IJBPAS, April, 2025, 14(4): 2325-2341

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

# BILOSOME: NOVEL VESICULAR CARRIER

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**Received 20th Dec. 2023; Revised 25th Jan. 2024; Accepted 6th July 2024; Available online 1st April 2025**

https://doi.org/10.31032/IJBPAS/2025/14.4.8956

## ABSTRACT

Oral administration remains the most popular means of drug administration as far as patient compliance is concerned. After oral administration, most of the biological therapeutics (proteins/peptides) and vaccines have poor performance due to poor solubility or degradation in the gastrointestinal tract (GIT). Tremendous research in the last decade has made the bilosomes now acts as potential carrier system to tremendous research. Bilosomes are niosome like colloidal carrier system containing bile salts. Bilosomeshave developed as a potential carrier system for transdermal and parenteral targeted drug delivery and oral vaccine delivery. The present article covers various aspects related to the novel vesicular system that is based on bile salts called bilosomes, for targeted drug delivery systems. It includes information related to introduction of bilosome, composition, material required, method of preparation, characterization methods, stability consideration, and applications.

**Keywords: Bile salts, Bilosomes, Vesicular carriers, Liposomes, Niosomes**

## 1. INTRODUCTION:

Bilosomes are closed bilayered vesicular carriers of lipids incorporating nonionic surfactants and bile salts. Their size ranges from 5-200 nm with spherical and both unilamellar and multilamellar vesicles **[1]**. Bilosomes were first explained by Conacher *et al*. from the University of Glasgow in 2001 **[2]**.

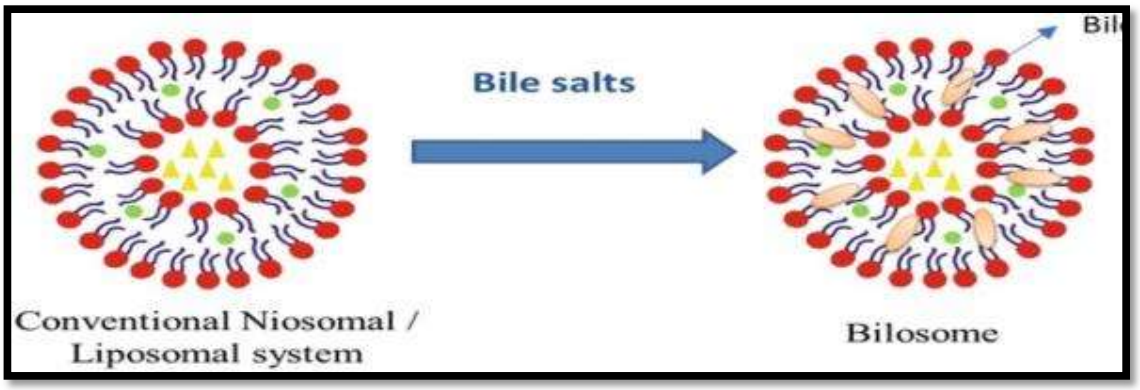



**Figure 1: Structure of bilosomes**

Oral delivery is the preferred route of drug intake considering the ease of administration and the patients' acceptance. The gastrointestinal tract acts as the substantial physical and a biochemical barrier for systemic availability of oral medicines due to highly acidic environment in the stomach and enzymatic degradations, variable intestine pH &mucus secretion, The oral bioavailability of drugs primarily depends on aqueous solubility and dissolution rate, permeability across biological membranes, pre-systemic metabolism and susceptibility to efflux mechanisms **[3]**. About 60–70% of drug molecules are insufficiently soluble in aqueous media and very low permeability. Bile acids are synthesized in liver and stored in gall bladder, and exist as ionized bile salts under physiological conditions. They are amphiphilic molecules that contain steroid nucleus with hydrophilic side chain containing hydroxyl group and a hydrophobic side chain containing methyl group. They play an important role in emulsifying and solubilizing dietary fats through the formation of mixed micelles. Hence bile salts increase the permeability of lipophilic drug molecules across the plasma membrane which results in increase in oral bioavailability of many biologically active molecules. Among different classes of colloidal systems, vesicular carriers have gained particular attention in delivering poorly soluble drugs and proteins/ peptides 20. Vesicular carriers comprise of unilamellar or multilamellar spherical structures formed of lipid molecules gathered into bilayers orientation and capable of encapsulating drug molecules **[4-6]**.

**Table 1: A Comparative account of different vesicular systems (liposomes, Niosomes and bilosomes) [7]**

| Characterization parameter | Liposomes | Niosomes | Bilosomes |
|---|---|---|---|
| Composition | Phospholipids With cholesterol and charge inducer | Nonionic Surfactant with cholesterol and charge inducer | Nonionic Surfactant and bile salt and charge induce |
| Chemical stability | Undergo Oxidative degradation Does Not | Does Not undergo oxidative degradation | Does not undergo oxidative degradation |

| | | | |
|---|---|---|---|
| Stability in simulated gastric fluid | Unstable | Unstable | stable |
| Stability in simulated intestinal fluid | Unstable | Unstable | stable |
| Antigen dose | Relatively high | Relatively high | Relatively low |
| Storage stability | Required Liquid nitrogen for storage | Special Conditions not required | Special Conditions not required |

**1.1 ADVANTAGES:**

- Bilosomes can allow small quantities of antigens so as to be effective and also help to increase the efficacy of antigens which are weak when injected.
- It is a non-invasive system that offers advantages in terms of user's acceptance and compliance and is less toxic and has wide range of therapeutic activity.
- Immune response can be manipulated by controlling size of the carrier vesicles.
- Bilosomes remove cold chain which is required for preparations such as vaccines.
- Antigens encapsulated or incorporated in polymerized liposomes, microspheres, nanoparticles or bilosomes can be protected from gastric acid and secreting enzyme.
- They can be easily stored after lyophilization (in the case of microspheres and nanoparticles) and do not need strict refrigeration storage conditions.
- Several multicomponent vaccine combinations can be carried out easily.
- No need of trained personnel during administration.
- Oral administration eliminates repeated dosing.
- Incorporation of antigens into biodegradable microspheres or microcapsules leads to prolonged and controlled delivery **[4]**.

**2. BILOSOMES:**

Bilosomes are composed of two layers:

• Innermost layer containing hydrophilic drugs and / or antigens

• Outermost layer containing bile salts and / or hydrophobic drugs

**2.1.Materials Used in the formulation of Bilosomes**

Materials used in bilosomes comprise of lipids, nonionic surfactants and bile salts.

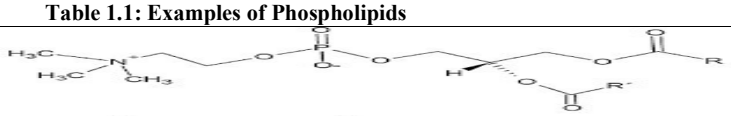
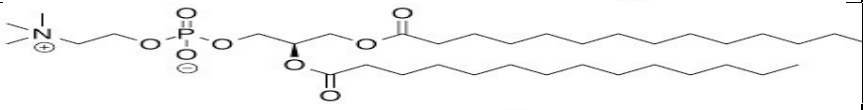
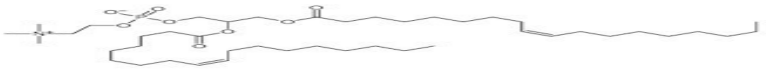
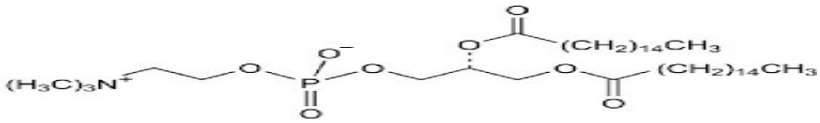
**2.1.1. Lipids:**

**2.1.1.1. Phospholipids-**

Phospholipids are unique and versatile molecules. These are naturally occurring components in cellular membranes and arranged as a lipid bilayer. Phospholipids play a significant role in the structure as well as functionality of

biological membranes. They are amphiphilic in nature and consist of a hydrophilic headgroup and a lipophilic/hydrophobic tail. Phospholipids have excellent biocompatibility and a especial amphiphilicity. Due to excellent biocompatibility and a especial amphiphilicity, phospholipids preferred as important pharmaceutical excipients and have a very wide range of applications in various drug delivery systems. Commonly used phospholipids that are used in bilosomes are as follows:

**Table 1.1: Examples of Phospholipids**

| | |
|---|---|
| **Soybean Phosphatidylcholine** |  |
| **DimyristoylPhosphatidylcholine** |  |
| **DilauroylPhosphatidylcholine** |  |
| **DioleoylPhosphatidylcholine** |  |
| **DipalmitoylPhosphatidylcholine** |  |

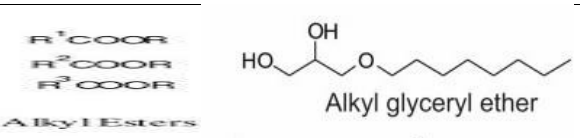
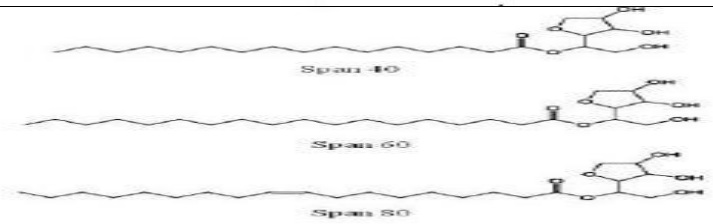
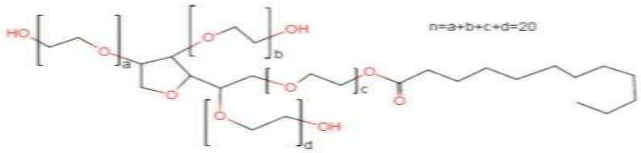
**2.1.1.2. Cholesterol-**

Cholesterol protects membrane lipid from chemical damage. It enhance the order of the lipid packing and lowers the lipid bilayer permeability in the liquid phase. It added to bilosomal membrane to prevent loss of large amount of active ingredients at high temperatures **[9]**.

**2.1.2. Nonionic Surfactants:**

Nonionic surfactants are widely used in the preparation of bilosomes because of their stability and compatibility properties compared to the anionic, cationic or amphoteric forms. Nonionic surfactants give minimal cellular irritation and sustain optimum pH which is close to physiological pH. It improves absorption of drugs and tissue specific action of drugs. They contribute to various roles such as permeability enhancers, solubilizers, emulsifiers and wetting agents.

**Table 2: Example of Nonionic Surfactant**

| | |
|---|---|
| **Polyoxyethylene 4 lauryl ethers** |  |
| **Alkyl esters and alkyl glyceryl ethers** |  |
| **Sorbitan fatty acid esters – Span 40, Span 60, Span 80** |  |
| **Polyoxyethylene fatty acid esters - Tween 20** |  |

### 2.1.3. Bile salt:

Bile acids are steroid acids found predominantly in the bile of mammals and other vertebrates **[10]**. Bile acids are synthesized by the liver. Secondary bile acids result from bacterial actions in the colon. In humans, taurocholic acid and glycocholic acid (derivatives of cholic acid) and taurochenodeoxycholic acid and glycochenodeoxycholic acid (derivatives of chenodeoxycholic acid) are the major bile salts **[11]**. Bile salts are primary component of bile and are needed by the body to help breakdown fats, aid digestion, absorb important vitamins and eliminate toxins. Bile salts are stored in your gallbladder when they're not being used. If your gallbladder is removed, it can lead to a bile salt deficiency **[12]**. Bile salts also enhance stability of bilosomes in simulated fluids, by causing repulsion between the bile salts present in the bilosomes and external bile salts in the gut lumen.

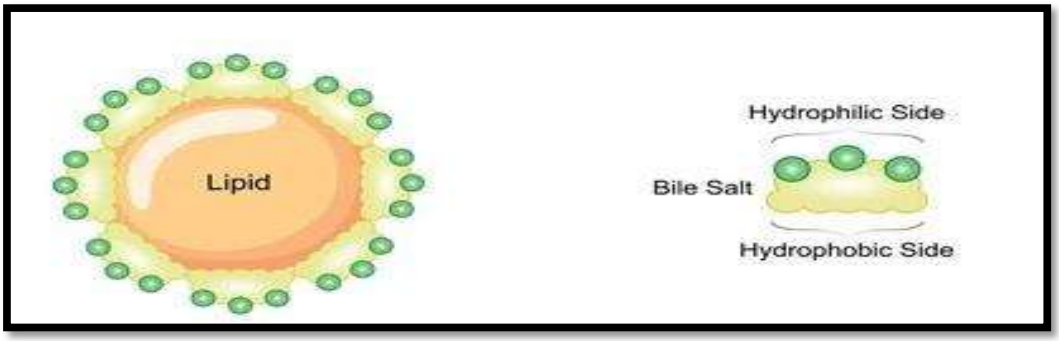
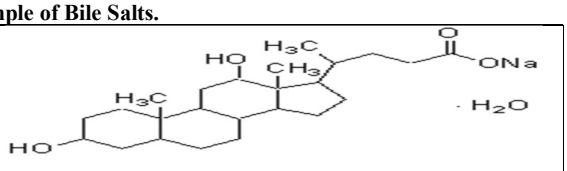
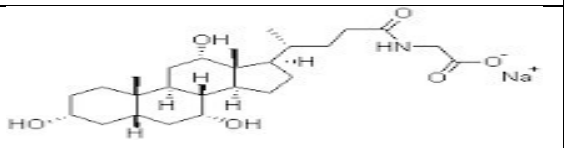
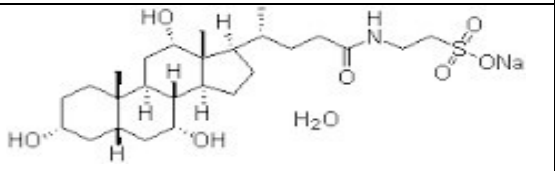
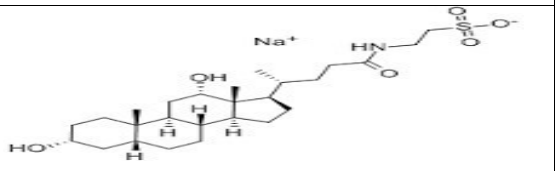



**Figure 2: Structure of Bile Salt**

Bile salts used in bilosomes are as follows: **[7, 13, 14]**

**Table 3: Example of Bile Salts.**

| | |
|---|---|
| **Sodium Deoxycholate (SDC)** |  |
| **Sodium Glycocholate (SGC)** |  |
| **Sodium Taurocholate (STC)** |  |
| **Sodium Taurodeoxycholate (STDC)** |  |

## 3. METHODS OF PREPARATION:

### 3.1. Thin-Film Hydration Method -

For preparation of antigen-containing bilosomes, surfactant, lipid components, cholesterol, and diacetyl phosphate (DCP) are dissolved together and evaporated at a low pressure. The thin layer thus formed is then hydrated with a buffer containing bile salt and antigen for creation of large multilamellar vesicles, further which are transformed into small unilamellar vesicles by extrusion. Thin-layer hydration is used to create bilosomes loaded with diphtheria toxoid Hepatitis B antigen BSA and tetanus toxoid **[15]**.

Advantage:

- Feasible on laboratory scale

• High entrapment efficiency in case of hydrophobic drugs

Disadvantage:

• High temperature exposure may lead to damage to phospholipid and/or drug

• Minimal encapsulation

• Difficult to scale up **[4]**.

**3.2. Hot Homogenization Method -**

In the hot homogenization process, bilosomes are prepared by melting the lipid components (cholesterol, monopalmitoyl glycerol, and DCP) at 140°C for 5 min, and then they are hydrated using a buffer solution. After the homogenization of this lipid mixture, bile salt solution is added to form a dispersion containing empty vesicles, and then it is again homogenized. Then antigen buffer solution is combined with the homogenate and protein entrapment is attained by constant freeze–thaw cycles **[16]**.

**Advantage:**

• Easy to handle

• It is a solvent free method

**Disadvantage:**

• Temperature induced degradation of drug **[4]**.

scattering and laser diffraction particle size analyzer **[20]**. Optically homogeneous square polystyrene cells are used, to analyze the particle size with

**3.3. Reverse phase evaporation method -**

Bilosomes with a triblock copolymer can be formulated using a different weight of sodium cholate using thin film hydration method. For stabilization of all nanocarriers, triblock copolymer pluronic P123 was used in a concentration of 0.6%. In a round-bottom flask, chloroform phosphatidylcholine, cholesterol and pluronic P123 were dissolved. Under reduced pressure by a rotary evaporator at 40°C with 80 rpm speed, the organic solvent was evaporated. Using 10 mL of distilled water containing sodium cholate the formed lipid thin film was hydrated. In order to strengthen the detachment of the lipid film, glass beads were added to the flask. The dispersion was stirred for 2 hours on the magnetic stirrer. To reduce the particle size the resulting suspension of multilamellar vesicles was further downsized using probe sonicator for about 15-20 min. These bilosomes were kept at 4°C. Using a similar procedure with the addition of curcumin to the lipid phase and methylene blue to the aqueous phase double-loaded vesicles were prepared.

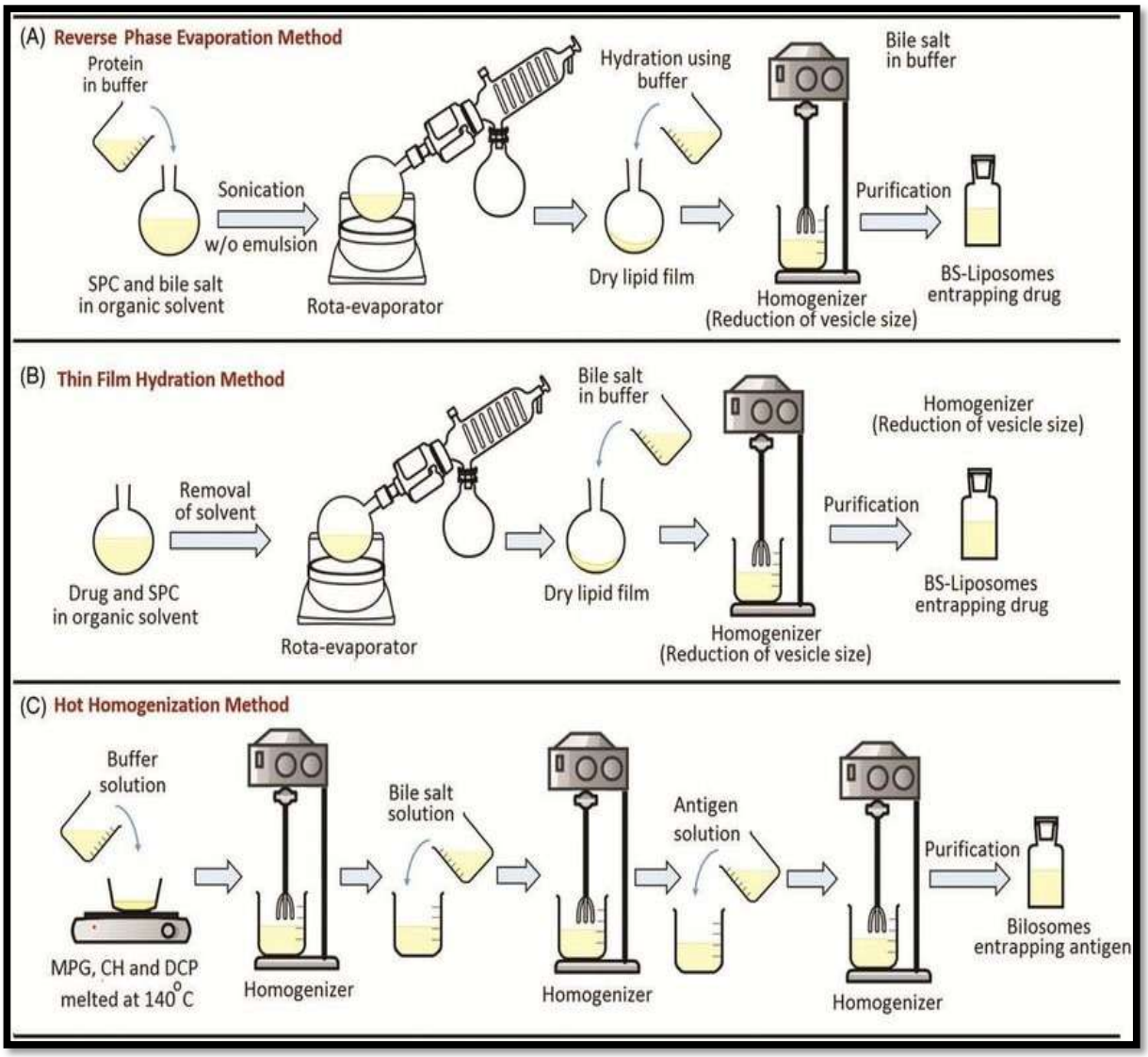



**Figure 3: Method of Preparation of Bilosomes [18]**

## 4. CHARACTERIZATION OF TECHNIQUES OF BILOSOMES:

### 4.1. In Vitro Characterization:

#### 4.1.1. Particle Size-

Particle size of bilosomes exerts substantial impact on their in vitro and in vivo performances. The vesicle size of bilosomes ranges from 90nm-3µm. Larger bilosomes vesicles (~6 µm versus 2 µm in diameter) showed increase in uptake within the Peyer's patches and were able to reduce median temperature differential change and promote a reduction in viral cell load in an influenza challenge study **[19]**. Particle size can be determined using dynamic light dynamic light scattering instrument **[21]**. In vitro and in vivo performances are greatly affected by the particle size of bilosomes **[22]**.

#### 4.1.2. Zeta Potential-

The overall charges acquired by the particles in a particular medium can be measured as zeta potential. Stability can be achieved if the vesicles are having a surface charge. Bilosomes are negatively charged due to the presence of bile salts that stimulated the zeta potential. Zeta potential is analyzed with the help of dynamic light scattering and electrophoretic mobility (EPM) measurements **[20]**. Due to electric repulsion between the particles, the zeta is around +30 mV and that system is considered to be the stable one.

**Table 4: Methods used to study morphology of bilosmes.**

| Method | Principle |
|---|---|
| Transmission electron microscope | It is used to ascertain the formation of BS-vesicles and visualize their morphology. This microscope works on principal that electron beam from electron gun is passed through ultra-thin section of the microscopic object & the image is magnified by electromagnetic fields [23] |
| Scanning electron microscope | A focused electron beam scans over a surface to create an image. The electrons in the beam interact with the sample & produce various signals. The signals can be used to obtain information of the surface topography and composition. |
| Cryo-electron microscopy | Cryo-EM, can directly visualize particles without any staining in a hydrated (albeit vitrified) state. That means that bilosomes can be imaged directly, and the bilosome structures are preserved very close to their native state in solution. The suspension has to form a thin film (usually) [24-29] |
| Freeze fracture microscopy | It is used for preparations that contain larger bilosomes (>500 nm). In freeze fracture, a thicker layer of the sample is quickly frozen, then fractured along its length which exposes surfaces with weak molecular interactions within lipid bilayer, followed by some etching that is water sublimation in the vacuum. The exposed surface is then metal shadowed and the replica eventually imaged in the electron microscope [30] |

**4.1.3. Ultracentrifugation-**

The ultracentrifuge works on the same principle as all other centrifuges. The working of an ultracentrifuge is based on the sedimentation principle. Sedimentation principle states that, the less dense particles slowly settle down as compared to the denser particles under gravity. An ultracentrifuge is a centrifuge that is optimized for spinning a rotor at very high speed and that can generate acceleration as high as 10 million grams (approximately 9800 km/s2). It is capable of separating unencapsulated drug in the drug-loaded bilosomes **[22]**.

**4.1.4. Polydispersity Index (PDI)-**

PDI can be measured using dynamic light scattering method. Due to the increase in a medium viscosity, there is an increase in bile salts content caused by vesicles enlargement bound with an increase in PDI. The degree of non-uniformity of particle size distribution is described by PDI. It is considered a homogenous population of phospholipid vesicles when the PDI value is 0.3 or below 0.3 **[31]**.

**4.1.5. Entrapment Efficiency Percent (EE%)-**

Entrapment efficiency is expressed as percent of the drug that is successfully entrapped/ encapsulated into the vesicles. Increase in the content of bile

salts simultaneously increases the drug entrapment efficiency and solubility in the dispersion medium **[1, 31]**. Also EE% increases with increase in the lipid content **[1, 31]**.

$$EE\% = \frac{\text{(Total amount of drug – Total amount of free drug) X 100}}{\text{Total amount of drug}}$$

Entrapment efficiency can determined using spectroscopic or chromatographic methods such as High Performance Liquid Chromatography and UV spectrophotometry.

**4.2. In Vivo Performance of Bilosomes:**

**4.2.1. Improvement in oral drug bio availability-**

In the literature, the integration of drugs or proteins into BS-liposomes substantially enhanced their bioavailability and in vivo efficacy. For example, orally administered SDC-liposomes loaded with fenofibrate displayed a 1.57-fold increase in bioavailability. In beagle dogs, liposomes have a higher bioavailability than conventional liposomes. Fenofibrate undergoes transmembrane absorption in BS-liposomes, based on the ultra-deformability of the vesicles introduced into the Peyer's patch through M-cells. The superiority of BS-liposomes compared to the marketed microemulsion formulation (Sandimmune Neoral) and conventional liposomes in increasing oral bioavailability of cyclosporine. Facilitated absorption of intact BS-liposomes have also been demonstrated. The increase in bioavailability of cyclosporine A was due to liposomal formulation rather than drug solubility **[18]**.

**4.2.2. Skin Irritability Test-**

Skin irritability test is performed to evaluate and observe any kind of skin irritation. The formulation is applied on the Wistarrats. The skin irritancy test is carried out as follows: The back of healthy rats is shaved in order to avoid peripheral damage 24 hours prior to the test. The rats may be divided into 2 groups; the first group is treated with no medication and the formulation is applied to the second group of rats .Both groups are examined for visual changes called erythema after 24, 48 and 72 hours after application. After observation according to the Draize scale, scores are given as follows:

1- Slight erythema (light pink)
2- Moderate erythema (dark pink)
3- Moderate to severe erythema (light red)
4- Severe erythema (extreme redness).

**4.2.3. Evaluation of anti-inflammatory activity-**

Evaluation of anti-inflammatory activity is carried out on the male Wistar rats (200 ± 50 g). This is carried out as follows: Each group contains six rats. Their backs are shaved. No medication may be given to the first group (negative control group). The rats of group two may be given the tablets. One of the group may be given the placebo and the last group can have a medication applied on their backs. Observation of all the groups is carried out simultaneously. 4% formaldehyde solution can be used to induce localized inflammation through sub-plantar injection of 0.1ml into one of the paw's footpad half an hour before the drug administration to ensure that maximum edema is obtained. The edema is then measured by plethysmometer at the 0, 1, 2, 4, 6, 24, 48 and 72 hour timestamps.

## 5. STABILITY CONSIDERATIONS OF BILOSOMES:

Stability studies (whether in process, in simulated fluids or during storage) play an important role in successful formulation and development of pharmaceutical systems. Denaturation of antigens in immunological preparations may cause inadequate immune response making the subject prone to diseases.

### 5.1. In Processing Stability-

In processing stability testing is done by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) which is a widely used procedure to separate proteins according to their electrophoretic mobility. SDS, as an anionic surfactant, is added to the protein substance to linearize proteins and impart negative charge and thus separate them according to the estimated size through electrophoresis. When the symmetrical position of bands between the pure and extracted antigens/proteins of bilosomes is compared and if it shows absence of additional bands then it confirms that the method of preparation and the quantity of bile salts did not cause any reversible aggregation or decomposition of the entrapped agents **[1]**.

### 5.2. Storage Stability-

Stability studies are done to explore the leaching of the entrapped agents from the vesicles during storage. Shukla *et al*., inspect the amount of diphtheria toxoid retained in the bilosomes after storage at refrigerated (5±3°C) and at room temperature (25±2°C) and 70% relative humidity. Around 94% of the antigen remained in the bilosomes stored at room temperature, for one month and more than 98% of the antigen was found in samples stored at refrigerated conditions **[20]**.

### 5.3. Kinetic Stability-

The kinetic stability of the bilosomes was evaluated by a Turbiscan Lab Expert optical analyzer. The working of analyzer based on multiple light scattering technologies that enables detection of different types of

formulation instability such as flocculation, sedimentation, coalescence or creaming. So, the turbidimetric method used for evaluation of in detail the backscattering profiles of the functional bilosome formulations. The dynamics of the processes in the formulation under investigation were determined at 0 day (freshly prepared bilosomes) and after 7 and 14 days of storage at 4 °C. A representative Turbiscan plot is shown in for the selected and optimized sample. The levels of the backscattering in percentage are marked on the ordinate axis, while the height expressed in mm is indicated on the abscissa axis. The x-axis corresponds to levels of the colloidal sample in the measurement vial. From the plot presented in. No significant changes after 14 days of storage time, indicates no evident particle growth or migration in the nanoparticle dispersion.

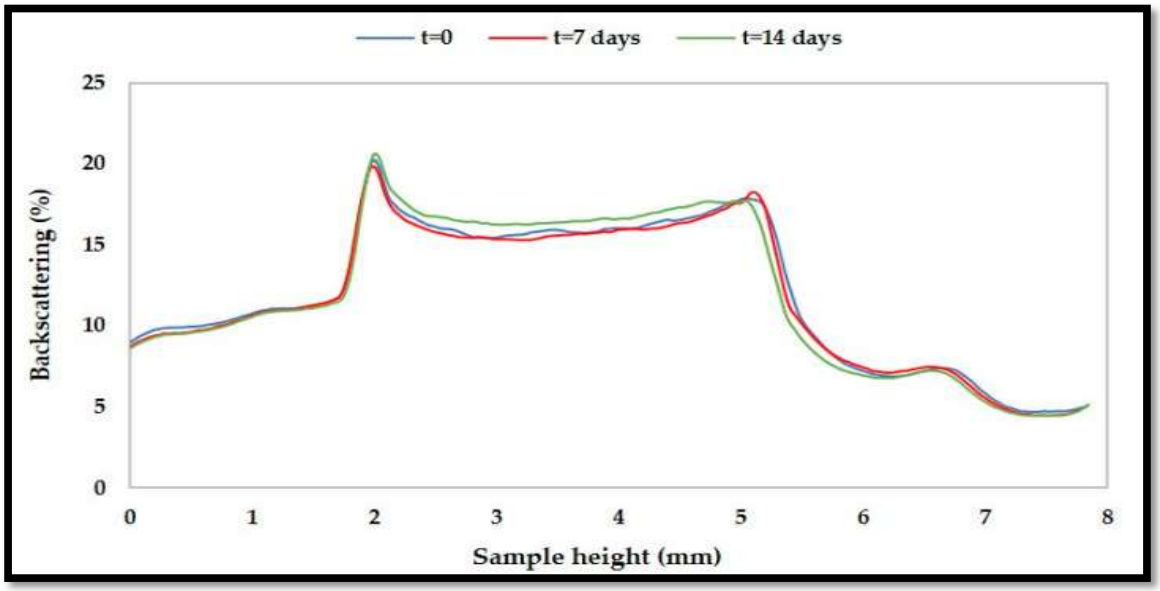



**Figure 4: Exemplary backscattering (%) profiles of the obtained functionalized bilosomes as a function of sample height (mm) analyzed over 14 days of storage. Blue line represents measurement at 0 day (freshly prepared bilosomes); red line was recorded after 7 days of their storage, while green line indicates data obtained after 14 days of storage**

## 6. APPLICATIONS OF BILOSOMES:

### 6.1. Bilosomes as Oral Drug Candidates-

To determine relative bioavailability of insulin based on the blood levels, recombinant human insulin (rhINS) loaded bilosomes incorporating different types of bile salts (sodium glycocholate, sodium taurocholate and sodium deoxycholate) were introduced into male Wistar rats. It was observed that the oral bioavailability of 8.5 % and 11% can be achieved by formulating bilosomes containing sodium glycocholate in non diabetic and diabetic rats respectively. These oral bioavailability values of insulin were found to be higher than the previously reported results. This proves the improved protective effect of

encapsulated rhINS against enzymatic degradation **[35, 36]**.

**6.2. Bilosomes in Transdermal Drug Delivery System-**

An investigation was carried out by Al-mahallawi *et al*., the results produced were that bilosomes portrayed the ability to increase transdermal transport of Tenoxicam (TX) which resulted in avoiding unnecessary GI side effects associated with oral administration **[22]**.

**6.3. Bilosomes in Ocular drug delivery-**

Liposomes loaded with tacrolimus have previously been shown to facilitate penetration across the cornea. However, there was too little transcorneal permeation to attain a therapeutic effect **[22]**.

**6.4. Bilosomes in Oral Immunization Against Hepatitis B-**

Arora *et al*. delineate oral immunization against Hepatitis B virus using mannosylatedbilosomes. The immune response was found to be significantly higher along with enhanced sIgA level at all local and distal mucosal sites as compared with bilosomes alone, whereas parenteral vaccine was unsuccessful at providing any considerable cellmediated response. Shukla *et al*. reported oral delivery of recombinant HBsAg using bilosomes **[37]**.

**7. FUTURE PERSPECTIVES:**

Bilosomes being surface modified carriers with anchoring ligands demonstrate their capacity for targeting specific immune cells. The bile salts and acids are available in low cost which helps in transforming the chiral carriers into the building blocks for targeting of novel drug carrier systems. Oriented research towards the selective transport of antigens to the intestinal lymphatic system and at cellular level by bilosomes is the primary need of today. The bilosomes deliver a wide range of antigens having various physicochemical properties, it also studies the instabilities in the GIT **[22]**.

Due to significant potential properties of bilosomes such as biocompatibility, stability and specificity as carriers for targeted delivery in vaccination, very soon bilosomes would contribute as major counteraction for dreadful and infectious diseases with eventual eradication of the same. Currently, it is utmost important for clinical researchers to apply the know-how of bilosomes for safe and effective trials in human subjects and reveal the exact immune mechanism on the oral administration of bilosomes.

**8. CONCLUSION:**

Based on the reviewed literature, bilosomes not only enhance the bioavailability of drugs but also increase efficacy of drugs and the ability to entrap proteins, peptides and antigens. The development of an effective oral delivery system for mucosal vaccines is a significant challenge for immunologists. In this regard, various lipid based delivery systems including bilosomes have been increasingly

studied and developed for oral immunization.

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