MINI REVIEW

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Bilosomes as a Versatile Drug Delivery System: Preparation Techniques and Biomedical Application

Heba A. Ghanem [a] *, Nashwa H. Abd Elwahab*[a], *Mamdouh Ghorab* [b], *Ali M. Nasr* [c,d], *Shadeed Gad* [b*]

[a] *Department of Pharmaceutics, Faculty of Pharmacy, Sinai University, Al Qantarah Sharq 41636, Egypt*
[b] *Department of Pharmaceutics, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt*
[c] *Department of Pharmaceutics, Faculty of Pharmacy, Port Said University, Port Said 42526, Egypt*
[d] *Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Galala University, New Galala 43713, Egypt*

Received: 10. 06. 2024

Revised: 01. 07. 2024

Accepted: 03. 07. 2024

*Correspondence Author:

Tel: +2 01003934422

E-mail address:

shaded_abdelrahman@pharm.suez.edu.eg

Abstract

Bilosomal nanostructures with bile salts incorporated into the lipid bilayer of the membrane are abundant. Drug-loaded bilosomes have received considerable attention as drug delivery systems for a variety of drugs. The properties of bile salts are crucial in drug delivery systems. One of the most fascinating research areas nowadays is the use of bilosomes to produce biotechnology products and innovative vaccines. thus,somes have thus been developed as a potential vesicular carrier system for oral vaccine delivery. Besides oral vaccination, oral drugs susceptible to lysis by natural bile salts can be incorporated into bilosomes. The possibility of using bilosomes for transdermal, ocular, and intranasal applications is a further benefit. The current article also discusses bilosome composition and formulation techniques. The impact of newly employed nonionic surfactants and the physicochemical properties of bile salts on the formulation of bilosomes as well as their benefits over traditional nanocarriers are also covered.

Keywords: Bilosomes, Drug Delivery System, Biomedical Application

1. Introduction

Different drug-delivery technologies have developed throughout the last few decades (Abbas, Gad, et al. 2021; Hussain et al. 2020; Abdellatif et al. 2022; Shewaiter et al. 2021; 2022). The use of drug-loaded bilosomes as drug-delivery systems has attracted a lot of attention (Rajput and Chauhan, n.d.; M. A. El-Nabarawi et al. 2020a).

Recently, bilosomes have been studied by numerous authors under a variety of scientific names, including nano bilosomes, bile salt (BS) stabilized vesicles, BS-reinforced liposomes, and liposomes containing BS (Y. Elnaggar 2015). Different types of bilosomes enabled targeted drug delivery (Rajput and Chauhan, n.d.). Because of their capacity to withstand gastrointestinal enzymes and provide

protection to the loaded active ingredient, bilosomes are used in the effective oral delivery of medications and vaccinations (Guan et al. 2016).

Liposomes are among the most extensively researched nanocarriers for drug delivery (P. Liu, Chen, and Zhang 2022; N. Wang, Chen, and Wang 2019; Crommelin, van Hoogevest, and Storm 2020; M. Li et al. 2019; A. Gouda et al. 2021). They are spherical vesicular structures composed of phospholipids with two hydrophobic tails and a hydrophilic head. Sizes of liposomes range from 30 nm to micrometers having a phospholipid bilayer that is 4-5 nm thick [5]. Liposomes have several advantages as a drug delivery technology, including excellent biodegradability and biocompatibility with low toxicity, self-assembly, easy removal from the body, and improved efficacy and bioavailability of encapsulated drugs (Andra et al. 2022). Most crucially, this kind of nano carrier's phospholipid bilayer and aqueous core allows for the encapsulation of pharmaceuticals that are both hydrophobic and hydrophilic as illustrated in Figure. 1 (A. Gouda et al. 2021; Nasr et al. 2022).

Liposomes lack chemical and physical stability, which can cause processes like aggregation, flocculation, or coalescence, which ultimately modify the size of the nanostructure (Olusanya et al. 2018; Yu et al. 2021). Bilosomes are flexible, nanoscale vesicles comprised of lipid, surfactant, and bile salt (Figure 1)(Zafar et al. 2021; M. A. El-Nabarawi et al. 2020a; Rajput and Chauhan, n.d.)It structurally resembles a liposome and guards the drug molecule against enzymatic breakdown to GIT. It was first introduced by Conacher et al. (Conacher, Alexander, and Brewer 2001a). By inducing vesicle membrane lysis, the bile salt present in the gastrointestinal tract (GIT) lowers the capacity of the conventional vesicle (Pavlović et al. 2018). It causes the drug to be released earlier than expected or a molecule that was previously confined before it could reach the site of action (Y. Elnaggar 2015). The use of bilosomes as a delivery system has gained wide acceptance as a way to get around the drawbacks of conventional nano-vesicular drug delivery systems (Pavlović et al. 2018; Y. S. R. Elnaggar et al. 2019a; Nemati et al. 2022; M. A. El-Nabarawi et al. 2020b).

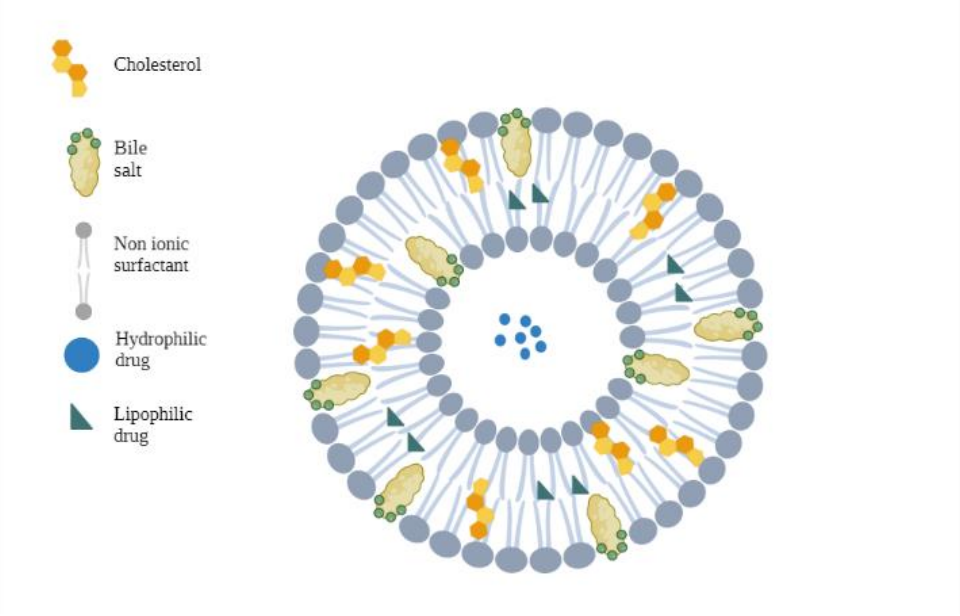

Figure 1. Bilayer structure of bilosome showing entrapment area of hydrophilic and lipophilic drug.

This article describes the various biomedical applications and routes of administration of bilosomes. The protocol for preparation of drug-loaded bilosomes *via* thin-film hydration, hot homogenization method, reverse-phase evaporation method and ethanol injection method. In addition, the critical aspects affect on physicochemical properties of bilosomes. This study investigates the various methods of bilosomes administration as well as their various therapeutic properties.

2. Biomedical uses for bilosomes and delivery methods

Bilosomes have been used as promising delivery carriers for a wide range of cargoes via a variety of administration routes. The following sections provide a brief discussion of the role of bilosomes in medication delivery for various therapeutic uses via a variety of administration routes**. (**Figure .2**)**

2.1. Oral delivery

Considering safety, convenience, and patient compliance, it is generally agreed that the oral route is the best way to administer medication (Abouelmaati et al. 2023). Drugs that are poorly soluble, unstable in the acidic environment of the stomach, and susceptible to the first pass effect, on the other hand, have poor oral bioavailability (Yadav et al. 2022; Wahab et al. 2022). One of the most innovative vesicular nanocarriers, bilosomes incorporate bile salts into the niosome membrane. Compared to previous nano-vesicular carrier systems, these are more ultra-deformable, elastic, and flexible. Bile salt in the GIT restricts the effectiveness of these typical nano vesicular carriers by triggering vesicle membrane distortion and lysis, which causes the encapsulated molecule to release early before reaching the intended location of the action (Nemati et al. 2022; C. Liu et al. 2022). Traditional nano vesicular carriers can't shield the medicine from intestinal bile salt, including liposomes and niosomes. Bilosomes or bile salt stabilized nano vesicular systems, were created to address problems with traditional nano vesicular carrier systems by integrating bile salts into the lipid bilayers of traditional nano vesicular systems. They repel the intestinal bile salts in the GIT due to this arrangement providing greater stability (Saifi et al. 2020). Various medications have been reported to be delivered orally using bilosomes in the literature such as (acyclovir, bioactive apigenin, eprosartan mesylate, risedronate, curcumin and torularhodin) (Zafar et al. 2021; Saifi et al. 2020; Ahad et al. 2018; Y. S. R. Elnaggar et al. 2019b; Hegazy et al. 2022; C. Liu et al. 2022). As illustrated in (Table).

2.2. Intranasal delivery

Drug administration using transdermal, topical, and ocular bilosomes has been used. The effects of intranasal administration of drug-loaded bilosomes on drug absorption and brain targeting, however, have not been thoroughly investigated. The nasal route has various disadvantages, including the small surface area of the nasal mucosa and insufficient drug absorption due to rapid clearance caused by ciliary activity. These restrictions can be reduced by using a drug carrier that is made properly (El Taweel et al. 2021). Different medications have been effectively delivered using bilosomes such as resveratrol and zolmitriptan (Abbas, Refai, et al. 2021a; El Taweel et al. 2021). El Taweel et al. reported that intranasal administration of zolmitriptan loaded through mucoadhesive, bilosomes gel provided direct nose-to-brain drug targeting with improved brain bioavailability. The newly created zolmitriptan formulation offered migraine sufferers an effective intranasal substitute with increased therapeutic effects (El Taweel et al. 2021). Abbas et al, demonstrated that resveratrol intranasally administered through brain targeting by the olfactory mucosa improved brain and memory functions, lowering levels of pro-inflammatory markers and suppressing the expression of NF-B and P38 (Abbas, Refai, et al. 2021a).

2.3. Topical delivery

Bile salts are contained in bilosomes, which contain non-ionic amphiphiles that resemble niosomes in closed, double-layered systems. With the aid of bile salts, bilosomes can be more flexible vesicles and pass through the stratum corneum to deliver medication to deeper skin layers . Some studies have demonstrated the significance of bilosomes in the successful administration of vaccinations and drugs both orally and topically (Abbas, Gad, et al. 2021; Salem et al. 2022).

Vesicular nanocarriers have received a lot of attention because of their potential to make drugs more soluble and to help them penetrate deeper layers of skin. By temporarily disrupting a highly structured lipid bilayer structure, the topical nanosized formulations may improve drug delivery (Bashir et al. 2021). A study on in vivo skin deposition found that Olmesartan from bilosomal formulation retained in the skin of rats more frequently than Olmesartan from transethosomes or drug dispersion (Albash et al. 2019). Comparing terconazole bilosomes to terconazole suspension, microbiological analysis showed that terconazole bilosomes effectively inhibited the growth of *Candida albicans*. Furthermore, due to their highly deformable characteristics and capacity to permeate the skin, terconazole bilosomes demonstrated

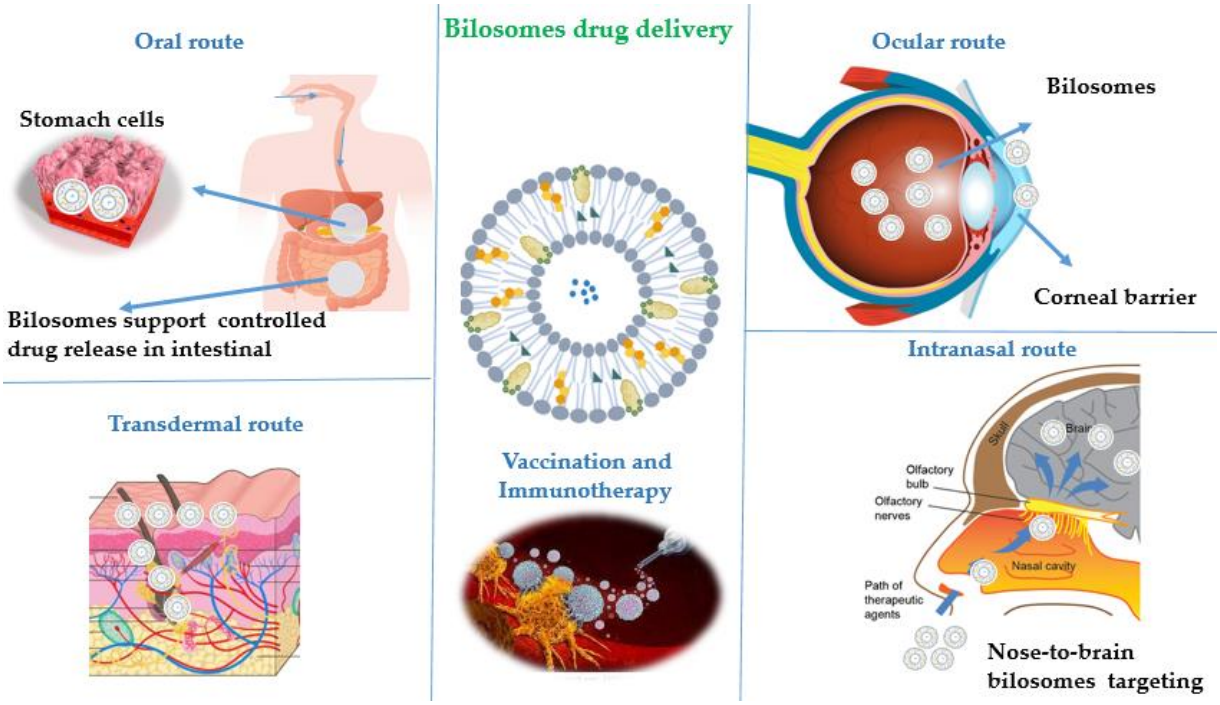

Figure 2. Bilosomes are effective drug delivery methods capable of loading both hydrophilic and lipophilic drugs with different delivery routes.

superior deposition in the skin when compared to conventional bilosomal formula and terconazole solution. Furthermore, terconazole bilosomes proved their dermatological safety upon application on the skin. Overall, terconazole bilosomes are promising vesicles for topical administration of terconazole for treating skin fungal infections (Mosallam et al. 2021a). Other examples are illustrated in (Table 1).

2.4. Ocular delivery

Due to the many anatomical barriers of the eye, including the layers of the cornea, sclera, and retina, as well as the lymphatic tear turnover, nasolacrimal drainage, and reflex blinking, ocular drug delivery is difficult (Attia and MacKay 2022). Because of their structure, the created bilosomal formulation offers promise as a nanocarrier for the ocular delivery of numerous medications (El Taweel et al. 2021; Mosallam et al. 2021a). Acetazolamide has been successfully delivered by bilosomes into the eyes to treat high IOP (Mohsen, Salama, and Kassem 2020). Agomelatine is a drug with two uses. When used orally, it acts as an antidepressant and when given topically to the eyes it acts as an antiglaucoma agent (Nemr, El-Mahrouk, and Badie 2022).

3. Factors affecting on physicochemical properties of bilosomes.

3.1. Lipids

3.1.1. Cholesterol

Bilosomes become more rigid when an amphiphilic molecule is inserted into the cellular membrane, with the hydroxyl groups towards the aqueous surface and the aliphatic chains lining up parallel to the acyl chains in the center of the bilayer (Palekar-Shanbhag et al. 2020). It affects the membrane's permeability and entrapment efficiency (Kumavat et al. 2021). Additionally, the average particle size (PS) significantly increased as a result of the increasing cholesterol molar ratio. According to reports, having a high level of cholesterol prevents the lipids in vesicles from packing tightly, which improves the distribution of the aqueous phase inside the liposomal vesicle and raises PS . An increase in the drug entrapped was accompanied by an increase in the cholesterol molar ratio. Cholesterol has been shown to increase the hydrophobicity and rigidity of lipidic bilayer membranes, resulting in increased membrane stability, which is accompanied by decreased drug permeability and hence greater drug retention.

Table 1. Different routes of administration by bilosomes**.**

Drug	Bilosomal composition	Method of preparation	Delivery route	Research outcome
Acyclovir	Span 60 and Tween 60 , cholesterol and Sodium glycocholate.	Thin film hydration technique	Orally	Compared to commercial formulation and ACV suspension, respectively, albino Wistar rats given with improved bilosomes demonstrated 2.5 and 4.3 fold better (p 0.05) relative bioavailability, as predicted (Saifi et al. 2020).
Eprosartan mesylate	Soybean phosphatidylcholine and sodium deoxycholate.	Thin film hydration technique	Orally	In vivo, a study performed on Wistar rats showed that the created formulation displayed renal protective effects by lowering oxidative stress and attenuating the increased expression of the transforming growth factor-1 (TGF β1) receptor, inducible nitric oxide synthase (iNOS), and angiotensin II type 1 receptor in streptozotocin-induced diabetic rats (Ahad et al. 2018).
Bioactive Apigenin (AG)	Cholesterol, Span 60 and sodium deoxycholate.	Thin film evaporation method	Orally	When compared to free AG dispersion, the optimized AG bilosomes increase relative bioavailability by 4.67-fold in Wistar albino rats. Increased bilosome uptake by Peyer's patch intestinal Micelles resulted in increased bioavailability (Zafar et al. 2021).
Risedronate (RS)	Soybean phosphatidylcholine, Cholesterol Sodium deoxycholate, sodium taurocholate and Sodium tauroglycocholate.	Thin film hydration technique and Reversed-phase evaporation technique	Orally	Anionic bilosomes outperformed typically used liposomes in lowering the toxicity of medicines administered orally. Anionic bilosomes addressed RS oral challenges by increasing intestinal permeability and decreasing intestinal toxicity. Although cationic bilosomes showed unexpected stability in GIT fluids and shelf life stability, their utility may be restricted by their induced oral toxicity (Y. S. R. Elnaggar et al. 2019b).
Curcumin (CUR)	Span 60, cholesterol, Sodium taurocholate and Sodium cholate.	Thin film hydration technique	Orally	In comparison to the uptake efficiency of CUR suspension, the cellular uptake efficiency of CUR via established Caco-2 cells was higher from TPGS-Bil and CUR-Bil. Following 48 hours of incubation of Doxorubicin-Resistant Breast Cancer (MCF-7/ADR) cell lines, TPGS-CUR-Bil demonstrated an excellent response in the form of a dominant reduction in IC50 value against multidrug-resistant (MDR) tumors (Hegazy et al. 2022).
Torularhodin	Lecithin, cholesterol, sodium deoxycholate and Tween80.	Thin film hydration	Orally	The AUC of bilosomes was 2.73 times that of liquid-lip in mice, and the t 1/2 value was up to 22.5 h, indicating that bilosomes might be used as a delivery method to increase the bioavailability of torularhodin (C. Liu et al. 2022).
Sofosbuvir	Span 60, L-a Phosphatidylcholine, and sodium taurocholate.	Thin film hydration	Orally	Results from in vivo tests showed that the produced formula might raise medication availability in the target organ. When compared to the corresponding drug solution, The availability of sofosbuvir in the liver was significantly increased by the galactose-anchored and taurocholate-stabilized bilosomes.

Zolmitriptan	Sodium deoxycholate, cholesterol and Span 40.	Thin film hydration technique	Intranasal	Sol-gel temperature for the bilosomal formulation mucoadhesive gel was 34.03 °C, and nasal mucociliary transit time was 22.36 min. Higher C max and AUC0- values, combined with longer tmax homogenized brain tissue values, demonstrated the gel's superiority over free bilosomal dispersion (El Taweel et al. 2021).
Resveratrol	Sodium deoxycholate, Span 60 and cholesterol.	Thin film hydration	Intranasal	Resveratrol is administered intranasally to the brain, where it is targeted by the olfactory mucosa to enhance brain and memory function, decrease pro-inflammatory marker levels, and reduce NF-B and P38 expression (Abbas, Refai, et al. 2021b).
Terconazole	Sodium taurocholate, Span 60, Tween 80, Cremophor EL, and Cremophor RH 40.	Thin film hydration	Transdermal	The ex vivo corneal permeation study of albino male rabbits revealed that the investigated PEGylated bilosome exceeded drug permeation using traditional bilosomes, niosomes, and drug suspension.
Olmesartan medoxomil	L-α phosphatidylcholine , Brij 20, Brij52, sodium deoxycholate, sodium taurocholate, Span 60, and cholesterol.	Thin film hydration	Transdermal	The findings proved that optimized olmesartan-Bil by eliminating its extensive first-pass metabolism and oral issues could be a promising TDDS for olmesartan (Albash et al. 2019).
Tenoxicam	Span 40, Span 60, or Span 80, cholesterol and sodium deoxycholate.	Thin film hydration followed by sonication	Transdermal	Bilosomes increase drug penetration and deposition in the skin, which is necessary for the transdermal distribution of tenoxicam, according to ex vivo skin permeation and in vivo skin deposition tests on male Wistar rats (Al-mahallawi, Abdelbary, and Aburahma 2015).
Lornoxicam	Sodium deoxycholate and soybean phosphatidylcholine.	Thin film hydration	Transdermal	LX-loaded bilosomes had been successful in enhancing the ex vivo skin permeability (326.101.57 g/cm2) (Ahmed, Kassem, and Sayed 2020).
Diacerein	Cholesterol, span 40, Span 60, sodium cholate, sodium taurocholate and sodium glycocholate.	Thin film hydration	Transdermal	Diacerein bilosomes had significantly more diacerein deposited than conventional niosomal formulations and drug suspensions (P 0.05). The AUC0 →10 for diacerein bilosome was approximately 3 folds higher than that of niosomal formulations that are typically used and nearly 6 folds higher than that of drug suspension (Aziz, Abdelbary, and Elassasy 2019).
Dapsone	Sodium taurocholate hydrate, sodium cholate hydrate, sodium deoxycholate,	Thin film hydration technique	Transdermal	About 1.5 times as much dapsone was retained in the skin after treatment with bilosomes as it was in the skin after treatment with dapsone alcoholic solution. Dapsone-loaded bilosomes showed normal histological structures characterized by the

	Span 60 and cholesterol.			absence of defects or inflammation (M. A. El-Nabarawi et al. 2020a).
Terbutaline sulfate (TBN)	Sodium deoxycholate, cholesterol, and soybean phosphatidylcholine.	Thin film hydration technique	Transdermal	Male Wistar rats had a relative bioavailability of TBN of about 233.62% from the transdermal TBNCTS-BLS gel compared to an oral solution of TBN of 83.64% from the transdermal TBN gel (El Menshawe et al. 2020).
Acetazolamide (ACZ)	Span 60, deoxycholic acid sodium salt, cholic acid sodium salt, taurocholic acid sodium salt and tauroglycocholic acid sodium salt.	Thin film hydration	Ocular	Researchers were able to show enhanced and sustained intraocular pressure (IOP) reducing abilities by contrasting the optimized ACZ bilosomal formulation to plain ACZ, commercially available dorzolamide eye drops, and commercially available ACZ oral tablets. An ocular Draize irritancy test was also used to confirm the safety of the improved bilosomal formulation after ocular instillation (Mohsen, Salama, and Kassem 2020).
Agomelatine (AGO)	Egg yolk L-a phosphorylcholine, sodium cholate, sodium deoxycholate, and sodium taurocholate.	A modified ethanol injection method	Ocular	The measured pharmacodynamic parameters for the AGO formula (%decrease in IOP max, T max, MRT, and AUC0-24h) versus the AGO solution in the in vivo study. These findings indicated that, when compared to the AGO solution, the optimized formula could result in a higher reduction in IOP and significantly increased AGO bioavailability(Nemr, El-Mahrouk, and Badie 2022).

3.1.2. Phospholipids

The biocompatibility of phospholipids with cellular membrane is quite good (J. Li et al. 2015). They have a self-assembling nature due to their amphiphilic nature, which causes wetting and emulsification. Due to their amphiphilic nature, phospholipids can form closed concentric bilayers when there is water present. Because they have high emulsifying properties, phospholipids can help to stabilize emulsions (Pavlović et al. 2018). Commonly used phospholipids in preparation of bilosomes (dicetyl phosphate, soybean phosphatidylcholine, dipalmitoyl phosphatidylethanolamine and soybean phosphatidylcholine) (Y. S. R. Elnaggar et al. 2019a; Zafar et al. 2021; Shukla et al. 2010; Jain et al. 2014b).

3.2. Nonionic Surfactants

Since nonionic surfactants are more stable and compatible than anionic, cationic, or amphoteric forms. They are frequently utilized in the manufacture of bilosomes (Kumar and Rajeshwarrao 2011). The type of surfactant significant influence on PS. The PS of the bilosomes was demonstrated to increase by lowering the HLB value from Span 60 (HLB 4.7) to Span 40 (HLB 6.7). The vesicles' ability to absorb more water as a result of increased surfactant hydrophilicity increased PS (Aziz, Abdelbary, and Elassasy 2019)The increase in the entrapment efficiency EE% occurred in the following order: Span 60 (C18) > Span 40 (C16) > Span 80 (C18). Span 60 (C18) and Span 40 (C16) share the same head group, but due to different alkyl chain lengths, they exhibit different levels of lipophilicity. Increasing alkyl chain length is often related to EE% rises as a result of enhanced surfactant lipophilicity. (Al-mahallawi, Abdelbary, and Aburahma 2015). The highest absolute zeta potential (ZP) was seen in bilosomes made with Span 60, which was likely due to its improved ability to encapsulate tenoxicam

when compared to the other surfactants under investigation (Al-mahallawi, Abdelbary, and Aburahma 2015).

3.3. Bile Salts

The membrane bilayer contains embedded bilosomal nanostructures with bile salts. Bile salts are steroidal-backboned surfactants that are present in nature that act as edge activators of the nanocarrier and have special self-organization properties (Waglewska, Pucek-Kaczmarek, and Bazylińska 2022). When absorption is hindered by factors like low membrane permeability or low water solubility, bile salts work as biosurfactants to enhance the bioavailability of the active payload (Pavlović et al. 2018). Hence could encourage numerous medications and drugs to have better bioavailability and longer half-lives. Many bile salts were used in preparing bilosomes sodium deoxycholate (SDC) sodium glycocholate (SGC) sodium taurocholate (STC) Sodium Taurodeoxycholate (STDC), sodium tauroglycocholate (STGC) and deoxycholic acid (DCA). The bile salt concentration have a significant factor in physicochemical properties (Qi et al. 2013). As bile salt concentration was raised, the PS of the bilosomes also increased. This could result from the used bile salt's anionic nature (sodium taurocholate) in which vesicles with significant negative ZP values were generated (Mosallam et al. 2021a). Due to the repulsion that developed between the bilayers, the size of the vesicles increased. It was found by S. Mosallam et al. (Mosallam et al. 2021a)Raising the bile salt reduced the EE%. The potential of mixed micelles leads to an increase in drug solubility in the dispersion medium when the bile salt content is raised, which lowers the EE%.

3.4. Edge Activators

The PS was found to be considerably influenced by edge activators. Many edge activators were used in preparing bilosomes such as (Cremophor EL, Cremophor RH 40, Brijo20 (polyoxyethylene (20) oleyl ether), Brij52 and Pluronic P123 (Abdelbary, Abd-Elsalam, and Al-mahallawi 2016). The PS of the bilosomes prepared with Cremophor RH 40 was significantly lower than those prepared with Cremophor EL. Both Cremophor RH 40 and Cremophor EL have hydrophilic and hydrophobic moieties in their structures. Both edge activators contain polyethylene oxide (PEO) units in their hydrophilic moiety, which serve as steric barriers to prevent the aggregation of nanoparticles. The fact that Cremophor RH includes 40 PEO units while Cremophor EL has 35 units may be the cause of the difference in stabilizing effectiveness and, subsequently, PS. (Madheswaran et al. 2014). Also type of edge activators was found to insignificantly influence the ZP (Abdelbary, Abd-Elsalam, and Al-mahallawi 2016). According to the results, EA type significantly affected the PS of the vesicles. In comparison to the Brij 20 formula, the PS of the formula containing Brij52 was greater. Pluronic P123 has emerged as one of the most promising amphiphilic block copolymers based on poly (ethylene glycol) (PEG) for controlled and targeted drug delivery (Waglewska, Pucek-Kaczmarek, and Bazylińska 2020).

4. Methods for preparation of bilosomes

The literature reports several techniques for preparing bilosomes, including thin-film hydration, ethanol injection, reverse-phase evaporation, hot homogenization, and proniosomal methods. This paper provides a summary of some of these techniques and a thorough explanation of the thin-film hydration and ethanol injection approach, which is regarded as an effective, straightforward, and easily scaleable technology.

4.1. Thin-film hydration

The thin-film hydration technique has long been utilized to load the lipophilic drug. (P. Liu, Chen, and Zhang 2022). This method was adopted to manufacture bilosomes (Dai et al., 2013; Guan P, Lu Y, Qi J, 2011). A mixture of organic solvents was used to dissolve the calculated amounts of phospholipids, bile salt, and drug (Figure. 3). Commonly used solvents (methanol, chloroform and dichloromethane) (Qi et al. 2013; Bashyal et al. 2018) . Solvent evaporation was carried out in a rotary evaporator with a vacuum at 60 °C for 2 h following the formation of the dry residue to ensure that all of the organic solvents were removed. After that, phosphate buffer saline was used to hydrate the produced film. Till milky dispersion was formed, the hydration was carried out in a rotary evaporator set at 600 rpm for 1 hour at 60 °C (Abdelalim, Abdallah, and Elnaggar 2020). After that, the latter was sonicated and subsequently homogenized to create the small unilamellar structure of BS (Guan et al. 2016; Zafar et al. 2021).

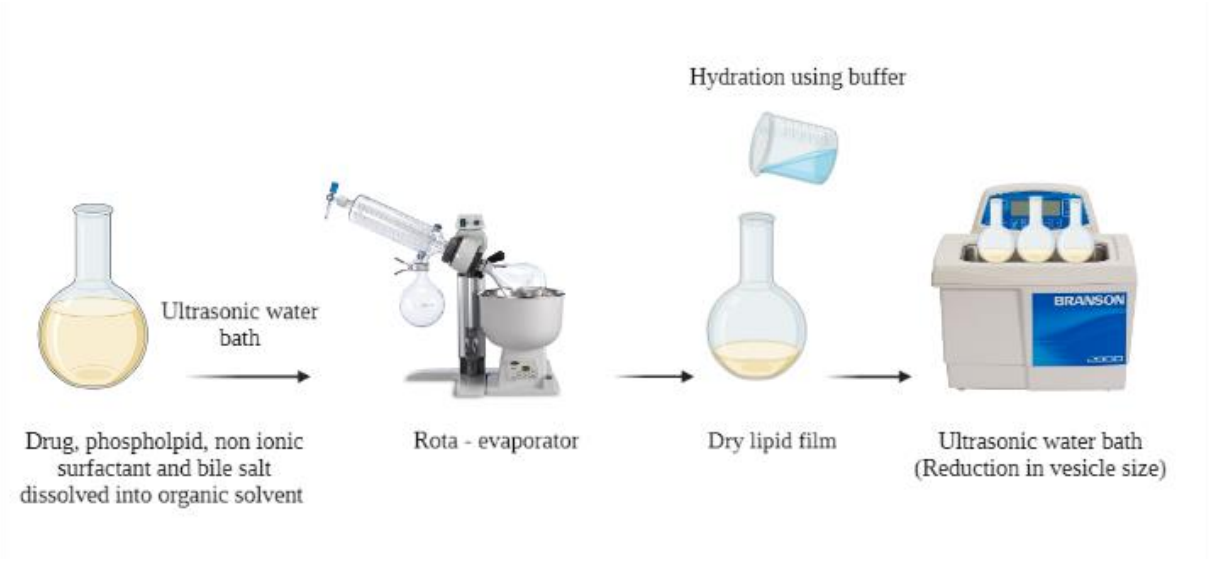

Figure 3. Thin-film hydration method.

4.2. Ethanol injection method

In summary, the predicted amounts of phospholipids, bile salt, and drug were dissolved in organic solvents. Phosphate buffer pH 7.4 was maintained at 60 ^{o}C and received the newly generated solution (Abdelbary, Abd-Elsalam, and Al-mahallawi 2016; L. Wang et al. 2021). A magnetic stirrer operating at 600 rpm was used to stir the mixture for at least an hour at room temperature in order to evaporate the solvent (Figure. 4).

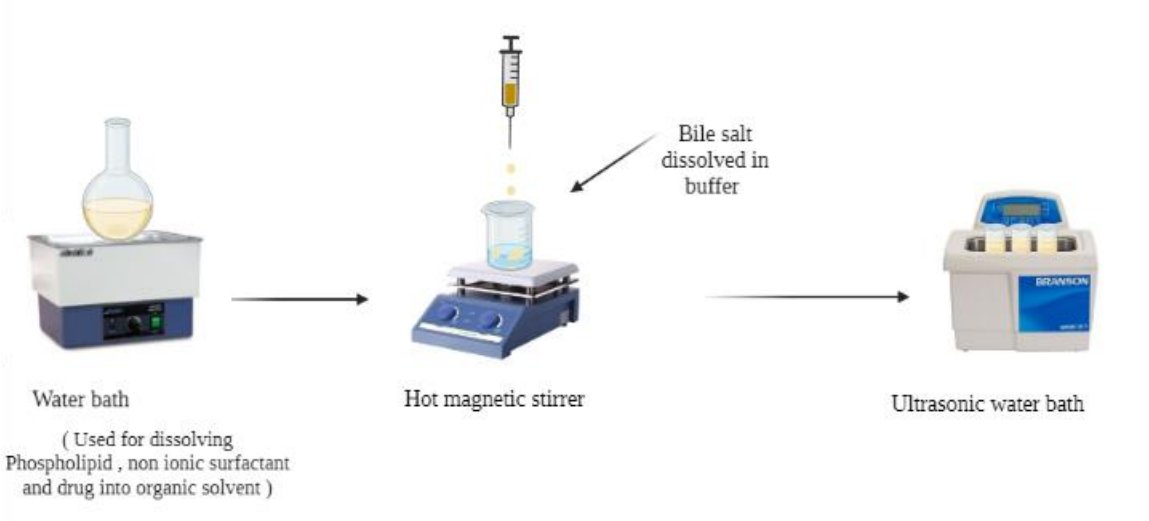

Figure 4. Ethanol injection method.

4.1. Reverse-phase evaporation method.

The reverse-phase evaporation process was used to produce bilosomes. This procedure involved dissolving phospholipids and bile salts in ethyl ether, mixing them with a drug-containing buffered solution, and then ultrasonically processing the mixture to produce a reverse w/o emulsion (Sun et al. 2010). Then, under lower pressure, the solvent was removed from the emulsion using a rota-evaporator. The created dry lipids were eventually hydrated by the buffer to produce homogenous aqueous vesicles dispersion, It was extruded through a high-pressure homogenizer to create a protein that was encapsulated in bilosomes (Figure. 5). In the first step, the poorly water-soluble drug was combined with the lipid components and dissolved in the organic solvent.

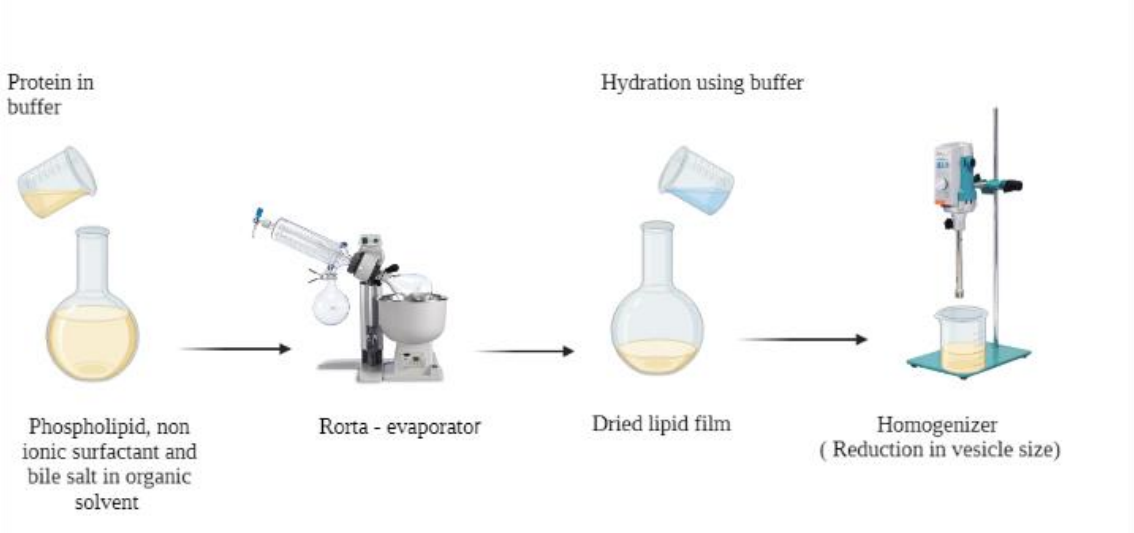

Figure 5. Reverse-phase evaporation method.

4.4. Hot homogenization method

To prepare bilosomes, the lipid components, namely monopalmitoyl glycerol, cholestrol, and DCP, were melted at 140 ºC for 5 min. Then, a buffered solution was used to hydrate them. The bile salt solution was then added after homogenizing the lipid mixture to create a dispersion that contained empty vesicles (Figure. 6). The homogenate was then mixed with the antigen buffered solution, and add or several cycles of freezing and thawing traditional homogenization was used to accomplish protein entrapment or several freeze–thaw cycles (Gebril et al. 2014). The gonadotropin releasing hormone (GnRH) immunogen and influenza A antigen were both entrapped to bilosomes using this technique (Gebril et al. 2014). It is important to note that the antigen was added only at the very end to reduce prolonged homogenization (D'Elia et al. 2019; Wilkhu et al. 2013).

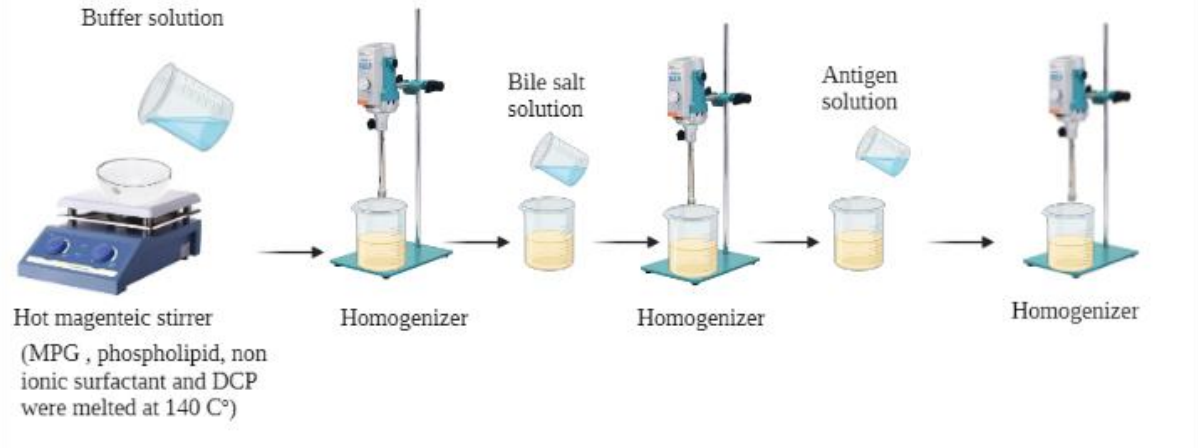

Figure 6. Hot homogenization method. Abbreviation MPG: mono palmitoyl glycerol and DCP: diacetyl phosphate

4.5. Proniosomal method

Liposomes should be handled more easily and should have greater long-term stability, Song et al. (2005) started the process of creating bile salts-containing proniosomes. The carrier, which is commonly sorbitol particles, is placed in a round-bottomed flask and vacuum-dried on a rota-evaporator in this method. Then, to load them onto sorbitol particles, a solution of PC, bile salt, and drug dissolved in an organic solvent was introduced drop by drop into the flask. To guarantee that all organic solvent residues had completely evaporated, the loaded sorbitol products were subsequently freeze-dried (fig .7). After manual agitation in water, the produced proliposomal powder was changed into bilosomes.

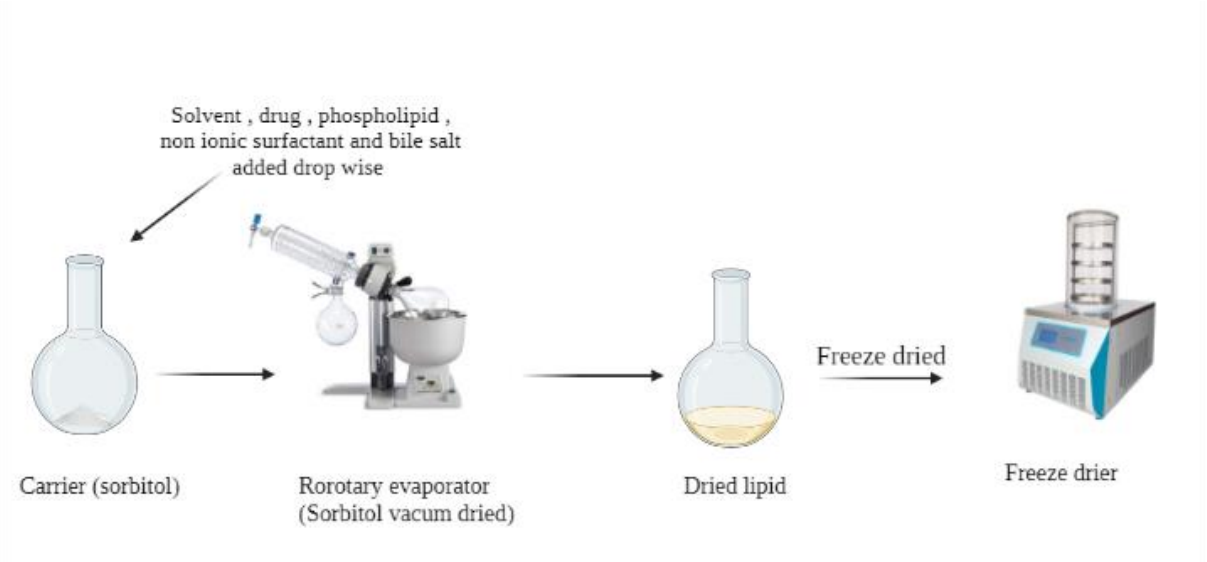

Figure 7. Proniosomal method

5. Bilosomal drug delivery in several diseases

Bilosomes' ability to carry drugs may be useful in treating autoimmune conditions, viral infections, and other infectious diseases, as well as cancer. It has undergone extensive development as a strategy for the controlled and targeted dispersion of active materials.

5.1. Bilosomes in cancer

One-sixth of all deaths worldwide in 2015 were due to cancer, which is among the most terrifying diseases in the world. It is also the second leading cause of illness-related death (A. M. Gouda et al. 2018; Kamel and Al-Amodi 2017). After cardiovascular illnesses, it is the second leading cause of death globally (Omran et al. 2021). Hepatocellular carcinoma, the most common kind of primary liver cancer, is responsible for approximately 75–85% of all liver malignancies (Rashed et al. 2020). For the treatment of liver cancer, (PIP) 3,5-bis(4-bromobenzylidene)-1-propanoylpiperidin-4-one, PIP-loaded BLs offer a potential oral drug delivery strategy. The therapeutic anticancer drug doxorubicin and parent curcumin suspension were more harmful to non-cancerous Wi-38 cells in this investigation, but synthetic PIP-loaded BLs were less toxic. In comparison to curcumin suspension and doxorubicin, the formed BLs also showed higher uptake, selectivity, and anticancer efficacy (Abbas et al. 2022). Currently, pancreatic cancer presents a major issue for the entire world (Hani et al. 2021). As a result, considerable effort has been expended in developing an effective treatment. A human cancer cell line called PNAC1 was utilized to test the newly created icariin-loaded bilosomes melittin (ICA-BM) formulation. This cell line is frequently used to evaluate the therapeutic potential of candidate molecules against pancreatic cancer. A bilosomes melittin carrier (ICA-BM) consequently greatly increased all parameters relating to ICA anticancer efficacy against cancer cells, including decreased IC50, increased anti-proliferative and pro-apoptotic/necrotic activities, and increased caspase-3 and p53 protein levels (Alhakamy et al. 2021). Curcumin was successfully loaded into bilosomes

covered in highly functionalized biomaterial (TPGS- Bil). Due to its highly functionalized capacity to attenuate the p-gp efflux pump, which is accountable for the most common mechanism of multidrug resistance (MDR) (expressed by almost all cancer cells), TPGS coating was able to increase cytotoxicity against doxorubicin-resistant Breast Cancer (MCF-7/ADR) cell lines (Hegazy et al. 2022).

5.2. Viral infection skin and other infectious diseases

The most current developments in the use of bilosomes to treat infectious disorders are covered in this section. The ability of nano-bilosomes loaded with acyclovir (ACV) to inhibit herpes simplex virus type-1 (HSV-1). According to the findings of this study, ACV bilosomes are potential drug-delivery vesicles for overcoming the problems of multiple-dose regimens, poor absorption, and low oral bioavailability of acyclovir (Saifi et al. 2020). The lipophilic DPS was successfully encapsulated using bilosomal systems with reasonable properties suitable for topical application. According to the findings, the amount of DPS retained in the bilosomes-treated skin was approximately 1.5 times greater than that retained in the DPS alcoholic solution-treated skin, demonstrating the prepared bilosomes' success in improving skin penetration and drug retention (M. A. El-Nabarawi et al. 2020a). A significant public health issue that threatens millions of individuals globally is the Hepatitis C virus (HCV) (Pérez-Ayala et al. 2011). In this study, BILS, a nanocarrier, was used to transport DAC to hepatocytes specifically for the treatment of hepatitis C. Because of this, the bioavailability of the DACPEG-BILS was comparable to that of the DAC-un PEG-BILS and the DAC suspension. The results also revealed that the DAC-PEG-BILS had a higher rise in DAC plasma concentration, a delayed rate of clearing it, and a prolonged release attribute in vivo. (M. El-Nabarawi et al. 2021). Terconazole (TCZ), an antifungal drug with low permeability characteristics, was loaded into highly deformable bilosomes (HBs) in the current study to improve its topical delivery. When compared to TCZ suspension, HB14 effectively inhibits the growth of *Candida albicans*, according to microbiological analysis. Additionally, HB14's superior skin deposition in comparison to conventional bilosomal formula and TCZ suspension was due to its highly deformable characteristics and ability to penetrate the skin (Mosallam et al. 2021b).

5.3. Autoimmune Diseases and Neurological Disorders

Selective serotonin reuptake inhibitors (SSRIs) are a class of antidepressants that include sertraline hydrochloride (SER) (Dimoula et al. 2022). The SER-loaded bilosomal formula was successful in this study. In vivo, absorption studies in rats revealed that SER-loaded bilosomal powder (FDN1) had a relative bioavailability% (Fr%) of 222% when compared to pure SER. As a result, the developed SER bilosomal system appears promising for SER oral administration in order to improve oral bioavailability while reducing gastric upset (Ismail et al. 2022). The most prevalent form of neurological illness is migraine, which is frequently accompanied by atypical serotonergic activity(Peterlin and Rapoport 2007). In this study, bilosomes loaded with zolmitriptan were produced using the thin film hydration method. This experiment resulted in successful brain targeting, as shown by greater DTE and positive DTP values in the manufactured gelling system (El Taweel et al. 2021). Alzheimer's disease (AD) is a neurodegenerative disorder of the central nervous system marked by increasing neuronal loss (El-Sayed and Bayan 2015). RES was successfully delivered to the brain through the olfactory mucosa utilizing CS-coated bilosomes and SPION-loaded CS-coated bilosomes on SA wafers as a result of this study. NF-B, P38, and pro-inflammatory marker expression can also be decreased by SPION-loaded bilosomes (Abbas, Refai, et al. 2021a). Tenoxicam is currently widely used to treat rheumatic diseases. In this study, the possibility of using bilosomes as a delivery system for transdermal TX was explored. Bilosomes improved drug penetration and deposition in the skin, which is necessary for transdermal TX delivery, according to ex vivo and in vivo studies on the topic (Al-mahallawi, Abdelbary, and Aburahma 2015).

5.4. Bilosomes in diabetes, hypertension, other diseases

Diabetes mellitus (DM) is a chronic metabolic disorder that affects millions of people around the world (Cole and Florez 2020; Almaimani 2021). This study found that the oral bioavailability of insulin was markedly increased by liposomes containing bile salts (BS-liposomes) (rhINS). Due to

their remarkable ability to protect cells from damages caused by bile salts and protease, Due to their longer GIT residence duration than standard liposomes, BS-liposomes were found to be more stable there (Niu et al. 2014). The current work demonstrates that different elastic bilosome derivatives loaded with cholic acid can be helpful for the buccal administration of insulin. Insulin was found to be most efficiently transported across TR146 cell layers by SDGC-lipo. Increasing insulin permeability in TR146 cells was also possible with the help of the potential candidates SC-lipo, SDTC-lipo, SGC-lipo, and STC-lipo (Bashyal et al. 2018). Because hypertension necessitates long and ongoing treatment, hypertension appears to be a disorder worthy of consideration in the development of a transdermal drug delivery system (TDDS) (Ahad et al. 2018). In this investigation, PBs were developed for transdermal OLM delivery. The results showed that by removing PB15's substantial first-pass metabolism and oral problems, it might be a possible TDDS for OLM (Albash et al. 2019). Renal fibrosis is the final pathological feature of chronic kidney disease (CKD) (Allison 2018). DCS-Lips and IGE have been developed successfully as a combination therapy for renal fibrosis. as a result of DCS-Lips improving EMO oral bioavailability and EMO-IGE restoring gut microbiota. All of these findings strongly suggest that our approach is novel and promising for the treatment of renal fibrosis through the use of combination therapy (Xu et al. 2022).

5.5. Vaccine delivery

The effectiveness of vaccines in preventing a wide range of diseases has been well demonstrated (Bouazzaoui et al. 2021). A variety of immunizations have been administered using bilosomes. It is always preferable to use oral vaccination rather than traditional vaccination (Jain et al. 2014a). According to Conacher et al., oral immunizations were administered using bovine serum albumin (BSA). It was discovered that the high antibody concentration against BSA produced by the orally administered bilosomal formulation was equal to that produced by systemic immunization (Conacher, Alexander, and Brewer 2001b). Conacher et al. described oral immunization using an influenza subunit vaccine and showed that, in comparison to systemic immunization, oral administration of the formulation resulted in high antibody titers and cell-mediated response. It was found that induction of T helper type 1 (Th1) and type 2 (Th2) responses (Conacher, Alexander, and Brewer 2001b). In addition to greatly increasing mucosal IgA production, Mann et al. improved bilosomes expressing A/Panama influenza hemagglutinin and shown that these bilosomes also significantly raised targeted systemic antibody production [89]. For oral HBsAg immunization, bile salt stabilized vesicles are a potential vehicle. However, bilosomes require a five-fold increase in entrapped dose to generate a comparable systemic antibody response, with the added benefit of secretory mucosal protection (Shukla et al. 2008). Arora et al. reported using mannosylated bilosomes for an oral hepatitis B virus vaccination. When compared to bilosomes alone, immune response was shown to be significantly stronger at all local and distal mucosal sites coupled with elevated sIgA levels, whereas parenteral vaccine failed to produce any appreciable cell-mediated response (Arora et al., n.d.). Mann et al. reported that oral immunization with TTx-loaded bilosomes significantly increased systemic and mucosal immunity. It was discovered that the TTx contained in bilosomes might induce a Th2 response characterized by systemic IgG1 (J. F. Mann et al. 2006). Katare and coworkers produced nano-bilosomes containing DTx produced by thin film hydration after oral immunization with varied dosages of DTx entrapped in nano-bilosomes. The high dose-loaded nano-bilosomes produced anti-DTx IgG levels in blood that were comparable to those produced by IM-administered, alum-adsorbed DTx. The orally taken nano-bilosomal DTx formulation produced serum antibody titers that were comparable to those of IM administered alum-adsorbed DTx at a fourfold higher dose and without the development of tolerance (Shukla, Singh, and Katare 2011).

6. Bilosome cytotoxicity

When used as penetration enhancers, bile salts have been found to irritate and be toxic. As a result of the fact that their enhancing effect involves destroying the intestinal epithelial barrier. It is crucial to do the cytotoxicity assay for the bilosomes. The assay to evaluate the safety of bilosomal preparations could be performed on any normal epithelial cell, including human fibroblasts. According to published findings, bilosomes are safe.

Conflicts of Interest: The authors declare no conflict of interest.

References:

Abbas, Haidy, Yasmin A. El-Feky, Majid Mohammad Al-Sawahli, Nehal M. EL-Deeb, Hala Bakr El-Nassan, and Mariam Zewail. 2022. "Development and Optimization of Curcumin Analog Nano-Bilosomes Using $2^{1}.3^{1}$ Full Factorial Design for Anti-Tumor Profiles Improvement in Human Hepatocellular Carcinoma: *In-Vitro* Evaluation, *in-Vivo* Safety Assay." *Drug Delivery* 29 (1): 714–27. https://doi.org/10.1080/10717544.2022.2044938.

Abbas, Haidy, Heba A Gad, Mohamed A Khattab, and Mai Mansour. 2021. "The Tragedy of Alzheimer's Disease: Towards Better Management via Resveratrol-Loaded Oral Bilosomes," 23.

Abbas, Haidy, Hanan Refai, Nesrine El Sayed, Laila Ahmed Rashed, Mohamed R. Mousa, and Mariam Zewail. 2021a. "Superparamagnetic Iron Oxide Loaded Chitosan Coated Bilosomes for Magnetic Nose to Brain Targeting of Resveratrol." *International Journal of Pharmaceutics* 610 (December): 121244. https://doi.org/10.1016/j.ijpharm.2021.121244.

Abdelalim, Lamiaa R., Ossama Y. Abdallah, and Yosra S.R. Elnaggar. 2020. "High Efficacy, Rapid Onset Nanobiolosomes of Sildenafil as a Topical Therapy for Erectile Dysfunction in Aged Rats." *International Journal of Pharmaceutics* 591 (December): 119978. https://doi.org/10.1016/j.ijpharm.2020.119978.

Abdelbary, Aly A., Wessam H. Abd-Elsalam, and Abdulaziz M. Al-mahallawi. 2016. "Fabrication of Novel Ultradeformable Bilosomes for Enhanced Ocular Delivery of Terconazole: In Vitro Characterization, Ex Vivo Permeation and in Vivo Safety Assessment." *International Journal of Pharmaceutics* 513 (1–2): 688–96. https://doi.org/10.1016/j.ijpharm.2016.10.006.

Abdellatif, Ahmed A. H., Asmaa T. Ali, Abdellatif Bouazzaoui, Mansour Alsharidah, Osamah Al Rugaie, and Nahla Sameh Tolba. 2022. "Formulation of Polymeric Nanoparticles Loaded Sorafenib; Evaluation of Cytotoxicity, Molecular Evaluation, and Gene Expression Studies in Lung and Breast Cancer Cell Lines," Nanotechnology Reviews, 11 (1): 987–1004. https://doi.org/10.1515/ntrev-2022-0058.

Abouelmaati, Ezzat Abdelziz Ali, Hoda A El-Guindy, Aziza Mahmoud, and Sanaa Hassan Mohamed El-malah. 2023. "MEDICATION ADMINISTRATION PRINCIPLES AND ERRORS AS PERCEIVED BY NURSES CARING FOR ELDERLY PATIENTS." *NILES Journal for Geriatric and Gerontology* 6 (1): 27–48.

Ahad, Abdul, Mohammad Raish, Ajaz Ahmad, Fahad I. Al-Jenoobi, and Abdullah M. Al-Mohizea. 2018. "Eprosartan Mesylate Loaded Bilosomes as Potential Nano-Carriers against Diabetic Nephropathy in Streptozotocin-Induced Diabetic Rats." *European Journal of Pharmaceutical Sciences* 111 (January): 409–17. https://doi.org/10.1016/j.ejps.2017.10.012.

Ahmed, Sadek, Mohamed Aly Kassem, and Sinar Sayed. 2020. "Bilosomes as Promising Nanovesicular Carriers for Improved Transdermal Delivery: Construction, in Vitro Optimization, Ex Vivo Permeation and in Vivo Evaluation." *International Journal of Nanomedicine* Volume 15 (December): 9783–98. https://doi.org/10.2147/IJN.S278688.

Albash, Rofida, Mohamed A. El-Nabarawi, Hanan Refai, and Aly A. Abdelbary. 2019. "Tailoring of PEGylated Bilosomes for Promoting the Transdermal Delivery of Olmesartan Medoxomil: In-Vitro Characterization, Ex-Vivo Permeation and in-Vivo Assessment." *International Journal of Nanomedicine* Volume 14 (August): 6555–74. https://doi.org/10.2147/IJN.S213613.

Alhakamy, Nabil A., Shaimaa M. Badr-Eldin, Waleed S. Alharbi, Mohamed A. Alfaleh, Omar D. Al-hejaili, Hibah M. Aldawsari, Basma G. Eid, et al. 2021. "Development of an Icariin-Loaded Bilosome-Melittin Formulation with Improved Anticancer Activity against Cancerous Pancreatic Cells." *Pharmaceuticals* 14 (12): 1309. https://doi.org/10.3390/ph14121309.

Allison, Susan J. 2018. "Targeting ARNT to Attenuate Renal Fibrosis." *Nature Reviews Nephrology* 14 (9): 535–535. https://doi.org/10.1038/s41581-018-0038-y.

Al-Mahallawi, Abdulaziz M, Aly A Abdelbary, and Mona H Aburahma. 2015. "Investigating the Potential of Employing Bilosomes as a Novel

Vesicular Carrier for Transdermal Delivery of Tenoxicam." *International Journal of Pharmaceutics* 485 (1–2): 329–40.

Al-mahallawi, Abdulaziz M., Aly A. Abdelbary, and Mona H. Aburahma. 2015. "Investigating the Potential of Employing Bilosomes as a Novel Vesicular Carrier for Transdermal Delivery of Tenoxicam." *International Journal of Pharmaceutics* 485 (1–2): 329–40. https://doi.org/10.1016/j.ijpharm.2015.03.033.

Almaimani, Riyad. 2021. "The Association between Oxidative Stress Marker Beta-2 Microglobulin and Type 2 Diabetes Mellitus in Context of Glycaemic Index." *Journal of Umm Al-Qura University for Medical Sciences* 7 (1): 28–31. https://doi.org/10.54940/ms74813000.

Andra, Veera Venkata Satya Naga Lakshmi, S. V. N. Pammi, Lakshmi Venkata Krishna Priya Bhatraju, and Lakshmi Kalyani Ruddaraju. 2022. "A Comprehensive Review on Novel Liposomal Methodologies, Commercial Formulations, Clinical Trials and Patents." *BioNanoScience* 12 (1): 274–91. https://doi.org/10.1007/s12668-022-00941-x.

Arora, Daisy, Bharat Khurana, Murugesan Senthil Kumar, and Suresh P Vyas. n.d. "Oral Immunization against Hepatitis B Virus Using Mannosylated Bilosomes," 8.

Attia, Sara Aly, and J. Andrew MacKay. 2022. "Protein and Polypeptide Mediated Delivery to the Eye." *Advanced Drug Delivery Reviews* 188 (September): 114441. https://doi.org/10.1016/j.addr.2022.114441.

Aziz, Diana E., Aly A. Abdelbary, and Abdelhalim I. Elassasy. 2019. "Investigating Superiority of Novel Bilosomes over Niosomes in the Transdermal Delivery of Diacerein: *In Vitro* Characterization, *Ex Vivo* Permeation and *in Vivo* Skin Deposition Study." *Journal of Liposome Research* 29 (1): 73–85.

https://doi.org/10.1080/08982104.2018.1430831.

Bashir, Mehreen, Junaid Ahmad, Muhammad Asif, Salah-Ud-Din Khan, Muhammad Irfan, Asim Y Ibrahim, Sajid Asghar, et al. 2021. "Nanoemulgel, an Innovative Carrier for Diflunisal Topical Delivery with Profound Anti-Inflammatory Effect: In Vitro and in Vivo Evaluation." *International Journal of Nanomedicine* Volume 16 (February): 1457–72. https://doi.org/10.2147/IJN.S294653.

Bashyal, Santosh, Jo-Eun Seo, Taekwang Keum, Gyubin Noh, Young Wook Choi, and Sangkil Lee. 2018. "Facilitated Permeation of Insulin across TR146 Cells by Cholic Acid Derivatives-Modified Elastic Bilosomes." *International Journal of Nanomedicine* Volume 13 (September): 5173–86. https://doi.org/10.2147/IJN.S168310.

Bouazzaoui, Abdellatif, Ahmed A. H. Abdellatif, Faisal A. Al-Allaf, Neda M. Bogari, Saied Al-Dehlawi, and Sameer H. Qari. 2021. "Strategies for Vaccination: Conventional Vaccine Approaches Versus New-Generation Strategies in Combination with Adjuvants." *Pharmaceutics* 13 (2): 140. https://doi.org/10.3390/pharmaceutics13020140.

Casares, Doralicia, Pablo V. Escribá, and Catalina Ana Rosselló. 2019. "Membrane Lipid Composition: Effect on Membrane and Organelle Structure, Function and Compartmentalization and Therapeutic Avenues." *International Journal of Molecular Sciences* 20 (9): 2167. https://doi.org/10.3390/ijms20092167.

Cole, Joanne B, and Jose C Florez. 2020. "Genetics of Diabetes Mellitus and Diabetes Complications." *Nature Reviews Nephrology* 16 (7): 377–90.

Conacher, Margaret, James Alexander, and James M. Brewer. 2001a. "Oral Immunisation with Peptide and Protein Antigens by Formulation in Lipid Vesicles Incorporating Bile Salts (Bilosomes)." *Vaccine* 19 (20–22): 2965–74. https://doi.org/10.1016/S0264-410X(00)00537-5.

Crommelin, Daan JA, Peter van Hoogevest, and Gert Storm. 2020. "The Role of Liposomes in Clinical Nanomedicine Development. What Now? Now What?" *Journal of Controlled Release* 318: 256–63.

D'Elia, Riccardo V., Stuart Woods, Wendy Butcher, Jonathan McGahon, Swapnil Khadke, Yvonne Perrie, E. Diane Williamson, and Craig W. Roberts. 2019. "Exploitation of the Bilosome Platform Technology to Formulate Antibiotics and Enhance Efficacy of Melioidosis Treatments." *Journal of Controlled Release* 298 (March): 202–12. https://doi.org/10.1016/j.jconrel.2019.02.002.

Dimoula, Anna, Dimitrios Fotellis, Evmorfia Aivalioti, Dimitrios Delialis, Alexia Polissidis, Raphael Patras, Nikolaos Kokras, and Kimon Stamatelopoulos. 2022. "Off-Target Effects of Antidepressants on Vascular Function and Structure." *Biomedicines* 10 (1). https://doi.org/10.3390/biomedicines10010056.

El Menshawe, Shahira F., Heba M. Aboud, Mohammed H. Elkomy, Rasha M. Kharshoum, and Amany M. Abdeltwab. 2020. "A Novel Nanogel Loaded with Chitosan Decorated Bilosomes for Transdermal Delivery of Terbutaline Sulfate: Artificial Neural Network Optimization, in Vitro Characterization and in Vivo Evaluation." *Drug Delivery and Translational Research* 10 (2): 471–85. https://doi.org/10.1007/s13346-019-00688-1.

El Taweel, Mai M., Mona H. Aboul-Einien, Mohammed A. Kassem, and Nermeen A. Elkasabgy. 2021. "Intranasal Zolmitriptan-Loaded Bilosomes with Extended Nasal Mucociliary Transit Time for Direct Nose to Brain Delivery." *Pharmaceutics* 13 (11): 1828. https://doi.org/10.3390/pharmaceutics13111828.

El-Nabarawi, Mohamed Ahmed, Rehab Nabil Shamma, Faten Farouk, and Samar Mohamed Nasralla. 2020a. "Bilosomes as a Novel Carrier for the Cutaneous Delivery for Dapsone as a Potential Treatment of Acne: Preparation, Characterization and *in Vivo* Skin Deposition Assay." *Journal of Liposome Research* 30 (1): 1–11. https://doi.org/10.1080/08982104.2019.1577256.

———. 2020b. "Bilosomes as a Novel Carrier for the Cutaneous Delivery for Dapsone as a Potential Treatment of Acne: Preparation, Characterization and *in Vivo* Skin Deposition Assay." *Journal of Liposome Research* 30 (1): 1–11. https://doi.org/10.1080/08982104.2019.1577256.

El-Nabarawi, Mohamed, Mohamed Nafady, Shahira elmenshawe, Marwa Elkarmalawy, and Mahmoud Teaima. 2021. "Liver Targeting of Daclatasvir via Tailoring Sterically Stabilized Bilosomes: Fabrication, Comparative In Vitro/In Vivo Appraisal and Biodistribution Studies." *International Journal of Nanomedicine* Volume 16 (September): 6413–26. https://doi.org/10.2147/IJN.S319255.

Elnaggar, Yosra. 2015. "Multifaceted Applications of Bile Salts in Pharmacy: An Emphasis on Nanomedicine." *International Journal of Nanomedicine*, June, 3955. https://doi.org/10.2147/IJN.S82558.

Elnaggar, Yosra S.R., Sara Omran, Heba A. Hazzah, and Ossama Y. Abdallah. 2019a. "Anionic versus Cationic Bilosomes as Oral Nanocarriers for Enhanced Delivery of the Hydrophilic Drug Risedronate." *International Journal of Pharmaceutics* 564 (June): 410–25. https://doi.org/10.1016/j.ijpharm.2019.04.069.

El-Sayed, Nesrine S., and Yasmeen Bayan. 2015. "Possible Role of Resveratrol Targeting Estradiol and Neprilysin Pathways in Lipopolysaccharide Model of Alzheimer Disease." In *GeNeDis 2014*, edited by Panayiotis Vlamos and Athanasios Alexiou, 107–18. Cham: Springer International Publishing.

Gebril, Ayman M., Dimitrios A. Lamprou, Manal M. Alsaadi, William H. Stimson, Alexander B. Mullen, and Valerie A. Ferro. 2014. "Assessment of the Antigen-Specific Antibody Response Induced by Mucosal Administration of a GnRH Conjugate Entrapped in Lipid Nanoparticles." *Nanomedicine: Nanotechnology, Biology and Medicine* 10 (5): e971–79. https://doi.org/10.1016/j.nano.2013.12.005.

Gouda, Ahmed M., Hoda A. El-Ghamry, Tahani M. Bawazeer, Thoraya A. Farghaly, Ashraf N. Abdalla, and Akhmed Aslam. 2018. "Antitumor Activity of Pyrrolizines and Their Cu(II) Complexes: Design, Synthesis and Cytotoxic Screening with Potential Apoptosis-Inducing Activity." *European Journal of Medicinal Chemistry* 145 (February): 350–59. https://doi.org/10.1016/j.ejmech.2018.01.009.

Gouda, Ahmed, Omar S. Sakr, Maha Nasr, and Omaima Sammour. 2021. "Ethanol Injection Technique for Liposomes Formulation: An Insight into Development, Influencing Factors, Challenges and Applications." *Journal of Drug Delivery Science and Technology* 61 (February): 102174. https://doi.org/10.1016/j.jddst.2020.102174.

Guan, Peipei, Yi Lu, Jianping Qi, and Wei Wu. 2016. "Readily Restoring Freeze-Dried Probilosomes as Potential Nanocarriers for Enhancing Oral Delivery of Cyclosporine A." *Colloids and Surfaces B: Biointerfaces* 144

(August): 143–51. https://doi.org/10.1016/j.colsurfb.2016.04.006.

Hani, Umme, Riyaz Ali M. Osmani, Ayesha Siddiqua, Shadma Wahab, Sadia Batool, Hissana Ather, Norhan Sheraba, and Ali Alqahtani. 2021. "A Systematic Study of Novel Drug Delivery Mechanisms and Treatment Strategies for Pancreatic Cancer." *Journal of Drug Delivery Science and Technology* 63: 102539. https://doi.org/10.1016/j.jddst.2021.102539.

Hegazy, Hanaa, Maha M. Amin, Walid Fayad, and Mohamed Y. Zakaria. 2022. "'TPGS Surface Modified Bilosomes as Boosting Cytotoxic Oral Delivery Systems of Curcumin against Doxorubicin Resistant MCF-7 Breast Cancer Cells.'" *International Journal of Pharmaceutics* 619 (May): 121717. https://doi.org/10.1016/j.ijpharm.2022.121717.

Hussain, Zahid, Hnin Ei Thu, Ibrahim Elsayed, Mohammed A.S. Abourehab, Shahzeb Khan, Mohammad Sohail, Rai Muhammad Sarfraz, and Muhammad Asim Farooq. 2020. "Nano-Scaled Materials May Induce Severe Neurotoxicity upon Chronic Exposure to Brain Tissues: A Critical Appraisal and Recent Updates on Predisposing Factors, Underlying Mechanism, and Future Prospects." *Journal of Controlled Release* 328 (December): 873–94. https://doi.org/10.1016/j.jconrel.2020.10.053.

Ismail, Aliaa, Mohammed Teiama, Basma Magdy, and Wedad Sakran. 2022. "Development of a Novel Bilosomal System for Improved Oral Bioavailability of Sertraline Hydrochloride: Formulation Design, In Vitro Characterization, and Ex Vivo and In Vivo Studies." *AAPS PharmSciTech* 23 (6): 188. https://doi.org/10.1208/s12249-022-02339-0.

Jain, Sanyog, Anura Indulkar, Harshad Harde, and Ashish K Agrawal. 2014a. "Oral Mucosal Immunization Using Glucomannosylated Bilosomes." *J. Biomed. Nanotechnol.*, 16. https://doi.org/10.1166/jbn.2014.1800.

Jain, Sanyog, Anura Indulkar, Harshad Harde, and Ashish K. Agrawal. 2014b. "Oral Mucosal Immunization Using Glucomannosylated Bilosomes." *Journal of Biomedical Nanotechnology* 10 (6): 932–47. https://doi.org/10.1166/jbn.2014.1800.

Kamel, Hala Fawzy Mohamed, and Hiba Saeed A. Bagader Al-Amodi. 2017. "Exploitation of Gene Expression and Cancer Biomarkers in Paving the Path to Era of Personalized Medicine." *Genomics, Proteomics & Bioinformatics* 15 (4): 220–35. https://doi.org/10.1016/j.gpb.2016.11.005.

Kumar, Gannu P., and Pogaku Rajeshwarrao. 2011. "Nonionic Surfactant Vesicular Systems for Effective Drug Delivery—an Overview." *Acta Pharmaceutica Sinica B* 1 (4): 208–19. https://doi.org/10.1016/j.apsb.2011.09.002.

Kumavat, Sonali, Pravin Kumar Sharma, Sweta S Koka, Ravi Sharma, Ashish Gupta, and G. N. Darwhekar. 2021. "A Review on Niosomes: Potential Vesicular Drug Delivery System." *Journal of Drug Delivery and Therapeutics* 11 (5): 208–12. https://doi.org/10.22270/jddt.v11i5.5046.

Li, Jing, Xuling Wang, Ting Zhang, Chunling Wang, Zhenjun Huang, Xiang Luo, and Yihui Deng. 2015. "A Review on Phospholipids and Their Main Applications in Drug Delivery Systems." *Asian Journal of Pharmaceutical Sciences* 10 (2): 81–98. https://doi.org/10.1016/j.ajps.2014.09.004.

Li, Mingyuan, Chunyang Du, Na Guo, Yuou Teng, Xin Meng, Hua Sun, Shuangshuang Li, Peng Yu, and Hervé Galons. 2019. "Composition Design and Medical Application of Liposomes." *European Journal of Medicinal Chemistry* 164: 640–53.

Liu, Chang, Yahui Guo, Yuliang Cheng, and He Qian. 2022. "Bilosomes: A Controlled Delivery System for the Sustained Release of Torularhodin during Digestion in the Small Intestine Both in Vitro and in Vivo." *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, August, 130055. https://doi.org/10.1016/j.colsurfa.2022.130055.

Liu, Peng, Guiliang Chen, and Jingchen Zhang. 2022. "A Review of Liposomes as a Drug Delivery System: Current Status of Approved Products, Regulatory Environments, and Future Perspectives." *Molecules* 27 (4): 1372. https://doi.org/10.3390/molecules27041372.

Madheswaran, Thiagarajan, Rengarajan Baskaran, Chul Soon Yong, and Bong Kyu Yoo. 2014. "Enhanced Topical Delivery of Finasteride Using Glyceryl Monooleate-Based Liquid Crystalline Nanoparticles Stabilized by Cremophor Surfactants."

AAPS PharmSciTech 15 (1): 44–51. https://doi.org/10.1208/s12249-013-0034-2.

Mann, Jamie F S, Valerie A Ferro, Alexander B Mullen, Lawrence Tetley, Margaret Mullen, Katharine C Carter, James Alexander, and William H Stimson. 2004. "Optimisation of a Lipid Based Oral Delivery System Containing A/Panama Influenza Haemagglutinin," 5.

Mann, Jamie FS, Hannah E Scales, Eisin Shakir, James Alexander, Katharine C Carter, Alexander B Mullen, and Valerie A Ferro. 2006. "Oral Delivery of Tetanus Toxoid Using Vesicles Containing Bile Salts (Bilosomes) Induces Significant Systemic and Mucosal Immunity." *Methods* 38 (2): 90–95.

Mohsen, Amira Mohamed, Abeer Salama, and Ahmed Alaa Kassem. 2020. "Development of Acetazolamide Loaded Bilosomes for Improved Ocular Delivery: Preparation, Characterization and in Vivo Evaluation." *Journal of Drug Delivery Science and Technology* 59 (October): 101910. https://doi.org/10.1016/j.jddst.2020.101910.

Mosallam, Shaimaa, Nermin M. Sheta, Ahmed Hassen Elshafeey, and Aly Ahmed Abdelbary. 2021a. "Fabrication of Highly Deformable Bilosomes for Enhancing the Topical Delivery of Terconazole: In Vitro Characterization, Microbiological Evaluation, and In Vivo Skin Deposition Study." *AAPS PharmSciTech* 22 (2): 74. https://doi.org/10.1208/s12249-021-01924-z.

———. 2021b. "Fabrication of Highly Deformable Bilosomes for Enhancing the Topical Delivery of Terconazole: In Vitro Characterization, Microbiological Evaluation, and In Vivo Skin Deposition Study." *AAPS PharmSciTech* 22 (2): 74. https://doi.org/10.1208/s12249-021-01924-z.

Nasr, Ali M., Fayrouz Moftah, Mohammed A. S. Abourehab, and Shadeed Gad. 2022. "Design, Formulation, and Characterization of Valsartan Nanoethosomes for Improving Their Bioavailability." *Pharmaceutics* 14 (11): 2268. https://doi.org/10.3390/pharmaceutics14112268.

Nemati, Mohadeseh, Anahita Fathi-Azarbayjani, Hani Al-Salami, Elmira Roshani Asl, and Yousef Rasmi. 2022. "Bile Acid-based Advanced Drug Delivery Systems, Bilosomes and Micelles as Novel Carriers for Therapeutics." *Cell Biochemistry and Function* 40 (6): 623–35. https://doi.org/10.1002/cbf.3732.

Nemr, Asmaa Ashraf, Galal Mohamed El-Mahrouk, and Hany Abdo Badie. 2022. "Hyaluronic Acid-Enriched Bilosomes: An Approach to Enhance Ocular Delivery of Agomelatine via D-Optimal Design: Formulation, *in Vitro* Characterization, and *in Vivo* Pharmacodynamic Evaluation in Rabbits." *Drug Delivery* 29 (1): 2343–56. https://doi.org/10.1080/10717544.2022.2100513.

Niu, Mengmeng, Ya'nan Tan, Peipei Guan, Lars Hovgaard, Yi Lu, Jianping Qi, Ruyue Lian, Xiaoyang Li, and Wei Wu. 2014. "Enhanced Oral Absorption of Insulin-Loaded Liposomes Containing Bile Salts: A Mechanistic Study." *International Journal of Pharmaceutics* 460 (1–2): 119–30. https://doi.org/10.1016/j.ijpharm.2013.11.028.

Olusanya, Temidayo, Rita Haj Ahmad, Daniel Ibegbu, James Smith, and Amal Elkordy. 2018. "Liposomal Drug Delivery Systems and Anticancer Drugs." *Molecules* 23 (4): 907. https://doi.org/10.3390/molecules23040907.

Omran, Ziad, Chris P. Guise, Linwei Chen, Cyril Rauch, Ashraf N. Abdalla, Omeima Abdullah, Ikhlas A. Sindi, et al. 2021. "Design, Synthesis and In-Vitro Biological Evaluation of Antofine and Tylophorine Prodrugs as Hypoxia-Targeted Anticancer Agents." *Molecules* 26 (11): 3327. https://doi.org/10.3390/molecules26113327.

Palekar-Shanbhag, Pradnya, Supriya Lande, Riya Chandra, and Drushti Rane. 2020. "Bilosomes: Superior Vesicular Carriers." *Current Drug Therapy* 15 (4): 312–20. https://doi.org/10.2174/1574885514666190917145510.

Pavlović, Nebojša, Svetlana Goločorbin-Kon, Maja Đanić, Bojan Stanimirov, Hani Al-Salami, Karmen Stankov, and Momir Mikov. 2018. "Bile Acids and Their Derivatives as Potential Modifiers of Drug Release and Pharmacokinetic Profiles." *Frontiers in Pharmacology* 9 (November): 1283. https://doi.org/10.3389/fphar.2018.01283.

Pérez-Ayala, A, JA Pérez-Molina, F Norman, M Navarro, B Monge-Maillo, M Díaz-Menéndez, J Peris-García, M Flores, C Cañavate, and R López-Vélez. 2011. "Chagas Disease in Latin American Migrants: A Spanish Challenge." *Clinical Microbiology and*

Infection 17 (7): 1108–13.

Peterlin, B Lee, and Alan M Rapoport. 2007. "Clinical Pharmacology of the Serotonin Receptor Agonist, Zolmitriptan." *Expert Opinion on Drug Metabolism & Toxicology* 3 (6): 899–911. https://doi.org/10.1517/17425255.3.6.899.

Qi, Jianping, Dai, Zhou, Yi Lu, Wei Wu, and Liu. 2013. "Liposomes Containing Bile Salts as Novel Ocular Delivery Systems for Tacrolimus (FK506): In Vitro Characterization and Improved Corneal Permeation." *International Journal of Nanomedicine*, May, 1921. https://doi.org/10.2147/IJN.S44487.

Rajput, Tanvi, and Meenakshi K Chauhan. n.d. "BILOSOME: A BILE SALT BASED NOVEL CARRIER SYSTEM GAINING INTEREST IN PHARMACEUTICAL RESEARCH." *Journal of Drug Delivery*, 13.

Rashed, Wafaa M., Mohamed Ahmed Mohamed Kandeil, Mohamed O. Mahmoud, and Sameera Ezzat. 2020. "Hepatocellular Carcinoma (HCC) in Egypt: A Comprehensive Overview." *Journal of the Egyptian National Cancer Institute* 32 (1): 5. https://doi.org/10.1186/s43046-020-0016-x.

Saifi, Zoya, Md. Rizwanullah, Showkat R. Mir, and Saima Amin. 2020. "Bilosomes Nanocarriers for Improved Oral Bioavailability of Acyclovir: A Complete Characterization through in Vitro, Ex-Vivo and in Vivo Assessment." *Journal of Drug Delivery Science and Technology* 57 (June): 101634. https://doi.org/10.1016/j.jddst.2020.101634.

Salem, Heba F, Mohammed M Nafady, Adel A Ali, Nermeen M Khalil, and Amani A Elsisi. 2022. "Evaluation of Metformin Hydrochloride Tailoring Bilosomes as an Effective Transdermal Nanocarrier." *International Journal of Nanomedicine* Volume 17 (March): 1185–1201. https://doi.org/10.2147/IJN.S345505.

Shewaiter, Mona A., Taha M. Hammady, Alaa El-Gindy, Sami H. Hammadi, and Shadeed Gad. 2021. "Formulation and Characterization of Leflunomide/Diclofenac Sodium Microemulsion Base-Gel for the Transdermal Treatment of Inflammatory Joint Diseases." *Journal of Drug Delivery Science and Technology* 61 (February): 102110. https://doi.org/10.1016/j.jddst.2020.102110.

Shewaiter, Mona A., Adli A. Selim, Yasser M. Moustafa, Shadeed Gad, and Hassan M. Rashed. 2022. "Radioiodinated Acemetacin Loaded Niosomes as a Dual Anticancer Therapy." *International Journal of Pharmaceutics* 628 (November): 122345. https://doi.org/10.1016/j.ijpharm.2022.122345.

Shukla, Anshuman, O.P. Katare, Bhupinder Singh, and Suresh P. Vyas. 2010. "M-Cell Targeted Delivery of Recombinant Hepatitis B Surface Antigen Using Cholera Toxin B Subunit Conjugated Bilosomes." *International Journal of Pharmaceutics* 385 (1–2): 47–52. https://doi.org/10.1016/j.ijpharm.2009.10.027.

Shukla, Anshuman, Kapil Khatri, Prem N Gupta, Amit K Goyal, Abhinav Mehta, and Suresh P Vyas. 2008. "Oral Immunization against Hepatitis B Using Bile Salt Stabilized Vesicles (Bilosomes)." *Journal of Pharmacy & Pharmaceutical Sciences* 11 (1): 59. https://doi.org/10.18433/J3K01M.

Shukla, Anshuman, Bhupinder Singh, and OP Katare. 2011. "Significant Systemic and Mucosal Immune Response Induced on Oral Delivery of Diphtheria Toxoid Using Nano-bilosomes." *British Journal of Pharmacology* 164 (2b): 820–27.

Sun, Jukui, Yingjie Deng, Siling Wang, Jinna Cao, Xiaofei Gao, and Xiaodong Dong. 2010. "Liposomes Incorporating Sodium Deoxycholate for Hexamethylmelamine (HMM) Oral Delivery: Development, Characterization, and in Vivo Evaluation." *Drug Delivery* 17 (3): 164–70. https://doi.org/10.3109/10717541003667764.

Waglewska, Ewelina, Agata Pucek-Kaczmarek, and Urszula Bazylińska. 2020. "Novel Surface-Modified Bilosomes as Functional and Biocompatible Nanocarriers of Hybrid Compounds." *Nanomaterials* 10 (12): 2472. https://doi.org/10.3390/nano10122472.

———. 2022. "Self-Assembled Bilosomes with Stimuli-Responsive Properties as Bioinspired Dual-Tunable Nanoplatform for pH/Temperature-Triggered Release of Hybrid Cargo." *Colloids and*

Surfaces B: Biointerfaces 215 (July): 112524. https://doi.org/10.1016/j.colsurfb.2022.112524.

Wahab, Shadma, Md P Ahmad, Arshad Hussain, and Shaik FA Qadir. 2022. "Nanomaterials for the Delivery of Herbal Bioactive Compounds." *Current Nanoscience* 18 (4): 425–41.

Wang, Li, Xin Huang, Huijuan Jing, Chaoyang Ma, and Hongxin Wang. 2021. "Bilosomes as Effective Delivery Systems to Improve the Gastrointestinal Stability and Bioavailability of Epigallocatechin Gallate (EGCG)." *Food Research International* 149 (November): 110631. https://doi.org/10.1016/j.foodres.2021.110631.

Wang, Ning, Minnan Chen, and Ting Wang. 2019. "Liposomes Used as a Vaccine Adjuvant-Delivery System: From Basics to Clinical Immunization." *Journal of Controlled Release* 303: 130–50.

Wilkhu, Jitinder S., Sarah E. McNeil, David E. Anderson, and Yvonne Perrie. 2013. "Characterization and Optimization of Bilosomes for Oral Vaccine Delivery." *Journal of Drug Targeting* 21 (3): 291–99. https://doi.org/10.3109/1061186X.2012.747528.

Xu, Zhishi, Yu Hou, Jiang Sun, Lin Zhu, Qibin Zhang, Wenjie Yao, Xudong Fan, Ke Zhang, Ji-gang Piao, and Yinghui Wei. 2022. "Deoxycholic Acid-Chitosan Coated Liposomes Combined with in Situ Colonic Gel Enhances Renal Fibrosis Therapy of Emodin." *Phytomedicine* 101 (July): 154110. https://doi.org/10.1016/j.phymed.2022.154110.

Yadav, Nisha, Deeksha Mudgal, Ritesh Anand, Simran Jindal, and Vivek Mishra. 2022. "Recent Development in Nanoencapsulation and Delivery of Natural Bioactives through Chitosan Scaffolds for Various Biological Applications." *International Journal of Biological Macromolecules*.

Yu, Ji Young, Piyanan Chuesiang, Gye Hwa Shin, and Hyun Jin Park. 2021. "Post-Processing Techniques for the Improvement of Liposome Stability." *Pharmaceutics* 13 (7): 1023. https://doi.org/10.3390/pharmaceutics13071023.

Zafar, Ameeduzzafar, Nabil K. Alruwaili, Syed Sarim Imam, Nasser Hadal Alotaibi, Khalid Saad Alharbi, Muhammad Afzal, Raisuddin Ali, et al. 2021. "Bioactive Apigenin Loaded Oral Nano Bilosomes: Formulation Optimization to Preclinical Assessment." *Saudi Pharmaceutical Journal* 29 (3): 269–79. https://doi.org/10.1016/j.jsps.2021.02.003