values <0.3 suggest narrow size distribution.[18]
		Hydrodynamic Diameter	Represents particle size in solution, including hydration shell, measured by DLS.[19]
2.	Drug Characterization	Entrapment Efficiency (EE%)	Determines how much drug is encapsulated within bilosomes; typically assessed by ultracentrifugation and UV-spectroscopy.[20]
		% Drug Loading	Ratio of drug to total bilosome mass; important for dose calculations.[20]
		Drug Release Profile	In vitro drug release measured over time in different media; helps simulate in vivo drug availability.[20]
3.	Structural & Morphological Analysis	Morphology & Structure (TEM)	Transmission Electron Microscopy confirms spherical shape, vesicle integrity, and uniform dispersion.[18]
		DSC	Differential Scanning Calorimetry assesses thermal behavior and drug-lipid interactions.[19]
		FTIR	Confirms drug-excipient compatibility and chemical integrity of the formulation.[18]
4.	Stability & Storage Evaluation	Stability Studies	Follows ICH cGMP guidelines under stress and storage conditions to assess physical and chemical stability.[20]
		Kinetic Stability	Assessed using backscattering profile to detect aggregation or sedimentation over time.[21]
		Deformability Index	Evaluates elasticity and penetration capacity of vesicles, especially for transdermal applications.[22]
		Temperature Stability	Studies under various temperatures (e.g., 4°C, RT, 45°C) for 1–3 months to assess leakage and aggregation. [22]
		Freeze-Thaw Stability	Subjected to multiple freeze–thaw cycles to check structural integrity. [22]
		Long-Term Storage Stability	Typically assessed over 6–12 months under controlled conditions to ensure drug retention and vesicle integrity.[21]

		pH Stability	Evaluated in different pH media (pH 1.2, 6.8, 7.4) to simulate GI and blood conditions.[21]
		Visual Appearance	Checked for changes in color, phase separation, turbidity, or precipitation.[20]
		Sedimentation Rate	Evaluates phase separation under gravity; indirect measure of physical stability.[20]
5.	Release and Permeability Studies	In Vitro Dissolution Study	Drug release profile assessed using Franz diffusion cell or dialysis bag methods.[21]
		Permeation Study	Performed using excised skin or membranes; flux and permeation coefficients calculated.[21]
6.	Safety and Toxicity Evaluation	Bio-Adhesive Study	Determines interaction with mucosal or epithelial surfaces; essential for nasal or oral routes.[19]
		Corneal Hydration Study	Assesses safety for ocular applications by measuring corneal water content.[20]
		Histopathological Study	Examines tissue sections post-treatment to evaluate toxicity or inflammation.[21]
		HET-CAM Study	Assesses irritation potential using chick embryo membrane model. [22]
		Isotonicity Test	Ensures the formulation is isotonic to avoid irritation upon administration. [20]
7.	Antimicrobial Activity & Effectiveness	MIC	Minimum Inhibitory Concentration test to find lowest concentration of drug inhibiting microbial growth.[21]
		Antimicrobial Activity	Evaluated via zone of inhibition or turbidity assays against standard microbial strains [22].

Applications of Bilosomes:

Bilosomes have emerged as promising nanocarriers in the pharmaceutical field due to their ability to encapsulate both hydrophilic and lipophilic drugs, protect them from harsh physiological environments, and enhance their absorption across biological membranes. These vesicular systems have been extensively explored for oral, transdermal, topical, ocular and nasal drug delivery, showing improved bioavailability, targeted delivery and reduced systemic toxicity compared to conventional systems. The incorporation of bile salts enhances membrane fluidity and permeation, making bilosomes especially suitable for drugs with poor solubility or stability. The following **table 4** represents a categorized overview of diverse therapeutic applications of bilosomes, their route of administration and various drugs that are administered.

Table 4: Overview of therapeutic applications of bilosomes, their route of administration, and drugs administered.

Sl. No.	Category	Types of Medication	Specific Use	Drugs example
1.	Oral route of administration	Antidepressant	Treatment of depression	Desvenlafaxine succinate [2]
		Anti-inflammatory	Systemic inflammation	NSAID [3]
		Type 2 Diabetes	Glycemic control	Metformin HCl [18]
		Antihistamines	Enhanced solubility and bioavailability	Antihistamines [20]
		Antiviral	Targeted delivery	Hepatic antiviral agents [19]
		Antibiotic	Improved permeability and retention	Ciprofloxacin, norfloxacin [3,18]
		Diabetic Nephropathy	Nephroprotective drug delivery	Bilosomal formulations for renal targeting [21]
		Hepatocellular Carcinoma	Polysaccharide delivery	Bioactive plant polysaccharides [22]
		Hepatoprotective Agent	Liver protection and regeneration	Silymarin [21]
2.	Transdermal, Topical & Ocular Applications	Antiarrhythmic	Localized cardiac support	Amiodarone [3]
		Antidiabetic	Sustained release and skin absorption	Metformin [18]
		Antifungal	Ocular or dermal fungal infections	Fluconazole, amphotericin-B [19,22]
		Acne Treatment	Local antibacterial therapy	Clindamycin [2]

		Osteoarthritis Management	Anti-inflammatory therapy	Diclofenac [19]
		5-HT3 Antagonist Delivery	Antiemetic therapy	Ondansetron [21]
		Musculoskeletal Disorders	Targeted topical anti-inflammatories	NSAIDs [3]
		NSAID	Non-irritant topical delivery	Ibuprofen, ketoprofen [3,19]
		Ocular Drug Delivery	Treatment of bacterial conjunctivitis	Moxifloxac [20]
3.	Oral Applications of Probilosomes	Bioavailability Enhancement	Hydrophobic drug absorption	Self-rehydrating bilosome systems [2]
		Immunosuppressant Delivery	Chronic immune therapy	Cyclosporine-loaded probilosomes[21]
		Antihypertensive Therapy	Oral delivery of lipophilic agents	Improved pharmacokinetics[20]
		Metabolic Disorders	Herbal bioactives for chronic disease	Flavonoids, alkaloids in probilosomes[22]

Future Perspectives:

Despite significant advancement in the arena of bilosome, there remains huge potential for further expansion. Surface modifications; integration of stimuli-responsive polymers, mucoadhesive agents, and targeted ligands into the parent nano-vesicular structure are being attempted for multifunctional, sustained and site-specific delivery of therapeutic moieties [7,8]. The emergence of hybrid bilosomes, incorporating both natural and synthetic components, could enhance vesicle stability, drug encapsulation efficiency, and control over release kinetics [14]. Scalability and long-term storage are critical for commercialization. Technologies such as spray-drying and microfluidics are showing promise in improving the physical stability and shelf-life of probilosomal formulations. Additionally, the development of standardized evaluation protocols and comprehensive toxicological assessments will be vital to gain regulatory acceptance and move bilosome-based therapies into clinical settings. The bilosomes can potentially deliver oral vaccines. They are found very efficient in gene delivery and cancer therapy. They are very much promising for oral and nasal immunization [18,19]. Furthermore, surface-modified bilosomes with targeting moieties such as peptides, antibodies, or folate conjugates may significantly improve site-specific delivery in oncology and chronic disease therapy. With continued interdisciplinary research and innovations in nanotechnology, bilosomes are poised to play a key role in the advancement of personalized and precision medicine, offering efficient, biocompatible, and patient-friendly alternatives for drug delivery.

CONCLUSION:

Bilosomes represent a promising class of nanocarrier system that offer enhanced drug delivery through improved stability, permeability of the nanocarrier translating into enhanced absorption and improved bioavailability. Their unique composition incorporating bile salts into the lipid layers, allows protection of encapsulated drugs from harsh biological environments and facilitates efficient transport across epithelial membranes owing to their super flexible membrane. The diversity of preparation methods and comprehensive evaluation tools have enabled the fine-tuning of bilosome characteristics for specific therapeutic needs. With demonstrated efficacy across multiple routes of administration and disease areas-including diabetes, depression, infection, and cancer-bilosomes have gained considerable attention for advanced therapies. As research advances, bilosomes are likely to play a vital role in the development of next-generation, patient-friendly, and personalized drug delivery platforms.

Acknowledgement: The authors gratefully acknowledge the support extended by the affiliated institution.

Conflict of interest: The authors declare no conflict of interest.

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