RESEARCH PAPER

Engineering Bilosomes for Enhanced Drug Delivery: Current Status and Future Prospects

Tharini Lakshmanan[1*], Muthukumaran Mylasalam[1*], Purnima Ashok[2], Rajesh Dhandapani[3], Senthil Periyasamy[1]

[1]School of Pharmacy, SBV Karaikal, Sri Balaji Vidyapeeth (Deemed to be University), Thiruvetakudi Post, Karaikal-609609, India

[2]Department of Pharmacology, East West College of Pharmacy, Off Magadi Road, Vishwaneedham Post, Anjananagar, Bengaluru 560091, India

[3]Steril Gene Life Sciences (P) Limited, Mangalam, Puducherry 605110, India

*Corresponding Author:

Dr. Muthukumaran Mylasalam; E-mail: muthuviyas@gmail.com

Ms. Tharini Lakshmanan; E-mail: tharinil24@gmail.com

Abstract:

Bilosomes are the bile salt-stabilized lipid vesicles used to tackle the main drawbacks of classical vesicular systems, in particular their poor stability in the gastrointestinal tract and inadequate mucosal delivery. By incorporating bile salts (e.g., sodium deoxycholate, sodium taurocholate) into the lipid bilayer, bilosomes gain increased membrane elasticity, which is vital for withstanding enzymatic and pH-mediated degradation/escalating epithelial permeation via membrane fluidization, as well as for transient tight-junction modulation. This review will focus on core phospholipid vesicles including its basic principles (Self-assembly, composition, critical quality attributes), improvement strategies (Polymer coating, PEGylation, Ligand functionalization, hybrid/stimuli-responsive system formation as well as optimized lipid–surfactant–bile salt complex), and preparation techniques (scalable microfluidics production with homogenization/sonication/extrusion post-sizing). Bilosomes work by enhancing mucosal adhesion, thereby increasing paracellular/transcellular drug transport and absorption via lymphatics, which helps avoid the first-pass effect and enables better intracellular delivery of macromolecules. It is useful in the case of oral administration of poorly bioavailable drugs, orally administered peptides/proteins/biologics, oral/intranasal vaccination with strong mucosal/systemic immune response, intranasal brain targeting, transdermal delivery, oncology agentive passive/ligand-mediated targeting, and the use of nucleic acid-based/biomacromolecular delivery systems. Finally, translational challenges in specificity, such as long-term stability, biological media variability, sensitivity, IVIVC gaps, scalable, reproducible manufacturing systems, safety/regulatory evaluation, multi-excipient system cost, further prospective directions, use of AI-guided formulation optimization techniques, real-time monitored continuous microfluidic production, and friendly bile salt mimetic development are discussed.

Key words: Bilosomes; bile salt–stabilized lipid vesicles; mucosal drug delivery; enhanced membrane elasticity; biotherapeutic delivery

How to cite this article: Lakshmanan T, Mylasalam M, Ashok P, Dhandapani R, Periyasamy S. Engineering bilosomes for enhanced drug delivery: current status and future prospects. Int J Drug Deliv Technol. 2026;16(73s): 607-621. DOI: 10.25258/ijddt.16.73s.67

1. Introduction:

Numerous progresses have been made in the field of vesicular drug delivery over the last few centuries, introducing many novel approaches to improve efficacy and patient compliance as well as to address liabilities of current-day drug therapy. The journey started with the creation of liposomes in 1960 century that were a breakthrough in encapsulating hydrophilic and lipophilic drugs in biocompatible lipid bilayers [1]. Liposomes were able to improve the way drugs work in the body by making them last longer, protecting them from breaking down, and helping them reach the right target. But despite these benefits, liposomes haven't been used as much in clinics as they could be, mainly because they have some problems: they aren't very stable, they're removed from the body too quickly by certain organs, and they can be broken down in the stomach and intestines [2]. These demerits gave rise to more stable alternatives like niosomes, transferosomes, ethosomes, and polymersomes [3]. Every subsequent vesicle system was designed specifically addressing certain issues, such as niosomes for improved stability plus lower cost, transferosomes for high elasticity, and ethosomes for superior transdermal permeation, etc [4]. However, these improved systems collectively faced some common problems, which included failure in maintaining integrity under harsh physiological conditions, besides the inability to deliver the entrapped moiety effectively through complex biological barriers [5]. One main point that prevents the majority of vesicle-based systems applications is that the human body contains numerous natural defense mechanisms, if compared with a simple barrier, e.g., acid gastric environment,

including digestive enzymes/mucosal barriers/bile salts/efflux transporters/-brain barrier, and as such [6]. Many highly promising drug candidates (e.g., peptides/proteins/vaccines/hydrophobic drugs/biologics) fail to reach their designated targets since they undergo quick degradation or have limited permeability Oral administration being most preferred patient compliant route faces challenges associated with vesicular carriers mainly transparent natural covering and premature release of content into systemic circulation making it worthless path despite excellent carrier performance Next-generation nanocarrier systems able to maintain their integrity under stress conditions at the mucosal site are required for efficient biodistribution at selected anatomic locations [7].

As with all other drug delivery systems currently available in the marketplace, the bilosomes are not alone. There are liposomes, micro- and nanoparticles, emulsions, and other systems used in various forms for drug delivery. The main novelty of bilosomes (**Figure 1**) is the inclusion of bile salts into the bilayer membrane. This not only results in increased stability, permeability, and interaction of the drug delivery system with its environment, but also targeted release of drugs [8]. The inclusion of bile salts not only protects the structure against gastric/ enzymatic degradation but also facilitates trans-epithelial transport through membrane fluidization and temporary modulation of tight junctions. These properties set bilosomes apart from conventional vesicles, making them excellent candidates for a variety of routes, including oral, nasal, transdermal, and systemic drug delivery. Bilosomes show great promise for oral vaccine delivery; they have repeatedly been shown, with M-cell targeting at Peyer's patches, to provide efficient antigen uptake and robust mucosal immune responses, as well as systemic immune reactions. Therefore, bilosomes become an attractive option for next-generation needle-free vaccination systems and universal immunization schedules.

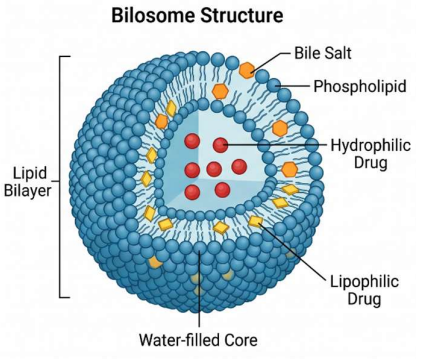

Figure 1: Structure of Bilosome

1.1. Rationale for Bilosomes in Advanced Drug Delivery:

Bilosomes are considered a better option not only because of their stability. But the composition of bilosomes is so versatile that you can encapsulate a wide range of molecules, such as small molecules, peptides, proteins, nucleic acids, natural products, and immunogens [8]. Recent progress in ligand functionalization, polymer coating, and stimuli-responsive engineering has enabled these vesicles to acquire multifunctional characteristics for a site-specific, receptor-mediated targeted drug-delivery system [9, 10]. Another fact is that, alongside conventional technologies, contemporary approaches such as microfluidics, AI-based formulation design, and nanohybrid systems are driving interest in this novel delivery vehicle called Bilosomes. As the regimen shifts toward precision medicine, biologics, and personalized therapy, bilosomes represent a highly versatile and smart approach to developing therapeutic vectors for future applications in disease course or in the development of patient treatment trajectories. This paper reviews the step-by-step progression of scientific morphing towards innovative lipidic vesicular carriers, bilosomes, reasons for their superiority, ways in which they work, and fabrication techniques that resulted in a multitude of pharmacological benefits across different delivery routes, latest inventions, and key case reports portraying the practical utility of this novel carrier platform. Moreover, it shall identify existing technical barriers to translation to the clinical arena, as well as upcoming challenges; namely, advanced, complex yet precision-driven engineering approaches, regulatory hurdles, and commercialization prospects will be laid out in the following sections. Building on the aforesaid information, this review aims to present lipidic nanostructures, Bilosomes, as among the foremost candidates for future vesicular carriers delivering improved therapeutic value in a targeted, systemic, and minimally invasive manner via drug administration route(s).

2. Fundamentals of Bilosomes:

Bilosomes are a special kind of carrier that comes in the form of tiny vesicles made from phospholipids, surfactants, and bile salts. Vesicles generated from bilayers (vesicles) can form a flexible and nanosized drug delivery system [11]. Incorporation of bile salts, such as sodium deoxycholate or sodium taurocholate, into the bilayer of vesicles makes bilosomes highly stable to enzymatic degradation, gastric and intestinal pH, and environmental conditions. These bile components enhance membrane fluidity and facilitate the flexible drug-containing bilosomes to cross mucosal barriers more efficiently than conventional systems (e.g., liposomes and niosomes). Bilosomes are highly stable and exhibit good mucoadhesive properties and targeted release at the mucosal surface. Encapsulation efficiency was high (**Table 1**). Bilosomes can be used for targeted oral, nasal, transdermal, and parenteral delivery systems. The bilosomes offer an efficient, targeted drug-delivery

system, since they have a native biological structure and good stability. Moreover, they exhibit good mucoadhesive properties and targeted release at the mucosal surface [9]. The new liposomal system has several advantages over previously developed vesicular systems, including increased stability and mucoadhesion, high drug entrapment efficiency, and improved activity. Therefore, the new system represents bilosomes as a promising drug delivery system [12].

Table 1: Liposomes, Niosomes, Transferosomes, and Other Nanocarriers in comparison with Bilosomes

Parameter	Liposomes	Niosomes	Transferosomes	Ethosomes	Bilosomes
Key Components	Phospholipids	Non-ionic surfactants	Phospholipids + edge activators	High ethanol content	Phospholipids + Surfactant + Bile salts
Stability in GI Tract	Poor	Moderate	Low–Moderate	Poor	Excellent
Membrane Flexibility	Moderate	Moderate	High	Very high	High (due to bile salts)
Permeability Across Mucosa	Low	Low	High	High	Very High
Entrapment Efficiency	Moderate	High	Moderate	Moderate	High
Ability to Withstand Bile Salts	Very poor	Poor	Poor	Poor	Excellent
Suitability for Oral Delivery	Limited	Moderate	Limited	Poor	Highly suitable
Use in Vaccination	Limited	Limited	Moderate	Limited	Excellent mucosal immunity
Industrial Scalability	Moderate	Good	Moderate	Moderate	Good
Overall Performance	Good	Good	High	High	Superior robustness + permeability

3. Engineering Strategies for Bilosome Design:

In general, a bilosome contains phospholipids, surfactants, and bile salts. The phospholipids, such as soya phosphatidylcholine, egg phosphatidylcholine, and hydrogenated lipids, are used to create, stabilize, and load drugs into vesicles. Phospholipids impart rigidity or fluidity to the bilosomes and also determine their biocompatibility [13]. In addition, phospholipids are often mixed with surfactants (e.g., Span, Tween, Poloxamer derivatives), which enhance bilayer flexibility, thereby reducing bilosome size and imparting greater fluidity to the bilayer (**Table 2** and **Figure 2**). Thus, the design of bilosomes can be manipulated to confer optimum characteristics to the system. Bile salts incorporated into the bilosome vesicular membrane provide the effect of bile salts on bilosomes, namely to increase acid stability and enzyme resistance, most importantly to enhance permeability across the trans-epithelial barrier [14]. The proportion of lipids, surfactants, and bile salts in the bilosome seems to be of importance for bilosome stability, where bile salts might need to be present in amounts just in excess of those required for membrane stabilization. Other important factors for optimization of bilosomes as delivery systems include particle size, zeta potential, and EE%. For lymphatic delivery, the size of bilosome vesicles is crucial. Efficient uptake of vesicles by lymphatic vessels, in addition to enhanced mucosal penetration, is observed for vesicles below 200 nm. The negative charge of bile salts can stabilize the zeta potential. The EE% can be optimized for drugs with varying hydrophobicity by optimizing lipid composition, bilosome preparation methodology, and bile salt concentrations. Also, an increase in EE% can be achieved by increasing the lipid concentration, increasing the sterol content (e.g., cholesterol), or optimizing the surfactant-to-lipid ratio. This method, in addition to facilitating an increase in drug loading, is effective for achieving sustained release formulations [15].

Table 2: Standard components of Bilosome delivery systems.

Component	Examples	Role in Bilosomes	Impact on Performance
Phospholipids	SPC, EPC, HSPC	Form bilayer structure	Determines rigidity, EE%, and stability
Surfactants	Span 60, Tween 80, Poloxamer 188	Improve membrane fluidity	Reduce particle size, enhance penetration
Bile Salts	SDC, STC, SC	Provide permeability & GI stability	Resist enzymatic degradation; improve uptake
Polymers	Chitosan, Alginate	Surface coating	Enhance mucoadhesion and controlled release
Ligands	Folic acid, Mannose, Transferrin	Active targeting	Increases cell-specific uptake
Cryoprotectants	Trehalose, Mannitol	Freeze-drying stabilizers	Prevent aggregation during storage

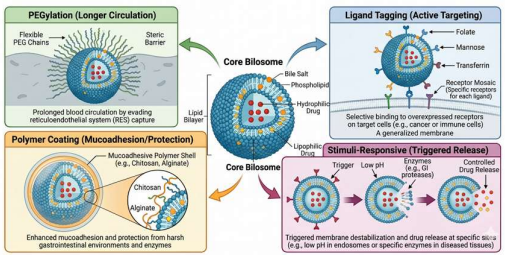

Figure 2: Engineering Strategies and Functionalization

3.1. Surface Modification and Functionalization Approaches:

Furthermore, surface modifications pose additional engineering challenges for rationally designing bilosomes to interact with desired biological entities and deliver drugs in an optimized and targeted fashion. PEGylation extends the half-life of bilosomes by preventing opsonization, while decoration with folic acid, mannose, transferrin, peptides, and antibodies enables targeted delivery to specific tissues or organs [16]. Polymers coated with biocompatible materials such as chitosan, alginate, and hyaluronic acid, in the case of bilosomal formulations, increase mucoadhesion; provide pH protection and influence the drug release rate. Another big step forward that was achieved thanks to stimuli-sensitive bilosomes is fine-tuning of drug release peculiarities: for example, by means of changes in pH levels, enzymic reactivity, redox conditions, or temperature levels fluctuations, and enabling the development of specific organ-targeted delivery systems [17]. Bilosome engineering encompasses many aspects, among which structural enhancement is one of the most important, as it affects storage stability and increases shelf life. It has been observed that lyophilization (freeze-drying), followed by the addition of cryoprotectants such as trehalose, sucrose, or mannitol, is widely practiced, converting them into free-flowing powders for reconstitution. On the other hand, spray drying is also employed for large-scale production, particularly for oral route dosage forms and inhalation powder formulations. In this context, lipids such as cholesterol appear to confer membrane rigidity, thereby diminishing the burst effect, while a polymer coating also ensures long-term stability under harsh gastrointestinal conditions [18].

3.3. Targeted Delivery Strategies (Ligand-tagged, Receptor-mediated, etc.):

These include the elicitation or enhancement of biological pathways that ensure target-specific delivery, such as trans-epithelial transport across epithelia (e.g., mucosal, alveolar), accumulation in macrophages, dendritic cells, or cancer cells. Targeted strategies are designed to attach drugs or genes specifically to certain cell types, thereby reducing nonspecific cytotoxicity and side effects. Bilosomes target Peyer's patches, thereby exploiting M-cell specificity to achieve effective vaccine uptake and initiate the immune response. Functionalized bilosomes can also cross the nasal–olfactory route towards brain drug targeting [19].

4. Methods of Bilosome Preparation:

There are different methods and excipients used for the formulation of bilosomes, as shown in **Tables 3 & 4** and **Figure 3,** which can be further grouped into categories based on their scalability, vesicle size control, drug-loading characteristics, and stability [20]. Amongst them, one of the most widely used methods is the Thin Film Hydration technique, which is also discussed in this paper. In this method, bile salts, Phospholipids, surfactants & hydrophobic drugs are dissolved in an organic solvent and then kept under reduced pressure to evaporate, forming thin films. Afterward, those films were hydrated in an aqueous medium containing hydrophilic drugs in case bilosomes were needed; this procedure has proven very effective. Even though it offers high entrapment efficiency for

both lipophilic and hydrophilic molecules, additional steps, such as sonication or homogenization, are required to reduce particle size [21].

Induced bilosome formation is a recognized technique, achieved by injecting a lipid–bile salt solution in ethanol into a preheated aqueous phase, allowing the solvent to diffuse rapidly and bilosomes to self-assemble. This process provides better control over particle size, a short processing time, and avoids the requirement for high temperatures; hence, it is suitable for thermolabile drugs [21]. Another method, called reverse-phase evaporation, is also useful for making bilosomes with a high success rate in trapping the desired substance. In this case, lipids and bile salts are first dissolved in an organic solvent and then mixed with water to create an emulsion. On drying of organic solvents containing cholesterol, vesicles are spontaneously formed, containing bilosomes of large internal volume. Their yield and purity are sensitive to the drying procedure, which requires careful control to avoid contamination by residues of organic solvents. Recent advances in nanotechnology, however, have opened up alternative approaches to bilosome formation using microfluidics. Using this technique, vesicles with bilosomes can be formed with controlled mixing and precise size measurement in the nanometer range. The microfluidic approach enables the production of uniform bilosomes of various sizes and has the potential for numerous drug delivery applications [22]. Microfluidics-based devices follow the fabrication principle of microfluidics that makes it feasible to produce bilosomes continuously with narrow bimodal distributions suitable for industrial manufacturing, since clinical translation context-sensitivity governing nanoparticles nucleation and vesicles generation is enforced, along with elimination of batch-to-batch variations usually encountered by conventional preparation protocols reduced scale operation also allows adoption of gentle processing conditions where biological entities such peptides proteins nucleic acids will remain unchanged [23].

Additional techniques, such as high-pressure homogenization, probe sonication, and extrusion, can be employed post-vesicle preparation to achieve a desirable vesicle size, greater homogeneity, and improved stability [24]. Down-sizing bilosomes is especially advantageous, as nano-bilosomes less than 100 nm are needed for mucosal or systemic administration, because the smaller the vesicles, the better the permeation and hence biodistribution. Each preparation method has its own merits and demerits; therefore, the type of drug, targeted vesicle properties, scalability requirements, and stability profile determine which method is chosen (**Table 3**). The versatility of preparation methods indeed yields a logically flexible, scheme-based technology that enables us to produce bilosomes with a wide range of applications [25].

Table 3: Bilosome Preparation Methods: Principles, Pros/Cons, Size Ranges

Method	Principle	Advantages	Limitations	Typical Size	Scalability
Thin-film hydration	Hydrate the dried lipid film with the aqueous phase of the bile salt	Simple; versatile; broad cargo	Batch variability; broader PDI	100–400 nm (post-sonication)	Good
Reverse-phase evaporation	W/O emulsion collapses into vesicles	High EE for hydrophilic drugs	Residual solvent; process complexity	150–500 nm	Moderate
Ethanol/Ether injection	Rapid injection of organic into aqueous	Continuous, low energy	Solvent removal; flammability	80–300 nm	Good
High-pressure homogenization	Intense shear downsizes vesicles	Narrow PDI; scalable	Heat stress; shear-sensitive cargos	80–200 nm	Excellent
Microfluidics	Laminar co-flow & controlled mixing	Precise size; reproducible	CapEx; channel fouling	50–200 nm	Excellent
Green methods	Solvent-free	Low residues;	Longer dev time;	100–300 nm	Moderate

(SCF/ melt)	or super critical CO_2	eco-friendly	equipment

Table 4: Common excipients in bilosomes and their roles

Class	Examples	Typical Level	Functional Role
Bile salts	Sodium deoxycholate; Sodium cholate; Sodium taurocholate	0.1–1% w/v	Membrane fluidization; GI stability; permeability enhancement
Surfactants (non-ionic)	Span 60/80; Tween 80; Brij 35/97	5–30% of lipid	Vesicle formation; size & PDI control; deformability
Phospholipids	Soy/Egg lecithin; DPPC; DSPC	40–70% of lipid	Bilayer matrix; lipophilic drug accommodation
Cholesterol	Cholesterol	10–40% of lipid	Membrane rigidity; leakage control; stability
Cryoprotectants	Trehalose; Mannitol; Sucrose	1–10% w/v (in cake)	Lyoprotection; redispersion after freeze-drying

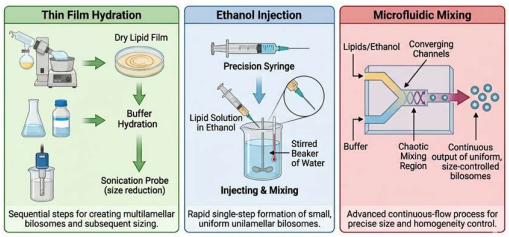

Figure 3: Bilosomes preparation methods overview

5. Characterization of Bilosomes:

To optimize the use of bilosomes across a variety of medicinal applications, it is imperative to characterize their physicochemical properties, as shown in **Table 5** [26]. One of the first and important parameters is particle size and size distribution, which can be determined by dynamic light scattering (DLS).

Particle size, size distribution, and surface properties are crucial parameters for molecules interacting with mucosal surfaces, tissue distribution, and lymphatic uptake. Nanosized bilayers are highly efficient at permeating bioactive agents through biological barriers. In this study, the formation of vesicles with a low PDI was a prerequisite for the formation of reproducible drug delivery systems [27]. Furthermore, the shape and size distribution of the particles can be characterized by TEM, SEM, and AFM images. Based on these qualitative studies, vesicles were formed, which guarantees the stability and uniformity of the studied drug delivery system. Another important parameter for particle stability is the zeta potential [28]. The zeta potential of bilosomes is generally negative and is due to the presence of bile salts, which promote electrostatic repulsion and facilitate sustained suspension stability. The EE% and drug loading capacity provide an index of drug incorporation into the vesicles; higher EE% indicates bilosomes that provide better-controlled delivery and appropriate dosing. Determination of entrapment efficiency involves spectrophotometric or chromatographic analysis after centrifugation or dialysis. It may be desirable for most drugs to be encapsulated within bilosomes to improve treatment outcomes, which depend on factors such as lipid type, drug properties, and production method [29].

These studies should be performed to check the stability of bilosomal formulations at different temperatures and humidity levels over time (accelerated and long-term stability) [30]. Following the study of advanced parameters such as particle size, zeta potential, drug leakage, and morphological alterations, many more stability questions could be asked [31]. There are also issues in bilosomes' stability when they are exposed to the simulated biological media, including stomach juice and intestinal fluid. This is important information when discussing oral treatment delivery systems. Next, based on this understanding, the development of *in vitro*, *in vivo* correlation can be initiated [32]. Finally, the implementation of *in vitro* drug release tests is evaluated using various approaches, including diffusion cell apparatuses, dialysis methods, and the Franz diffusion cell system. Studies of drug release kinetics from bilosomal gels aim to determine the release type: immediate, sustained, or controlled. But they can also help to get some information about the mechanism of drug diffusion from the matrix [33, 34].

Such models are also employed to evaluate the *in vitro* permeability of nanocarriers through different barriers. For this purpose, models of animal tissue slices, such as rat intestine, nasal mucosa, and porcine skin, can be used [35]. These models allow

us to predict the permeability, distribution, bio-accumulation, and interaction of active substances with biological membranes. For example, it is possible to determine the Cmax and Tmax parameters, which characterize the maximum concentration of the drug in the blood and the time to reach it, and AUC and bioavailability, which characterize the total amount of the drug in the bloodstream and the fraction of the administered dose introduced into systemic circulation. For this purpose, models of traditional drugs can be used, and the results can be used to assess how much better the new bilosomal formulation is compared to the traditional one [36]. *In vivo* tracking of a drug following its administration to an organism by imaging techniques: fluorescence and radiolabelling. These tests give us an insight into how our bilosomal formulation works. They are important for assessing the quality of our formulation and evaluating whether it is a good drug-delivery system [37].

Table 5: Characterization Panel and Acceptance Considerations

Attribute	Method	Typical Acceptance
Size & PDI	DLS	100–200 nm; PDI ≤ 0.2–0.3
Zeta potential	Laser Doppler	≤ −20 mV (route-dependent)
Morphology	Cryo-TEM/AFM	Unilamellar/multilamellar as designed
Entrapment efficiency	Ultrafiltration + HPLC/UPLC	≥ 60–90% (cargo-dependent)
In vitro release	Biorelevant media; dialysis	Model fit + sink maintenance
Residual solvents	GC/HS-GC	Within ICH Q3C limits

6. Mechanisms of Bilosome-Mediated Drug Delivery:

Furthermore, the drug can be entrapped as bioactive particles within the bilosome formulation, which can then be targeted to the specific site of action by interacting with the epithelial layer [26, 38]. The presence of bile salts in bilosome formulations increases their stability and effectiveness by enabling targeted delivery to the epithelial layer [39]. Sodium deoxycholate forms pores in the bilosome membrane; therefore, drug-containing bioactive particles entrapped within it can easily pass through the epithelial layer. Bile salts also incorporate into the bilayer, thereby stabilizing it by fluidizing the membrane and increasing its flexibility. Bile salts also alter the lipid packing of the membrane, resulting in paracellular and transcellular delivery of the drug through the temporary loosening of epithelial tight junctions. Bilosomes are a novel type of drug delivery system consisting of mesoporous silica particles coated with bile salts. This delivery system has potential for drug delivery across various types of epithelial barriers. To improve drug delivery across various bioenvironments, a novel bilosome delivery system leverages the biological activity of bile salts to enhance drug delivery. This study investigates the mechanism of action of bilosomes and their interactions with human and animal bodies to improve the design of targeted delivery systems. The inclusion of bile salts into the bilosome not only enhances drug delivery but also improves the stability of mesoporous silica vesicles, allowing them to adopt a more elastic form that can permeate throughout the GI tract and other mucous membranes. Such bilosomes have the potential to develop into effective drug delivery systems, thereby opening new avenues for systems with reduced toxicity and improved clinical efficacy for a variety of diseases [40]. The use of bilosomes as a drug delivery system is emerging and gaining popularity among pharmaceutical scientists for formulating novel approaches to reduce side effects and achieve enhanced therapeutic effects. Further studies are required to establish bilosomes as an effective drug delivery system through an in-depth understanding of their behavior and interactions with biological systems [41].

Bilosomes have greater absorption into the body than conventional liposomes and have gained attention as alternative systems. The interaction of bilosomes with epithelial cell membranes is due to their amphiphilic nature and the negative surface charge of bile acids, which facilitates their interaction with lectin-like receptors on epithelial cell surfaces. Due to their membranous nature, they are highly flexible and therefore easily penetrate the biological barriers, such as mucosae. The mucoadhesive properties of bilosomes enable the formulation to remain in contact with the mucosa for longer periods and to gradually release its contents into the absorption tissue. Thus, bilosomes can be used as effective delivery systems for drugs and nutrients for nasal, oral, and transdermal applications [28].

Besides its role in the immune system, the lymphatic system is also important for the uptake of lymphatic agents, including oral vaccines and drugs with poor bioavailability in the gastrointestinal tract [42]. The bile salts in the bilosomes enhance uptake of the lipid vector through M cells in Peyer's patches in the small intestine [43]. These absorbed bilosomes are then translocated to the underlying immune tissues, where the antigen is processed and immune responses generated. The bilosome system has been explored for oral vaccine delivery and administration of immunomodulators [44]. The study revealed better performance of the bilosome system compared to conventional systems [45].

Additionally, oral delivery of bilosomes containing drugs induced enhanced mucosal and systemic immune responses. In addition, drug delivery to the lymphatic system may also avoid first-pass metabolism [46]. In addition to the benefits of a bilosome mentioned above, these vector systems can deliver drugs into cells, where they become active. Such intracellular release may allow drugs to interact with subcellular targets. The mechanism by which vesicles are taken up by cells via bilosomes involves endocytosis [47]. In addition, the bile salt surface on vesicles may promote fusion with cell membranes. This could result in either the vesicles being taken completely into the target cell and degraded, or the vesicles fusing with the cell membrane, with part of the vesicle rupturing and releasing the drug into the cytosol. Several therapeutic peptides and proteins, as well as various nucleic acids, are currently unsuitable for oral administration because they are degraded in endosomes and fail to reach their site of action [48]. Furthermore, the release of the drug into the cytosol would enable bioactive macromolecules to interact with their subcellular sites of action. All the properties mentioned above - namely, membrane permeation, mucosal adhesion, lymphatic targeting, and intracellular delivery - offer excellent solutions for delivering drugs to overcome difficult barriers to drug delivery. The bilosomes offer an efficient and reliable solution for delivering drugs successfully across these and other barriers to bioavailability [41] (**Figure 4**).

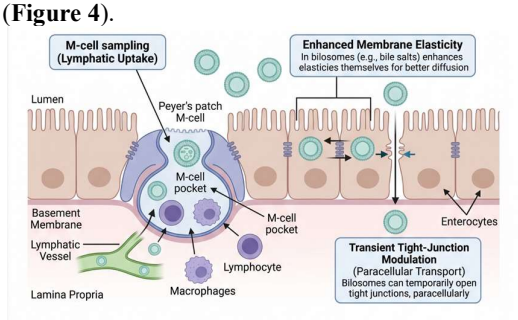

Figure 4: Delivery Mechanisms of Bilosomes

7. Applications of Bilosomes in Drug Delivery:

Bilosomes have significant potential for dermal, parenteral, nasal, and oral delivery. One of the key areas where poorly bioavailable drugs could benefit is oral drug delivery [25, 49]. Most conventional vesicular systems suffer from disadvantages such as rapid disruption by stomach acidity or enzymatic instability in the intestinal environment. However, incorporating bile salts into bilosomes stabilizes the carriers in the gastrointestinal tract. This property facilitates increased absorption of drugs such as peptides, proteins, lipid-soluble drugs, as well as BCS Class II and Class IV drugs, including NSAIDs [50]. Their ability to avoid first-pass metabolism via lymphatic uptake significantly enhances oral bioavailability, making bilosomes a suitable candidate for drugs that undergo hepatic metabolism. Moreover, bilosomal formulation has shown strong promise for delivering the active principles and phytochemicals of medicinal plants, owing to its ability to create a protected microenvironment and provide sustained release [51].

Vaccine delivery and immunomodulation are other areas that show potential for this type of system [9]. Bilosomes, for instance, can efficiently target antigens across the gastrointestinal mucosa and subsequently specifically target M cells in Peyer's patches, resulting in strong mucosal and systemic immune responses [43]. Therefore, they are a good choice for oral vaccines against viruses, bacteria, and parasites [52]. Hypothetically, it combines enhanced antigen stability with help in controlling the immune response; thus far, bilosomes have enabled the delivery of vaccines without needles, which are also patient-friendly compared to available alternatives. Several studies have shown that their immunogenicity is much better than that demonstrated by conventional oral vaccine formulations. In addition to oral vaccines, bilosomes have been tested for nasal vaccination, where they facilitate antigen uptake via the olfactory and respiratory epithelium, thereby promoting strong IgA-mediated mucosal immunity [53, 54].

On the same note, skin delivery of drugs is also possible with bilosomes, and literature has shown remarkable progress with them. They make use of their flexible, bile salt–rich membranes to pass through the stratum corneum more effectively than niosomes or conventional liposomes on the other hand [55]. This enables the application of anti-inflammatory, anti-infective, and cosmetic agents directly into the dermis [40]. Furthermore, bilosomes have been regarded as important for intranasal drug delivery, especially in the treatment of CNS diseases. These compounds, referred to as permeability-enhancing agents, permeate the nasal mucosa to reach the brain via the olfactory pathway, which is currently regarded as the most effective route for drug delivery to bypass the blood–brain barrier. As such, these compounds have a huge impact on the therapy of neurodegenerative disorders, migraine, and CNS infections [56-58].

Moreover, the potential of using Bilosomes to deliver anticancer drugs has also been explored. Such systems may offer both passive and active targeting of tumor cells [59]. Furthermore, conjugation of ligands to the Bilosome surface provides targeted delivery of the drug to its sites of action. The encapsulated anticancer agents act as effective chemotherapeutics and are expected to reduce systemic side effects through sustained release. In addition, the encapsulation of biomacromolecules, such as DNA, siRNA, microRNA, genes, oligonucleotides, proteins (e.g., for gene therapy, enzyme replacement therapy,

vaccines, or immunostimulatory therapy) has been explored. Bilosomes represent an innovative approach and strong contenders for next-generation drug delivery systems across various therapeutic domains (**Figure 5**) [48, 60, 61].

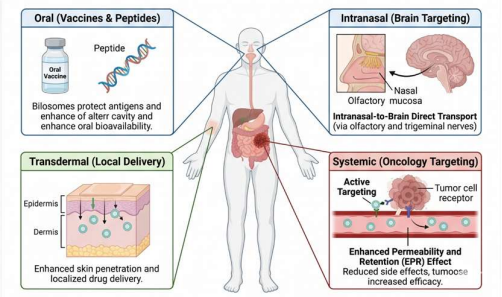

Figure 5: Applications of Bilosomes

8. Recent Advancements and Case Studies:

Recent advances in bilosome technology have enabled potential applications in the development of novel drug delivery systems. Recent developments, including new approaches to formulation design, surface modifications, and the engineering of hybrid systems to incorporate novel parameters, have opened avenues for bilosome technology. These are summarized in **Table 6**. The term nanobilosomes was therefore coined to define such more technologically advanced bilosomes, which are ultrafine (nanosize), highly membrane plastic (enhanced parameter), and therefore display enhanced permeation across biological membranes such as the mucus membrane, greatly enhanced uptake into lymphatics, and enhanced entrapment and controlled release of bioactive molecules [12, 62]. Nanobilosomes are therefore applicable for oral (vaccines, nutraceuticals, pharmaceuticals) and intranasal (aerosols or sprays of vaccines, nutraceuticals, pharmaceuticals) delivery of biologic molecules, proteins, peptides, and other sensitive bioactive molecules that require targeted release to achieve therapeutic efficacy. Another area of application for ligand-functionalized bilosomes is targeted delivery [63]. By incorporating ligands into the bilosome system, it is possible to achieve targeted delivery of drugs to the site of action via specific receptors or biomarkers. A large number of ligands have been studied in this context for their efficacy in achieving targeted delivery of drugs and bioactive molecules, including folic acid, mannose, RGD peptides, and various biological molecules such as transferrin and antibodies. The development of targeted delivery systems using bilosome carriers has been found to be extremely effective for cancer therapy, infectious diseases, and some neurological disorders [64-66].

Novel hybrid systems of hybrid bilosomes (hybrid polymer bilosomes), and hybrid bilosome systems (with metal and/or inorganic nanoparticles) that contain temperature or bioresponsive solubilizing agents for on-demand drug release into the bioenvironment offer immense potential [67-69]. The incorporation of pH-responsive polymer coatings into bilosome-hybrid systems could enable the development of efficient, targeted drug-delivery systems that release drugs rapidly at the tumor site. Incorporation of chitosan in bilosome-hybrid systems has significantly enhanced the mucoadhesive and permeation-enhancing properties of nasal and intestinal bilosomes. The combination of microfluidics with bilosome technology also offers several advantages, including ease of scale-up production of monodisperse vesicles of controlled composition and size. This progress greatly decreases batch-to-batch variability and makes bilosomal formulations closer to the requirements of the clinical translation system [70-72].

Different case reports further demonstrate the potential of liposomes in therapeutics. Bilosomes used for oral vaccine delivery against hepatitis B, influenza, tetanus toxoid, and enteric infections are among the many that have shown strong mucosal as well as systemic immune responses, performing much better than most conventional antigen-delivery systems [73, 74]. Similarly, bilosomal formulations of anti-diabetic, anti-inflammatory, and antihypertensive agents have shown significant improvements in oral bioavailability and often outperform some recently developed liposomal or niosomal carriers. In oncology, folate-functionalized bilosomes loaded with doxorubicin, paclitaxel, or curcumin at the preclinical level demonstrated a higher tumor uptake coupled with lower systemic toxicity. Additionally, intranasal bilosomes for the delivery of drugs such as donepezil, rivastigmine, and insulin showed remarkable potential for the treatment of neurodegenerative diseases by bypassing the blood-brain barrier and enabling a faster onset of action [60, 75].

In simple terms, the rapid advances and successful applications serve as evidence of the versatility and transformative impact bilosomes have as next-generation nano-carriers. They can be used via various administration routes, are well-suited to both small molecules and biologicals, and can benefit from modern development strategies, all of which place these carriers at the forefront of novel drug delivery research. With further advancement in this area, it is anticipated that bilosome-based therapeutics will rapidly enter clinical use [76].

Table 6: Representative bilosomal formulations and outcomes

Drug/ Antigen	Route	Model/ Study	Key Outcome	Inference
Insulin	Oral	Rodent	Improved hypoglycemia; ↑ bioavailability	Bile salt-stabilized vesicles

				protect the peptide
Rutin	Oral	Rodent (nephrotoxicity)	↑ Oral exposure; nephroprotection	Superior to niosomes/free drug
Lornoxicam	Transdermal	Rodent/ Ex vivo skin	↑ Permeation; sustained analgesia	Non-irritant bilosomal gel
Nimodipine	Transdermal	Ex vivo/in vivo	↑ Dermal deposition; controlled release	Bilosomal gel platform
Tetanus toxoid	Oral vaccine	Preclinical	Robust mucosal sIgA/systemic IgG	POC oral immunization

9. Challenges and Limitations:

Although bilosomes have several advantages, several issues should be addressed before bilosomal technology becomes a general clinical practice [49]. The stability of the formulation remains a key issue, as bilosomes, like other vesicular systems, can begin to aggregate or leak the encapsulated drug, and their size can increase during storage [77]. It is known that, despite bile salts supporting membrane strength, a delicate balance between membrane flexibility and integrity prevents destabilization of membranes under physiological conditions [40]. Furthermore, changes in bile salt concentration, lipid composition, or surfactant type may cause considerable variation in bilosome properties, which require consistency from batch to batch, leading to scale-up and industrial manufacturing challenges [78]. However, making high-quality bio-ink in large amounts is really tough. This problem worsens when you try to use specialized methods such as microfluidics or lyophilization [79].

In addition to the structural attributes of bilosomes, the complex system's interactions with the biological environment could be another limiting factor in bilosome formulation [80]. In addition to increasing membrane permeability and stabilizing vesicles in the digestive tract, bile salts can cause membrane disruption, cytotoxicity, and rapid vesicular disassembly, especially when the concentration exceeds a critical level. Exogenous components such as bile salts and digestive enzymes in the GI tract can nonspecifically interact with the bilosomal support system, potentially affecting drug delivery and the activity of the delivered drug [81, 82]. An important distinction exists between in vitro and in vivo performance of oral bilosomal formulations for which *in vitro–in vivo* correlations (IVIVC) do not exist. While several mechanisms have been proposed for enhanced lymphatic uptake, mucosal adhesion, and intracellular targeting and uptake, these areas are exciting but require more mechanistic studies to provide a basis for rational formulation development [83].

The processes of regulation and safety have also been cited as barriers [84]. This includes the fact that bilosomes may contain excipients such as phospholipids, surfactants, bile salts, and ligands, all of which require toxicological evaluation [85]. It is not known if there will be safety concerns after prolonged oral administration, especially with oral vaccines or chronic therapies) since they are not fully established [86]. In addition, animal-derived bile salts can pose problems with immunogenicity, variability/ regulatory compliance; thus, it is necessary to consider synthetic or recombinant alternatives. Moreover, clinical data on respace-to-bilosome-based therapies are scarce; most studies are preclinical, and only a few have entered the early clinical stage. As a result, it becomes impossible to apply the findings to a wider range and complicates the development of standard manufacturing process requirements and the establishment of quality control procedure systems [39, 87, 88].

Apparently, there are two main problems: the cost and the difficulty of reproducing the products. The use of high-purity lipids, specifically formulated for that purpose and produced using controlled methods, elevates formulation costs, ultimately impeding commercial scalability. However, stability studies and storage optimization for long-term use are essential for bilosomal products, especially those destined for less developed countries. Future challenges, to be addressed by the combined efforts of formulation scientists, regulators, and translational researchers, will involve the development and maturation of bilosomes as a drug delivery system [30, 89].

10. Future Prospects:

Bilosomes are likely to remain a popular drug delivery system in the future due to rapid technological advancement and the pursuit of new materials [90]. The future is very promising for bilosomes, as nanotechnology, polymer science, and formulation design are advancing at a much faster pace than ever before [91]. A possible upcoming trend can be formulated as an increase in complexity of AI-guided optimization, in which predictive ML

models identify suitable ranges for optimal lipid-to-bile salt ratios, surfactant combinations, and process parameters to achieve desired particle sizes and digestive kinetics [92, 93]. These software tools might significantly reduce development time and costs associated with the empirical trial-and-error method, enabling quicker emergence of bilosome-based treatment designs. Additionally, equipping these microfluidic systems with real-time monitoring will support continued high-quality manufacturing processes that have hitherto been elusive to scale-up; this development will eventually facilitate industrial production of bilosomal vaccines, biologics, and small-molecule drugs [23].

Novel materials have been investigated for bile salts and amphiphile analogs. From a materials perspective, the development of next-generation bile salt mimetics to control membrane fluidity, permeability, and drug-release patterns is an important area of study [39, 94]. Given limitations of parent natural bile salts in terms of variability, irritancy, and regulatory issues, "moving up the shelf" to mannosylated glycolipids and other versatile materials offers promise for bile acid-derived drug delivery systems. In addition to the development of novel materials, the field of "stimuli-responsive" or "smart" bilosome systems is important for the future development of drug delivery technologies as targeted systems for site-specific delivery to solid tumors, sites of inflammation, or within cells via endocytosis, followed by targeted release of the drug to achieve therapeutic effects while avoiding toxicity due to systemically released active therapeutic agent [70, 95].

Targeted delivery to specific tissues and cells is another area of development for bilosomes. In addition to vaccines, bilosomes could be used for gene and nucleic acid delivery, including siRNA, mRNA, plasmid DNA, and CRISPR-associated biologics [96]. The bile salts incorporated into the bilosome structure serve as internal stabilizers, enhancing stability and facilitating the delivery of labile genetic materials. In addition to vaccine delivery, targeted delivery to specific tissues and cells using modified ligands can be achieved by surface-modifying bilosomes to deliver drugs to specific sites of action, including cancer, neurodegenerative diseases, and site-specific immunomodulation. Targeted delivery to the brain or to specific tumor microenvironments using receptor-specific ligands, along with improved delivery across the blood-brain barrier, will open new avenues for precision oncology, neurodegenerative disease therapy, and targeted immunomodulation [73, 97].

Bilosomes have considerable potential for clinical and commercial development. At present, there is a need to develop and characterize bilosomes to improve stability. In addition, regulatory guidelines, safety, and human efficacy need to be addressed. To address these issues, standards need to be established for testing and characterization, as well as for scale-up. Toxicity studies need to be conducted. However, it is predicted that they will be a successful oral delivery technology for vaccines and chronic care medications, including personalized or designer medications. Currently, bilosomes are likely to remain the vector of choice for delivering small payloads in the short term [87, 98]. However, as the engineering and manufacturing of this microencapsulated delivery system advances, other vectors are likely to challenge for leadership in the medium term. Furthermore, as more preclinical studies progress to human clinical trials, bilosomes are expected to become a mainstream delivery system in the longer term.

Conclusion:

Bilosomes are among the most effective vesicular carrier systems, and are quite novel in their design and properties. Thus, using bile salt-enriched membranes can enhance gut membrane integrity while simultaneously improving mucosal permeability to drug particles, which can be impeded by these barriers in oral and mucosal drug delivery systems. Their benefits are not confined to solidity only: bilosomes represent an adaptable encapsulation system for small molecules, BCS II/IV drugs, peptides, proteins, vaccines, and other biologics, as well as permit a controllable release profile and improved absorption coupled with synergistic effects of membrane fluidization, tight-junction modulations, mucoadhesion, and lymphatic uptake. Bilosome functionalities have evolved further through more advanced processes, including Polymer Coating (chitosan/alginate/HA), PEGylation, Ligand-Targeting (Folate, Mannose, Transferrin), peptides/antibodies, and Hybrid/Stimuli-responsive delivery systems, for Oncology TSDs, Targeted Intranasal Brain Delivery, and Immunomodulation Applications (OSI). This is in conjunction with the various new preparative techniques employed in the manufacturing process, including microfluidic techniques used alongside controlled post-sizing procedures for scalable production. In order for these advantages to be realized, bilosome translators in the routine clinical practice should tackle the following bilosome-specific challenges: maintaining long-term stability without leakage/aggregation, guaranteeing consistency of batch-to-batch performance during the scale-up process, interpreting changes in functionality of complex biological fluids, enhancing IVIVC for oral systems, and meeting safety and quality expectations of multi-component nanocarriers from regulatory agencies. Looking ahead, AI/ML-based formulation optimization is one such advancement that will bring benefits. Continuous microfluidic manufacture with real-time

monitoring and next-generation bile salt mimetics are expected to be very important factors, too. By introducing standardized characterization, robust toxicology profiles, and well-designed human studies, bilosomes can advance from a promising preclinical system to a clinically impactful platform for oral vaccines, chronic therapies, and precision-targeted drug delivery.

Acknowledgments:
The authors would like to thank the managements of Sri Balaji Vidyapeeth (Deemed to be University), Puducherry, and East West College of Pharmacy, Karnataka, for supporting this project by providing the necessary facilities.

Author contributions:
Conceptualization: TL and MM; Formal Analysis: PA, SP and RD; Writing original draft preparation: TL; Writing review and editing: MM; Project administration: MM and PA. All authors have read and agreed to the published version of the manuscript.

Funding:
No external funding was received.

Data availability:
The manuscript and supplementary files include all the data generated during the study.

Ethics approval and consent to participate:
Not applicable.

Consent for publication:
Not applicable.

Competing interests:
The authors declare no competing interests.

References:

1. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. Journal of molecular biology. 1965;13(1):238-IN27.
2. Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. Nature reviews Drug discovery. 2005;4(2):145-60.
3. Uchegbu IF, Vyas SP. Non-ionic surfactant based vesicles (niosomes) in drug delivery. International journal of pharmaceutics. 1998;172(1-2):33-70.
4. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes—novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. Journal of controlled release. 2000;65(3):403-18.
5. Bleul R, Thiermann R, Maskos M. Techniques to control polymersome size. Macromolecules. 2015;48(20):7396-409.
6. Banks WA. Characteristics of compounds that cross the blood-brain barrier. BMC neurology. 2009;9(Suppl 1):S3.
7. Mehrotra S, Bg PK, Nayak PG, Joseph A, Manikkath J. Recent progress in the oral delivery of therapeutic peptides and proteins: overview of pharmaceutical strategies to overcome absorption hurdles. Advanced Pharmaceutical Bulletin. 2023;14(1):11.
8. Norheim G, Høiby EA, Caugant DA, Namork E, Tangen T, Fritzsønn E, Rosenqvist E. Immunogenicity and bactericidal activity in mice of an outer membrane protein vesicle vaccine against Neisseria meningitidis serogroup A disease. Vaccine. 2004;22(17-18):2171-80.
9. Conacher M, Alexander J, Brewer JM. Oral immunisation with peptide and protein antigens by formulation in lipid vesicles incorporating bile salts (bilosomes). Vaccine. 2001;19(20-22):2965-74.
10. Kesharwani P, Jain K, Jain NK. Dendrimer as nanocarrier for drug delivery. Progress in Polymer Science. 2014;39(2):268-307.
11. Singh B, Bandopadhyay S, Kapil R, Singh R, Katare OP. Self-emulsifying drug delivery systems (SEDDS): formulation development, characterization, and applications. Critical Reviews™ in Therapeutic Drug Carrier Systems. 2009;26(5).
12. Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, et al. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics. 2018;10(2):57.
13. Katare OP, Raza K, Singh B, Dogra S. Novel drug delivery systems in topical treatment of psoriasis: rigors and vigors. Indian journal of dermatology, venereology and leprology. 2010;76:612.
14. Azeem A, Anwer MK, Talegaonkar S. Niosomes in sustained and targeted drug delivery: some recent advances. Journal of drug targeting. 2009;17(9):671-89.
15. Ensign LM, Cone R, Hanes J. Oral drug delivery with polymeric nanoparticles: the gastrointestinal mucus barriers. Advanced drug delivery reviews. 2012;64(6):557-70.
16. Yu B, Tai HC, Xue W, Lee LJ, Lee RJ. Receptor-targeted nanocarriers for therapeutic delivery to cancer. Molecular membrane biology. 2010;27(7):286-98.
17. Sosnik A, Seremeta KP. Polymeric hydrogels as technology platform for drug delivery applications. Gels. 2017;3(3):25.
18. Vehring R. Pharmaceutical particle engineering via spray drying. Pharmaceutical research. 2008;25(5):999-1022.
19. Kamaly N, Yameen B, Wu J, Farokhzad OC. Degradable controlled-release polymers and polymeric nanoparticles: mechanisms of controlling drug release. Chemical reviews. 2016;116(4):2602-63.
20. Mozafari MR. Bioactive entrapment and targeting using nanocarrier technologies: an introduction. Nanocarrier technologies: Frontiers of nanotherapy. Springer; 2006. p. 1-16.
21. Sharma A, Sharma US. Liposomes in drug delivery: progress and limitations. International journal of pharmaceutics. 1997;154(2):123-40.

22. Szoka Jr F, Papahadjopoulos D. Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation. Proceedings of the national academy of sciences. 1978;75(9):4194-8.
23. Hood RR, DeVoe DL. High-throughput continuous flow production of nanoscale liposomes by microfluidic vertical flow focusing. small. 2015;11(43):5790-9.
24. Munir M, Zaman M, Waqar MA, Hameed H, Riaz T. A comprehensive review on transethosomes as a novel vesicular approach for drug delivery through transdermal route. Journal of Liposome Research. 2024;34(1):203-18.
25. Sonawane R, Harde H, Katariya M, Agrawal S, Jain S. Solid lipid nanoparticles-loaded topical gel containing combination drugs: an approach to offset psoriasis. Expert opinion on drug delivery. 2014;11(12):1833-47.
26. Allen TM, Cullis PR. Liposomal drug delivery systems: from concept to clinical applications. Advanced drug delivery reviews. 2013;65(1):36-48.
27. Desai MP, Labhasetwar V, Amidon GL, Levy RJ. Gastrointestinal uptake of biodegradable microparticles: effect of particle size. Pharmaceutical research. 1996;13(12):1838-45.
28. Honary S, Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems-a review (Part 2). Tropical journal of pharmaceutical research. 2013;12(2):265-73.
29. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, et al. Liposome: classification, preparation, and applications. Nanoscale research letters. 2013;8(1):102.
30. FDA U. ICH Q1A (R2): Stability testing of new drug substances and products. FDA, MD, USA. 2003.
31. Fonte P, Araújo F, Reis S, Sarmento B. Oral insulin delivery: how far are we? Journal of diabetes science and technology. 2013;7(2):520-31.
32. Dressman JB, Reppas C. In vitro–in vivo correlations for lipophilic, poorly water-soluble drugs. European journal of pharmaceutical sciences. 2000;11:S73-S80.
33. Ritger PL, Peppas NA. A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. Journal of controlled release. 1987;5(1):37-42.
34. Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. Mechanisms of solute release from porous hydrophilic polymers. International journal of pharmaceutics. 1983;15(1):25-35.
35. Balimane PV, Han Y-H, Chong S. Current industrial practices of assessing permeability and P-glycoprotein interaction. The AAPS journal. 2006;8(1):1.
36. Alshawwa SZ, Kassem AA, Farid RM, Mostafa SK, Labib GS. Nanocarrier drug delivery systems: characterization, limitations, future perspectives and implementation of artificial intelligence. Pharmaceutics. 2022;14(4):883.
37. Kaurav H, Tripathi M, Kaur SD, Bansal A, Kapoor DN, Sheth S. Emerging trends in bilosomes as therapeutic drug delivery systems. Pharmaceutics. 2024;16(6):697.
38. Yadav VK, Shukla AK. Specialized Drug Delivery Systems. Novel Carrier Systems for Targeted and Controlled Drug Delivery. 2024:391.
39. Hofmann AF, Hagey L. Bile acids: chemistry, pathochemistry, biology, pathobiology, and therapeutics. Cellular and molecular life sciences. 2008;65(16):2461-83.
40. Cevc G. Lipid vesicles and other colloids as drug carriers on the skin. Advanced drug delivery reviews. 2004;56(5):675-711.
41. Bozzuto G, Molinari A. Liposomes as nanomedical devices. International journal of nanomedicine. 2015:975-99.
42. Pandey V, Kohli S. Lipids and surfactants: the inside story of lipid-based drug delivery systems. Critical Reviews™ in Therapeutic Drug Carrier Systems. 2018;35(2).
43. Neutra MR, Kozlowski PA. Mucosal vaccines: the promise and the challenge. Nature reviews immunology. 2006;6(2):148-58.
44. Carreira B, Acurcio RC, Matos AI, Peres C, Pozzi S, Vaskovich-Koubi D, et al. Nanomedicines as multifunctional modulators of melanoma immune microenvironment. Advanced Therapeutics. 2021;4(1):2000147.
45. Venugopalan S, Mariajohn R, Chavda VP, Gondaliya P. Nanoparticle-based vaccines and future vaccine technologies. 2024.
46. Porter CJ, Charman WN. Intestinal lymphatic drug transport: an update. Advanced drug delivery reviews. 2001;50(1-2):61-80.
47. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. Nano-enabled medical applications. 2020:61-91.
48. Kabanov A, Gendelman HE. Nanomedicine in the diagnosis and therapy of neurodegenerative disorders. Progress in polymer science. 2007;32(8-9):1054-82.
49. Fadaei MR, Mohammadi M, Fadaei MS, Jaafari MR. The crossroad of nanovesicles and oral delivery of insulin. Expert Opinion on Drug Delivery. 2023;20(10):1387-413.
50. Parida P, Prusty AK, Prusty RK, Jena BR, Panda D, Das S. Overcoming hurdles in oral protein and peptide drug delivery: Strategies for future prospective. Advancement in Animal Handling and Generative AI for Pre-clinical Studies. CRC Press; 2025. p. 28-41.
51. Srivastava AK, Singh D, Singh RK. Drug-delivery systems of phytochemicals as therapeutic strategies in cancer therapy. Elsevier; 2023.

52. Holmgren J, Czerkinsky C. Mucosal immunity and vaccines. Nature medicine. 2005;11(Suppl 4):S45-S53.
53. Plotkin S. History of vaccination. Proceedings of the National Academy of Sciences. 2014;111(34):12283-7.
54. Read RC, Naylor SC, Potter CW, Bond J, Jabbal-Gill I, Fisher A, et al. Effective nasal influenza vaccine delivery using chitosan. Vaccine. 2005;23(35):4367-74.
55. Cevc G, Vierl U. Nanotechnology and the transdermal route: A state of the art review and critical appraisal. Journal of controlled release. 2010;141(3):277-99.
56. Lochhead JJ, Thorne RG. Intranasal delivery of biologics to the central nervous system. Advanced drug delivery reviews. 2012;64(7):614-28.
57. Hanson LR, Frey WH. Intranasal delivery bypasses the blood-brain barrier to target therapeutic agents to the central nervous system and treat neurodegenerative disease. BMC neuroscience. 2008;9(Suppl 3):S5.
58. Dhuria SV, Hanson LR, Frey II WH. Intranasal delivery to the central nervous system: mechanisms and experimental considerations. Journal of pharmaceutical sciences. 2010;99(4):1654-73.
59. Danhier F, Feron O, Préat V. To exploit the tumor microenvironment: Passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. Journal of controlled release. 2010;148(2):135-46.
60. Peer D. Harnessing RNAi nanomedicine for precision therapy. Molecular and cellular therapies. 2014;2(1):5.
61. Nowacek A, Gendelman HE. NanoART, neuroAIDS and CNS drug delivery. Nanomedicine. 2009;4(5):557-74.
62. Altamimi MA, Hussain A, Mahdi WA, Imam SS, Alshammari MA, Alshehri S, Khan MR. Mechanistic insights into luteolin-loaded elastic liposomes for transdermal delivery: HSPiP predictive parameters and instrument-based evidence. ACS omega. 2022;7(51):48202-14.
63. Đorđević S, Gonzalez MM, Conejos-Sánchez I, Carreira B, Pozzi S, Acúrcio RC, et al. Current hurdles to the translation of nanomedicines from bench to the clinic. Drug delivery and translational research. 2022;12(3):500-25.
64. Siafaka PI, Üstündağ Okur N, Karavas E, Bikiaris DN. Surface modified multifunctional and stimuli responsive nanoparticles for drug targeting: current status and uses. International journal of molecular sciences. 2016;17(9):1440.
65. Karra N, Benita S. The ligand nanoparticle conjugation approach for targeted cancer therapy. Current drug metabolism. 2012;13(1):22-41.
66. Tu Q, Zhang Y, Liu R, Wang J-C, Li L, Nie N, et al. Active drug targeting of disease by nanoparticles functionalized with ligand to folate receptor. Current medicinal chemistry. 2012;19(19):3152-62.
67. Mura S, Nicolas J, Couvreur P. Stimuli-responsive nanocarriers for drug delivery. Nature materials. 2013;12(11):991-1003.
68. Rout SR, Gowtham K, Sheikh A, Parvez S, Dandela R, Kesharwani P. Recent advances and future prospective of hybrid drug delivery systems. Hybrid Nanomaterials for Drug Delivery. 2022:357-74.
69. Tan S, Li X, Guo Y, Zhang Z. Lipid-enveloped hybrid nanoparticles for drug delivery. Nanoscale. 2013;5(3):860-72.
70. Bae YH, Park K. Targeted drug delivery to tumors: myths, reality and possibility. Elsevier; 2011. p. 198-205.
71. Paresishvili T, Kakabadze Z. Challenges and opportunities associated with drug delivery for the treatment of solid tumors. Oncology reviews. 2023;17:10577.
72. Steichen SD, Caldorera-Moore M, Peppas NA. A review of current nanoparticle and targeting moieties for the delivery of cancer therapeutics. European journal of pharmaceutical sciences. 2013;48(3):416-27.
73. Pouton CW, Porter CJ. Formulation of lipid-based delivery systems for oral administration: materials, methods and strategies. Advanced drug delivery reviews. 2008;60(6):625-37.
74. Porter CJ, Pouton CW, Cuine JF, Charman WN. Enhancing intestinal drug solubilisation using lipid-based delivery systems. Advanced drug delivery reviews. 2008;60(6):673-91.
75. Daka A, Peer D. RNAi-based nanomedicines for targeted personalized therapy. Advanced drug delivery reviews. 2012;64(13):1508-21.
76. Alshehri S, Imam SS, Rizwanullah M, Akhter S, Mahdi W, Kazi M, Ahmad J. Progress of cancer nanotechnology as diagnostics, therapeutics, and theranostics nanomedicine: preclinical promise and translational challenges. Pharmaceutics. 2020;13(1):24.
77. Fonte P. Lívia Barroso", Cláudia Viegas"*", João Vieira", Cíntia Ferreira-Pêgo*, J oyce Costa". Journal of Food Engineering. 2021;295(1):110451.
78. Desai N. Challenges in development of nanoparticle-based therapeutics. The AAPS journal. 2012;14(2):282-95.
79. Abdelwahed W, Degobert G, Stainmesse S, Fessi H. Freeze-drying of nanoparticles: formulation, process and storage considerations. Advanced drug delivery reviews. 2006;58(15):1688-713.
80. Maher S, Leonard TW, Jacobsen J, Brayden DJ. Safety and efficacy of sodium caprate in promoting oral drug absorption: from in vitro to the clinic. Advanced drug delivery reviews. 2009;61(15):1427-49.
81. Salama NN, Eddington ND, Fasano A. Tight junction modulation and its relationship to drug delivery. Advanced drug delivery reviews. 2006;58(1):15-28.

82. Deng F. Transport Pathways of Bile Acid-Modified Nanoparticles in Apical Sodium-Dependent Bile Acid Transporter Expressing Cells. The University of Utah; 2022.
83. Varkouhi AK, Scholte M, Storm G, Haisma HJ. Endosomal escape pathways for delivery of biologicals. Journal of Controlled Release. 2011;151(3):220-8.
84. Miao J, Gao P, Li Q, He K, Zhang L, Wang J, Huang L. Advances in nanoparticle drug delivery systems for anti-hepatitis B virus therapy: a narrative review. International Journal of Molecular Sciences. 2021;22(20):11227.
85. Knop K, Hoogenboom R, Fischer D, Schubert US. Poly (ethylene glycol) in drug delivery: pros and cons as well as potential alternatives. Angewandte chemie international edition. 2010;49(36):6288-308.
86. Clayton EW, Rusch E, Ford A, Stratton K. Adverse effects of vaccines: evidence and causality. 2012.
87. Ventola CL. Progress in nanomedicine: approved and investigational nanodrugs. Pharmacy and Therapeutics. 2017;42(12):742.
88. Jacob S, Varkey NR, Boddu SH, Gorain B, Rao R, Nair AB. Advances in Lipid-Polymer Hybrid Nanoparticles: Design Strategies, Functionalization, Oncological and Non-Oncological Clinical Prospects. Pharmaceuticals. 2025;18(12):1772.
89. Moghimi SM, Hunter AC, Murray JC. Long-circulating and target-specific nanoparticles: theory to practice. Pharmacological reviews. 2001;53(2):283-318.
90. Hari Priya VM, Ganapathy A A, Veeran MG, Raphael M S, Kumaran A. Nanotechnology-based drug delivery platforms for erectile dysfunction: addressing efficacy, safety, and bioavailability concerns. Pharmaceutical Development and Technology. 2024;29(9):996-1015.
91. Khan FA. Major nano-based products: nanomedicine, nanosensors, and nanodiagnostics. Applications of Nanomaterials in Human Health. Springer; 2020. p. 211-28.
92. Vamathevan J, Clark D, Czodrowski P, Dunham I, Ferran E, Lee G, et al. Applications of machine learning in drug discovery and development. Nature reviews Drug discovery. 2019;18(6):463-77.
93. Elbadawi M, Gaisford S, Basit AW. Advanced machine-learning techniques in drug discovery. Drug Discovery Today. 2021;26(3):769-77.
94. JG Marin J, IR Macias R, Briz O, M. Banales J, J. Monte M. Bile acids in physiology, pathology and pharmacology. Current drug metabolism. 2016;17(1):4-29.
95. Pasupathy R, Pandian P, Selvamuthukumar S. Nanobubbles: a novel targeted drug delivery system. Brazilian Journal of Pharmaceutical Sciences. 2022;58:e19608.
96. Garg S, Kumar M, Maurya R, Alam MI, Karwal A, Yadav AK, et al. Specialized Drug Delivery Systems: An Overview. Novel Carrier Systems for Targeted and Controlled Drug Delivery. 2024:391-458.
97. Lochhead JJ, Davis TP. Perivascular and perineural pathways involved in brain delivery and distribution of drugs after intranasal administration. Pharmaceutics. 2019;11(11):598.
98. Deng Z, Siraj AA, Lowry I, Ruan E, Patel R, Gao W, et al. Nanoparticle-based delivery systems for synergistic therapy in lung cancers. Bioengineering. 2025;12(9):968.