A Review on Engineered Bilosomes Vesicular Carrier Containing in Situ Gel for Sustained Ocular Delivery

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Abstract:

The treatment of ocular diseases, especially glaucoma, requires effective drug delivery systems that overcome the challenges of poor bioavailability, rapid clearance, and frequent dosing. Timolol Maleate, a commonly prescribed beta-blocker for glaucoma management, faces these issues due to its rapid elimination from the ocular surface. Recent advancements in drug delivery systems have led to the development of bilosomes—a vesicular carrier system combining liposomes and bile salts—which, when combined with in situ gel formulations, offer enhanced drug retention and sustained release. This review discusses the formulation, properties, and potential of engineered bilosome carriers incorporated with in situ gel for sustained ocular delivery of Timolol Maleate, focusing on their efficacy in improving therapeutic outcomes for ocular diseases.

Keywords*: ocular delivery, bioavailability, beta blocker, rapid elimination, drug retention, therapeutic outcomes, sustained release*

INTRODUCTION

Background On Ocular Drug Delivery Challenge

Ocular drug delivery remains a significant challenge in pharmaceuticals research due to the unique anatomical and physiological characteristics of the eye (Gaudana, R et al 2010). The major problem in the ocular drug delivery system with the eye drops is their fast and extensive elimination from the eye which cause extensive loss of the drug from the eye (Patel, A et al 2013). However, the poor drug retention and permeation results in the erratic bioavailability of the drug. Despite these there are numerous physiological barriers which plays significant roles in poor drug retention which includes the blood retinal barrier, blood aqueous barrier and corneal barrier (Akhter, M.H et al 2022).

CHALLENGES CAN BE BROADLY CLASSIFIED AS:

- Anatomical Barriers
- Physiological Barriers
- Pharmacokinetic Barriers

- **Anatomical Barrier:**

  1) Corneal Barrier: the cornea of the eye consists of mainly three layers: the epithelium, stroma, and endothelium. The epithelium layer of eye is lipophilic, so it restricts hydrophilic drug, while the stroma is hydrophilic drug, which limits the lipophilic drug penetration. (Ahmed S et al 2022).

  2)Scleral Barrier: this barrier is made up of thick collagen fibers in the Sclera which limits the diffusion of large and hydrophobic molecules into the eye cavity. (Jitendra et al 2011).

  3)Vitreous Humor: This is gel-like substance in the posterior segment which presents a barrier to drug diffusion, particularly in large molecules. (Gaudana et al 2010).

  4) Blood Ocular Barriers: **1)** Blood Aqueous Barrier: it is formed by the tight junctions in the ciliary epithelium and iris vessels, which restricts the drug entry into the anterior chamber of the eye. (Cunha-Vaz, J. G. 2004).

  2) Blood Retinal Barrier: This is one of type of anatomical barrier which maintains the retinal environment by preventing systemic drugs from reaching to the retina (Del Amo et al 2008).

- **Physiological Barriers:**

  1) Tear Film Dynamics: Due to the tear drainage and the blinking of the eye the constant turnover of the tear film rapidly removes the drug from the ocular surface and which limits their contact time. (Bron, A. J., Tiffany et al 2004)

  2)Lacrimal Drainage: The drainage of the significant portion of the administered drug into the nasal cavity helps in reducing the ocular bioavailability of the drug and potentially causing systemic side effects. (Urtti, A.2006).

  3)Enzymatic Degradation: There are various enzymes present in the tears and ocular tissues which metabolize the drug and helps in reducing the efficacy of the drug. (Edel Hauser, H. F. 2006).

- **Pharmacokinetic Barriers:**

  1) Low Bioavailability: Even less than of 5% of drugs administered as an eye drops reach the anterior segment of the eye and even less reaches to the posterior segment of the eye. (Urtti, A.2006).

  2)Short Half Life: Most of the drugs are cleared or moved quickly out from the ocular surface or vitreous which decreases the half-life of drug or requires frequently administration of the drug. (Patel, A. et al 2013).

  3) Systemic Absorption: Many of the drugs get drains into the nasal cavity where they get absorbed through nasal mucosa or conjunctival vessels which may lead to the systemic side effects. (Novack, G.D. 2009**)**

Brief Overview of Bilosomes and Their Importance in Eye Treatment

Recent advancements in drug delivery techniques and materials science have led to the development of novel therapeutics for treating ocular diseases. Currently, the primary methods of delivering drugs to the eye include topical application, redistribution into ocular tissues following systemic administration and targeted intraocular or periocular injections. However, drug transport to the desired ocular tissues is hindered by various precorneal dynamics and static ocular barriers. Conventional ocular drug delivery systems face challenges such as low bioavailability due to limited corneal retention time and the rapid clearance of administered drugs through the nasolacrimal drainage system. Additionally, maintaining therapeutic drug level in target tissues over an extended period remains difficult. To address these challenges and improve drug delivery to the anterior segment of the eye, topical gelling systems are been investigated. Incorporating drug -loaded bilosomes into an in-situ gel will help to enhance trans corneal drug permeation and improved ocular bioavailability. (Ahmad, M. Z et al 2013).

There are various different vesicular drug delivery systems which have been fortunate process to increase the therapeutic performance and stability of many drugs. These systems involve liposomes, noisomes, ethosomes and bilosomes etc.

Bilosomes are an advanced form of drug delivery system and a type of vesicular drug delivery system which is made up from non-ionic surfactants and bile salts. They are spherical, bilayered structures that resembles as liposomes but are more stable due to presence of bile salts which helps to enhance their resistance to brutal the conditions like enzymatic activity etc. Bilosomes are novel vesicular carriers composed of bile salts and phospholipids, forming stable, non-ionic surfactant vesicles and an advanced form of delivery system which increases the bioavailability and stability of therapeutic agent. (Alsaidan, O. A.

et al 2024).

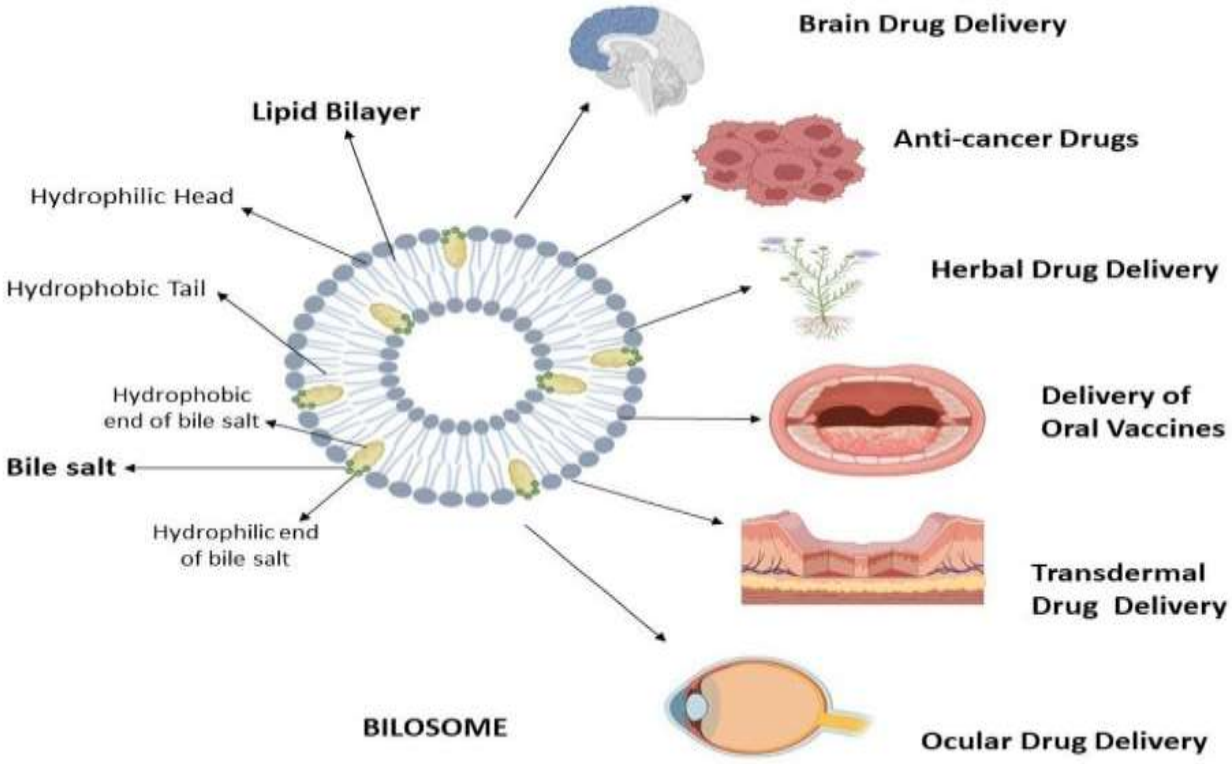

Figure-1 Bilosomes Structure (Kaurav et al 2024)

KEY FEATURES OF BILOSOMES IN OCULAR DELIVERY OF DRUG

1. **Enhanced Drug Stability:** The bilosomes protects the sensitive drugs from degradation in the ocular environment, including the enzymatic activity and oxidative stress. (Wang *et. al* reported that The EGCG bilosome was developed and described, as well as its stability under various conditions of storage (pH, NaCl concentration, and temperature) as well as in gastrointestinal fluid was assessed and compared with liposomes and niosomes. Among the various formulations, EGCG niosomes exhibited the greatest pH stability, but the presence of sodium cholate diminished the stability of bilosomes in acidic environments. The stability of EGCG was markedly enhanced in the presence of salt ions (0–100 mM NaCl) and at varying temperatures (25 °C, 37 °C) when administered as niosomes and bilosomes) (Wang et al 2024). (https://doi.org/10.1002/adfm.202403142).

2. **Improved Bioavailability**: Bioavailability can be improved by two ways, namely, by maximizing drug permeation or absorption and by minimizing precorneal drug loss and the use of drug delivery systems, which provide the controlled and continuous delivery of ophthalmic drugs to the pre- and intraocular tissues.[16] (Sakr et al., (2023) prepared betaxolol hydrochloride-loaded highly permeable ocular bilosomes (HPOBs) to combat glaucoma. BH-loaded HPOBs were made with a modified ethanol injection technique. BH permeability was 2.2 times higher in HPOB-4 than in the commercial eye drop.

Therefore, by increasing BH's transcorneal penetration, the overall results showed the potential application of HPOBs in efficiently delivering hydrophilic molecules (Sakr et al.,2023) (https://doi.org/10.1016/j.jddst.2023.104363).

3. **Prolonged Retention Time:** Bilosomes adhere to the ocular surface, increasing the residence time of drugs and reducing the frequency of administration. (Nabarawi et al., (2019) prepared and characterized the bilosomes as a novel carrier for the cutaneous delivery for dapsone (DPS). The amounts of DPS retained in the skin treated with DPS-loaded bilosomes and DPS alcoholic solution after 24 hours were determined to be 170.57 ± 55.12 and 120.24 ± 10.7 µg/mL, respectively. This indicates that the drug retention in the skin treated with bilosomes was approximately 1.5 times greater). (Nabarawi et al., (2019) (https://doi.org/10.1080/08982104.2019.1577256).

4. **Biocompatibility:** The ingredients used in formulation of bilosomes such as bile salts, are naturally occurring substances which make them more biocompatible with the ocular surface in treatment of eye disease. (A timolol maleate proniosomal gel was created by dispersing proniosomes using an in-situ gelling process, after which different amounts of cholesterol, lecithin, span 60, Brij 72, and Tween 80 were added. The FTIR analyses revealed no signs of interaction between the drug and the excipients, indicating that they are compatible. At the end of 12 hrs., formulations T2 and T10 had 99.98 percent and 99.90 percent drug release, respectively. (Lokapur et al., 2022) (https://doi.org/10.1080/08982104.2019.1577256)

5. **Controlled Release:** The Bilosomes enables the sustained or controlled release of drug by maintaining therapeutic drug levels for extended period and reducing side effects. (Ramadan et al., (2020) characterized the timolol maleate niosome formulations for the treatment of glaucoma. TM niosome were created using a variety of non-ionic surfactant combinations, the most sustained release of timolol maleate from the niosome (96% and 97.10%, respectively) within a 24-hour period. Niosomal gel formulations exhibited a delayed and sustained release of the drugs. The bioavailability of the test formula was 1.6 times greater than that of the commercially available Timogel (Ramadan et al., 2020) (https://doi.org/10.1080/08982104.2019.1577256)

6. **Reduced Irritation:** The bilosomes is composed of biocompatible materials that are gentle on the ocular surface and minimizing irritation and enhance patient compliance. (Nemr et al., (2022) prepared Hyaluronic acid-enriched bilosomes to improve the ocular delivery of agomelatine. AGO bilosomes were created using a modified ethanol injection approach. The Optimal bilosome formula is safe for the eye and does not induce ocular irritation or blurred vision, as confirmed by measures of pH and Refractive index in conjunction with the histopathological examination (Nemr et al., 2022). https://doi.org/10.1080/10717544.2022.2100513).

7. **Versatile Drug Encapsulation:** the bilosomes are suitable for encapsulating hydrophilic and lipophilic drugs which makes the dug more adaptable for various ocular conditions.

(Mohsen et al., (2020) developed acetazolamide loaded bilosomes for improved ocular delivery. Span 60, cholesterol, and several bile salts (sodium cholate, sodium deoxycholate, sodium taurocholate, and sodium tauroglycocholate) were used in two molar ratios (1:1:0.1 and 1:1:0.2) to make CZ bilosomes using the thin film hydration method. Under transmission electron microscopy, the produced vesicles showed spherical morphology, high entrapment efficiency (69.03–74.24%) (Mohsen et al., (2020) (https://doi.org/10.1016/j.jddst.2020.101910).

Importance of bilosomes in eye treatment

Bilosomes play a significant role in the eye treatments due to their unique properties that improve the efficacy of ocular drug delivery. Their importance can be highlighted in several ways.

1. **Enhance Drug Uptake**: In the treatment of eye diseases, the bilosomes helps to increase the absorption or as well as improve the bioavailability of drug by ensuring that a higher concentration of the medication reaches to the targeted tissue and improving therapeutic outcomes.(Sakr et al.,2023) (https://doi.org/10.1016/j.jddst.2023.104363).
2. **Regulate Drug Delivery**: Bilosomes allow for the gradual, sustained, controlled and as well as modulate release of drug, which helps in maintaining therapeutic drug levels over an extended period and reducing the need for frequent dosing. (Nemr et al 2022) (https://doi.org/10.1080/10717544.2022.2100513).
3. **Boost transcorneal penetration:** Bilosomes facilitate the increasing of drug permeation from the cornea which helps to enabling better drug penetration into the eye. This is crucial for treating deeper tissues such as retina which otherwise difficult to reach. (Wang et al 2024) (https://doi.org/10.1002/adfm.202403142).
4. **Prolonged Contact Time:** The use of bilosomes in eye treatment can extend the duration of time and drug stays in contact with the corneal surface which improve drug absorption and reduce the risk of drug washout. (Ramadan et al., 2020) (https://doi.org/10.1080/08982104.2019.1577256)
5. **Ensure safety and comfort:** Bilosomes are generally safe and non-irritating, and by making them suitable for long term use in sensitive areas such as eye, without causing discomfort or adverse reactions.(Mohsen et al., (2020) (https://doi.org/10.1016/j.jddst.2020.101910).
6. **Targeted treatment:** By improving the drug retention and penetration, bilosomes helps to deliver the drug more effectively to specific ocular sites, by optimizing the treatment of conditions like glaucoma, dry eye, or retinal diseases.(Sakr, M. G., et al.2023) (https://doi.org/10.1016/j.jddst.2023.104363).
7. **Improved Stability:** In the treatment of eye, the bilosomes structure provides increased stability for encapsulated drugs, and protecting them from degradation and ensuring sustained therapeutic effect.(Nabarawi et al., (2019) (https://doi.org/10.1080/08982104.2019.1577256).

8. **Bile Salts Functionality:** Bile salts improve drug penetration into the ocular tissues by modulating membrane permeability without causing significant damage. (Alsaidan, O. A. et al 2024) (https://doi.org/10.3390/gels8110687).
9. **Therapeutic Application;** The bilosomes have been explored for the treatment of eye with various diseases or conditions including glaucoma, cataracts and many infections in the eye.(Nemr, A et al. (2022) (https://doi.org/10.1080/10717544.2022.2100513).
10. **Non-invasive Delivery:** The bilosomes provide a non-invasive drug delivery option compared to systemic injections or implants which enhance patient compliance in ocular treatment.[15] (Wang, Y. et al. 2024). (https://doi.org/10.1002/adfm.202403142)

Composition and Structure of Bilosomes

Bilosomes are tiny artificial structures made to mimic the natural layers of cell membranes. They are made from two main ingredients: phospholipids (which are a key part of cell membranes) and bile salts (which help with digestion). The similarity in their structure to natural membranes, bilosomes can be used to deliver drugs or generic material to specific cells or tissues in the body more effectively. This makes them useful for improving the delivery and effectiveness of medicine or treatments. (Kothawade et al 2023).

Bilosomes are specialized vesicular systems composed of bile salts, lipids, and other excipients. The structure of bilosomes is similar to that of liposomes but incorporates bile salts, which provide unique stability and functional properties. (Nayak, D et al 2023).

Components:

1. **Lipids as component of bilosomes**
2. **Non- ionic Surfactant as component of bilosomes**
3. **Bile salts as component of bilosomes**

Lipids as component of bilosomes: Bilosomes are special structures made from phospholipids and cholesterol**.** Phospholipids are highly compatible with biological membranes, making them ideal for use in these systems. These molecules have both water- attracting(hydrophilic) and water repelling(hydrophobic) properties which is a feature called Amphiphilicity. This allows them to naturally form layered structures called concentric bilayers. These bilayers help in wetting and emulsifying substances. (Jana et al 2022).

Common phospholipids used in bilosomes include dicetyl phosphate, soyabean phosphatidylcholine, and monopalmitoyl glycerol. These phospholipids are selected for their ability to support the self-assembly process and form stable bilayer membranes. Cholesterol is another key component of bilosomes. It also has amphiphilic property. Cholesterol molecules position themselves within the bilayer, with their hydroxyl group (which are hydrophilic) facing the water, and their aliphatic chains (which are hydrophobic) aligning with the fatty acid tails in the bilayer's core. This strategic positioning stabilizes the bi layer structure. Cholesterol can cause phase separation in bilayers made of multiple lipid types. This means it can create

distinct regions within the membrane with different properties. Additionally, cholesterol can influence the behavior of proteins embedded in the membrane, causing them to change shape or move within the bilayer. One most important effect of adding cholesterol is the enhancement of bilosomes rigidity. Cholesterol helps to stabilize the bilayer by filling gaps between phospholipids molecules and making the membrane less fluid and more durable. This increased rigidity is critical for the structural integrity of bilosomes. (Kaurav et al 2024) (Jana et al 2022).

Nonionic surfactant as component of bilosomes: Nonionic surfactants are commonly used in making bilosomes because they are more stable and compatible compared to types of surfactants, such as anionic, cationic, and amphoteric ones. These surfactants are gentle on cells, causing less damage to red blood cells (less hemolytic) and are better at maintaining the stability and integrity of bilosomes. These compounds act as solubilizers, wetting agent, emulsifier, and permeability enhancers. They also inhibit P-glycoprotein, preventing drug efflux from cells and facilitating enhanced drug absorption and targeted delivery to specific tissues. [26] (Nemr et al 2024)**.** Non-ionic surfactants possess both polar (hydrophilic) and non-polar(lipophilic) regions, which contribute to their interfacial activity. Their entrapment efficiency depends on factors like the chain length and the size of the hydrophilic head groups. For instance, surfactants with stearyl chains(C18) demonstrate higher entrapment efficiency than those with shorter lauryl chains(C12). To maximize the entrapment efficiency of water-soluble drugs, a blend of tweens along with long alkyl chains and large hydrophilic components, combined with cholesterol in a 1:1ratio, is often employed. The hydrophilic-lipophilic balance (HLB) value of surfactants is critical in regulating drug entrapment within vesicles. Surfactants with HLB value around 8.6 are ideal, offering the highest entrapment efficiency, while lower HLB values (e.g. 1.7) result in reduced efficiency. Conversely, surfactants with very high HLB value (14-17) are unstable for bilosomes vesicle production. (D. Nayak et al 2023).

Bile Salts as a component of bilosomes: Nonionic surfactants are commonly used in making bilosomes because they are more stable and compatible compared to types of surfactants like sodium glycocholate (SGC), sodium deoxycholate (SDC), and sodium taurocholate (STC)for the production of bilosomes is primarily due to their distinct physiochemical properties, which enhance the efficiency of drug delivery systems. The repulsion induced by the interaction of internal bile salts within bilosomes and external bile salts maintaining bilosomes integrity. (Abdel-moneum et al 2023)

These bile salts play a significant role in-

1. Solubilization: They improve the solubility of lipophilic drugs, making them more bioavailable for absorption. (Ali et al 2024)
2. Stabilization of Bilosomes: By introducing steric and electrostatic repulsion, bile salts enhance the stability and flexibility of bilosomes structure. (Nayak et al.2023)
3. Drug Encapsulation and Release: Their amphiphilic nature allows them to form mixed micelles with lipids, facilitating the effective encapsulation and controlled release of drug. (Elebyary et al 2024**)**

4. Resistance to Degradation: Bilosomes incorporate these bile salts exhibit enhanced resistance to enzymatic degradation, improving the delivery of the drug. (Elebyary et al 2024)

Advantages of Bilosomes in Ocular Drug Delivery

1. **Improved Bioavailability of Drug:** In ocular system many drugs do not pass thorough cell membranes and even get breakdown before absorption, so the bilosomes increase the effectiveness of lipophilic drugs by protecting them from breaking down and make it easier for the drugs to pass through cell membranes, improving their absorption in the body. (Chauhan et al 2017).
2. **Drug Protection:** Bilosomes protect drugs from being broken down by enzymes and other natural process in the ocular system. This helps the drugs stay effective for longer. By delivering drugs directly to the target cells or tissues, bilosomes also reduce the chances of the drugs affecting to other part of system which lower the risk of toxicity and other side effects. (Kumar, S. et al 2016)
3. **Biocompatibility:** Bilosomes are considered safe for the ocular system and as well as body because they are made from natural components like phospholipids, which are found in the body, and bile salts. These materials are familiar to ocular system, which reduces the risk of harmful reactions and without unwanted side effects. (Pandey, P. 2023)
4. **Targeted delivery of Drug:** Bilosomes are highly effective for delivering drugs to the eyes because they allow targeted delivery of the drug. This means the medicine or drug id directed precisely to the affected area in the eye, and improving treatment results. By focusing on the target, bilosomes reduce waste and minimize side effects and making the treatment safer and more efficient. (Shah, N.et al. 2018)
5. **Prolonged Retention time on ocular surface:** Bilosomes helps in keeping the drug on the eye's surface or ocular surface for a longer time because they have a protective structure that prevents the drug from washing away from the surface of eye quickly. This prolonged retention allows the drug to stay in contact with the eye for an extended period, and improving the absorption and making the treatment more effective. (Batur, E et al 2024).
6. **Protection from enzymatic degradation and tear dilution:** Bilosomes protect drugs in the eye by forming a shield around them. This shield prevents enzymes in the eye from breaking down the drug and also protects it from being diluted or washed away by tears, which results that the drug stays stable and effective for a longer time and improving the ability to treat eye conditions. (Mahmoud, H. A., et al. 2020)
7. **Versatility in encapsulating various type of drug**: Bilosomes are versatile because they can carry different types of drugs, including water loving(hydrophilic) and fat loving(lipophilic) ones. Their structure allows them to hold and protect these drugs effectively. This flexibility makes bilosomes suitable for delivering a wide range of drugs and medicines and improving their stability and ensuring they reach their target in the system (Prajapati, S. K., & Patel, K. P. 2015)

Preparation Methods of Bilosomes and Factors influencing bilosomes:

The methods of preparation are-

1. Film Hydration Method
2. Reverse Phase Evaporation Method
3. Ether Injection Method

- **Film Hydration Method:** Bilosomes are commonly prepared using the film hydration method, a simple and adaptable technique. This method involves hydrating a thin film of phospholipids and bile salts, leading to the self-assembly of bilosomes. The process starts by creating a thin layer of these components. A solution containing phospholipids and bile salts is spread onto a glass plate, then evaporated under a laminar flow hood or in a desiccator. This step removes the solvent, leaving a dry, uniform film. To initiate hydration, a buffer solution is added to the dried film, often combined with the material to be encapsulated, such as antibiotics or genetic material. Hydration can be enhanced by sonicating the glass plate or gently swirling it to ensure even distribution. During hydration, the phospholipids and bile salts reorganize into bilosomes. This self-assembly process is influenced by factors such as temperature and mixing rate, which can be adjusted to optimize bilosomes formation. Once the bilosomes have formed, they are collected through centrifugation or ultracentrifugation. The resulting bilosomes are then analyzed for key characteristics, including size, stability, and encapsulation efficiency. These parameters are critical for ensuring the bilosomes are suitable for their intended application. (Sharma, M., et al. 2022) (https://doi.org/10.22270/jddt.v12i4.5420).

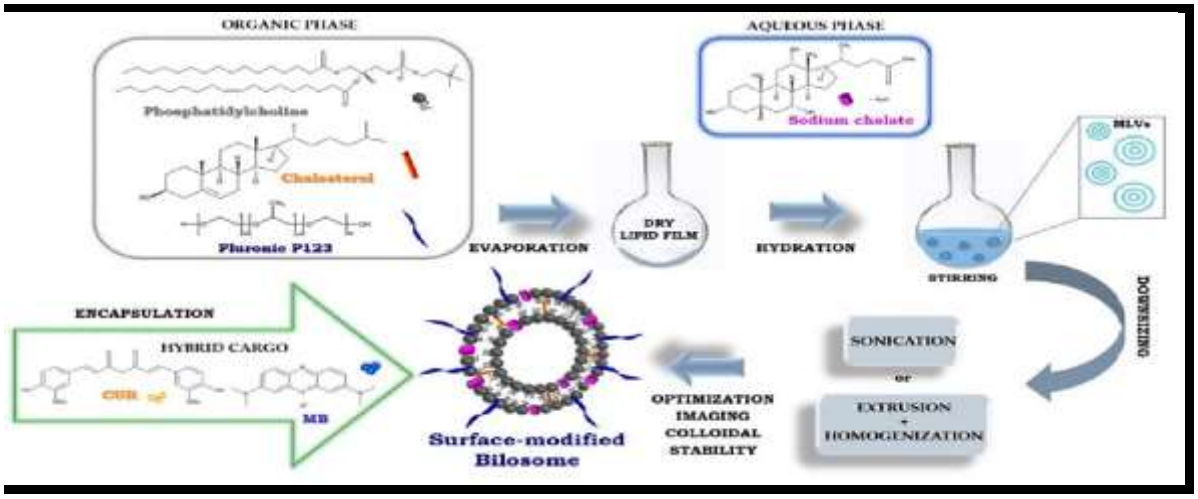

Figure- Film Hydration Method(Ahmad et al 2020)

- **Reverse Phase Evaporation Method:** The film reverse phase evaporation method is a widely used technique for preparing bilosomes and this method also known by reverse phase evaporation, similar to the film hydration method but with key differences. It involves the use of organic solvents methanol or chloroform to dissolve phospholipids

and bile salts. This solution is then combined with water, and the organic solvent is removed under low pressure, leaving a thin layer of phospholipids and bile salts at the interface of the organic and aqueous phases.

The encapsulated material, such as a drug or genetic substance, is added to the aqueous phase and stirred gently to form bilosomes. The resulting bilosomes are then separated through centrifugation or ultracentrifugation. This method is particularly effective for producing multilamellar vesicles (MLVs), which closely resemble natural bilayers. Additionally, using organic solvents can enhance the solubility and stability of the encapsulated material, protecting it from degradation. However, the reverse phase evaporation method has limitations. The use of organic solvents can pose safety risks and affect the characteristics of the bilosomes. Also, not all substances are compatible with this technique, so adjustments may be necessary to optimize results based on the properties of the encapsulated material. While this method is a common choice for bilosomes preparation, other methods, such as liposome extrusion, lipid film hydration, and microfluidic techniques, are also used depending on the application and desired outcomes. (Palekar-Shanbhag el at 2020).
https://doi.org/10.2174/1574885514666190917145510

- **Ether Injection Method:** The ether injection method is a simple and effective technique used to prepare bilosomes. In this method, phospholipids and bile salts are dissolved in an aqueous solution at an appropriate pH and temperature. An organic solvent, such as ether, is then injected into the aqueous solution using a syringe or similar device. The mixture is stirred or sonicated, causing the phospholipids and bile salts to self-assemble into bilosomes. The bilosomes are then separated using centrifugation or ultracentrifugation. Once isolated, the size, stability, and encapsulation efficiency of the bilosomes are evaluated. This method is particularly effective for creating multilamellar vesicles (MLVs), which closely mimic natural bilayers. Additionally, the use of organic solvents can improve the solubility of the encapsulated material and protect it from degradation. However, like other methods, the ether injection technique has its limitations. Organic solvents can be hazardous and may influence the properties of the bilosomes. Additionally, this method may not be suitable for all types of encapsulated substances. Therefore, the process may need to be adjusted to suit the specific requirements of the material being encapsulated.

  It’s important to note that the ether injection method is just one of many approaches for preparing bilosomes. Other techniques, such as lipid film hydration, liposome extrusion, and microfluidic methods, are also widely used, depending on the application and desired outcomes. (Kumar, S. et al 2023). https://doi.org/10.2147/JNN.S389101

Characterization of Bilosomes:

1. **Dynamic Light Scattering (DLS):** DLS measures the size and size distribution of bilosomes by analyzing how laser light scatters when it hits the particles in a suspension. This non-destructive technique also helps assess the stability of the bilosomes. (Khan, M. R., et al. 2021) https://doi.org/10.1016/j.jddst.2020.102211

2. Transmission Electron Microscopy (TEM): TEM uses an electron beam to capture images of bilosomes. It reveals their size, shape, and structural details like layers or flaws in the bilayer structure. (Walewska, E.et al 2020). (https://doi.org/10.3390/nano10122011)
3. Zeta Potential: This technique measures the surface charge of bilosomes, helping to understand their stability. Positively charged particles repel each other, while negatively charged ones attract, influencing particle behavior. (Gupta, D. K et al 2022)https://doi.org/10.1016/j.ijpharm.2022.114650
4. Differential Scanning Calorimetry (DSC): DSC studies thermal properties like melting and crystallinity of bilosomes. It helps determine lipid types and how compactly they are packed. (Almalki, A. H., Zidan et al 2023) (https://doi.org/10.3390/polym14194184)
5. Fourier-Transform Infrared Spectroscopy (FTIR): FTIR analyzes the chemical composition of bilosomes using infrared light. It identifies lipid types, chemical interactions, and structural changes over time or under different conditions. (Beloqui, A. et al 2019) (https://doi.org/10.1016/j.jconrel.2019.07.021)
6. Nuclear Magnetic Resonance (NMR) Spectroscopy: NMR provides insights into the molecular structure and interactions within bilosomes, such as lipid packing and their interactions with other molecules.(Nemr, N et al 2022) (https://doi.org/10.1080/10717544.2022.2100513)
7. X-Ray Diffraction (XRD): XRD examines the crystallinity and structural organization of bilosomes, including how the lipids are arranged. (Shakeel F et al 2018)((https://doi.org/10.1080/10717544.2017.1412196)
8. Size Exclusion Chromatography (SEC): SEC separates particles based on size, helping determine the size distribution and polydispersity (variation in particle sizes) of bilosomes. (Kumari, S. et al 2021) (https://doi.org/10.3390/gels8070418)
9. Fluorescence Spectroscopy:
10. This technique uses fluorescent dyes to study interactions between bilosomes and molecules like drugs. It helps locate drugs within bilosomes and evaluate their stability. (Mosallam et al 2021) (https://doi.org/10.1208/s12249-021-01983-9)

Applications of Bilosomes in Eye Treatment:

- **Treatment of cataract:** Bilosomes have potential in the treatment of cataract due to their unique properties as a drug delivery system. Cataracts are caused by the clouding of the eye's natural lens, which leads to vision impairment. Currents treatments often rely on surgery, but innovative drug delivery systems like bilosomes can provide non-invasive option to manage or prevent cataracts. Bilosomes can contribute by enhanced drug delivery, increasing penetration of drug, protection of encapsulated drug, sustained release of drug. (Al Shuwaili et al 2016) (https://doi.org/10.18433/J3ZP5M)

Management of Glaucoma: Bilosomes are tiny carriers made from natural components like bile salts and lipids. They are used to deliver drug or medicines more effectively in glaucoma treatment, which is a condition where high pressure in the eye can damage vision. Bilosomes protect the drug by making shield over the drug and do not break down too quickly, which increase the better absorption of drug and with reduced side effects

which improves the effectiveness of glaucoma treatment, providing better results with less medication (El-Zaafarany et al 2022). (https://doi.org/10.1016/j.ijpharm.2022.121697)

- **Addressing diabetic retinopathy:** Bilosomes are small, protective carriers made of natural substances like bile salts and lipids. They help in the treatment of diabetic retinopathy, a condition caused by high blood sugar levels which damage the blood vessels in the retina. Bilosomes helps in this treatment by targeted delivery of drug, enhanced absorption of drug by increased penetration of drug through eye tissues. Bilosomes also protect the drug from breaking down before it reaches retina and also decrease the side effects. This makes treatment for diabetic retinopathy more effective and safer(Nagai, N. et al 2018)(https://doi.org/10.2147/OPTH.S178355)
- **Age -related macular degeneration (AMD):** Bilosomes are tiny, protective carriers made from natural fat-like molecules(lipids) that help to deliver drug such as eye. AMD is a common eye condition that affects the macula (a small but critical part of the retina responsible for sharp, central vision so bilosomes will improve the delivery of drug to retina and targeted delivery of drug, by better absorption and protection of drug with controlled release of drug. This technique can treat the AMD more effectively and compatible for patients. (Varela-Fernández et al 2020) (https://doi.org/10.3390/pharmaceutics12030269)
- **Chronic dry eye and other surface diseases:** Bilosomes are tiny carriers made from natural fat-like molecules that help deliver medicine directly to the eye. In chronic dry eye and other surface eye diseases, bilosomes improve treatment by making medications more effective and longer-lasting. Bilosomes helps by Protecting Medicine, Better Penetration, Longer Action, Reduced Irritation. By improving drug delivery and effectiveness, bilosomes offer a better way to manage chronic dry eye and other surface eye conditions, providing relief and protecting the eye's health.(Aggarwal et al 2017) https://doi.org/10.1080/10717544.2017.1344332)

Challenges and Limitations:

1. **Variability in preparation conditions:** Variability in preparation conditions refers to the differences in how bilosomes are made, such as the ingredients, methods, and environmental factors used during production. These variations can create challenges in the ocular system because of Inconsistent Quality (Differences in preparation can lead to bilosomes that vary in size, shape, or stability, which can affect how well they work in delivering drugs to the eye), Reduced Effectiveness (If the bilosomes aren't consistently prepared, they may not be able to carry or release the drug properly, reducing their ability to treat eye conditions effectively), Reproducibility Issues (Making bilosomes with the same properties every time can be difficult, which is a problem when creating medicines that need to work the same way for every patient).In simple terms, variability during the preparation of bilosomes makes it harder to ensure they will always work as expected for eye treatments.(Singh et al 2023). (https://doi.org/10.1007/s13346-023-01215-x)

2. **Scalability and manufacturing challenges:** Bilosomes face challenges in scalability and manufacturing because making them in large amounts while maintaining quality is difficult. So, it effects as having Complex Production Process (Bilosomes require precise conditions and steps to ensure they are the right size, shape, and stable enough. Scaling up these processes for mass production can be tricky), Equipment and Cost (Producing bilosomes at a large scale often needs specialized equipment and materials, which can be expensive and hard to set up). Quality Control (Ensuring that every batch of bilosomes meets the same quality standards is challenging, especially when production increases. Even small variations can affect how they work). Reproducibility (Large-scale production makes it harder to recreate the exact conditions used in small-scale lab settings, leading to potential differences in the final product). In simple terms, making bilosomes in large quantities while keeping them effective and consistent is a big challenge due to the complexity and costs involved. (Garala et al 2021). (https://doi.org/10.2174/1567201818666210114104002)
3. **Regulatory considerations and clinical trials:** Bilosomes face challenges with regulatory approval and clinical trials in the ocular system because they are a newer drug delivery technology. So, it has Strict Regulations (Regulatory agencies like the FDA have strict rules to ensure that new treatments are safe and effective. Since bilosomes are relatively new, there isn't much existing data, making the approval process more complex and time-consuming). Safety Concerns (For use in the eyes, which are very sensitive, there needs to be strong evidence that bilosomes won't cause irritation, infection, or other side effects. This requires extra safety studies). Complex Testing (Clinical trials must show that bilosomes can deliver drugs effectively to the eyes without harm. Designing these trials is complicated and expensive because of the unique nature of the eye and the need for specialized tests). Lack of Established Guidelines (Since bilosomes are an emerging technology, there aren't well-established guidelines for their evaluation, making it harder to meet regulatory expectations). In simple terms, getting bilosomes approved for use in eye treatments is difficult because of strict safety requirements, expensive testing, and a lack of clear rules for new technologies. (Zhang et al 2022). (https://doi.org/10.1016/j.addr.2022.114207)

Future Directions and Research Opportunities:

1. **Innovations in Bilosomes Technology**: Innovation in bilosomes technology offers exciting future directions and research opportunities for improving eye treatment by ensuring better drug delivery, long lasting effect of drug, targeted material, combining therapies, personalized medicine etc. By addressing these opportunities, bilosomes technology could revolutionize how we treat eye diseases, making therapies more effective, comfortable and accessible. (Varela-Fernández et al 2021). (https://doi.org/10.3390/pharmaceutics13091387)
2. **Potential for Combination Therapies:** The potential for combination therapies in the ocular system offers exciting future directions and research opportunities. By Treating multiple conditions Combination therapies like glaucoma or infections more effectively, better results (Using multiple drugs together can enhance their effectiveness by targeting

different aspects of a disease or working in synergy), Simplified treatments (Combining drugs into a single dose reduces the need for multiple eye drops, making treatment easier and more convenient for patients). Custom solutions (Research is focused on tailoring combinations to specific eye conditions or individual patient needs for better outcomes). Fewer side effects, Advanced drug delivery. These therapies could transform eye care by making treatments more efficient, effective, and user friendly. (Bhatia et al 2022) https://doi.org/10.1016/j.ejpb.2022.01.012)

3. **Ongoing research and development efforts**: Ongoing research and development efforts in the ocular system focus on finding better ways to treat eye diseases and improve patient care. These efforts open up future directions and opportunities by Improved drug delivery, new materials (safe and more comfortable), long lasting treatments, smart technologies (innovation like systems that release drugs based on trigger like temperature, pH, or time are being developed to improve precision and convenience) (Gulati et al 2021) (https://doi.org/10.2174/2211738509666210106153729)
4. **Future Prospects in the field of ocular drug delivery:** The field of ocular drug delivery has a bright future, with research focused on improving how medicines are delivered to the eye. Current treatments, like eye drops or injections, can be inconvenient or less effective because the eye's natural barriers limit drug absorption. Future advancements aim to overcome these challenges with innovative approaches, including Sustained-Release Systems (Developing implants, contact lenses, or nanoparticles that slowly release medication over time, reducing the need for frequent dosing), Minimally Invasive Methods (Using microneedles or advanced eye drops that penetrate deeper into the eye for better treatment outcomes),Gene and Cell Therapies(Exploring ways to use genetic material or cells to treat eye diseases at their root cause, potentially offering long-term or permanent solutions),Smart Devices(Creating systems that monitor eye health and deliver drugs only when needed, improving personalized treatment),Better Drug Formulations(Designing drugs that stay longer on the eye's surface or target specific parts of the eye more effectively).These advancements aim to make treatments more effective, safer, and more convenient, improving the quality of life for patients with eye conditions. (Lajunen et al 2023) (https://doi.org/10.1016/j.addr.2023.114771)

Conclusion

1. In conclusion, engineered bilosomes show great promise as an innovative solution for eye treatments. These specialized vesicles can improve the delivery of drugs to the eye, offering advantages like better stability, controlled release, and enhanced absorption. Their ability to target specific areas within the eye could lead to more effective treatments with fewer side effects. Overall, bilosomes represent a promising future in the field of ocular drug delivery, offering potential benefits for patients and advancing eye care technology. (Das, S et al 2024).

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