IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)
An International Open Access, Peer-reviewed, Refereed Journal

BILOSOMES AS NON-INVASIVE DRUG DELIVERY SYSTEM

[1]Ms. Supriya S. Jana, [2]Ms. Mrunmayi D. Lad, [3]Ms. Suranya Subramanian, [4]Dr. Dhanashree P. Sanap

Bharati Vidyapeeth's College of Pharmacy,

Sector-8, C.B.D. Belapur, Navi Mumbai, Maharashtra – 400614, India.

Abstract: Vesicular delivery of drugs has gained more popularity due to its stability and improved therapeutic efficacy. Vesicles being a bilayer structure, can be used to incorporate both, hydrophilic and lipophilic drugs. Bile salts are mostly used to increase bioavailability, enhance antigen delivery by offering protection, retention, and premature release of antigen. Preparation of bile salts-containing vesicles are thin-film hydration method, hot homogenization method, reverse phase evaporation method. Bilosomes have proved to be one of the very effective methods of enhancing the bioavailability and efficacy of drugs. The ability to entrap various proteins, antigens, and peptides are exceptional.

Keywords: **Bilosomes, vesicular carriers, vaccine delivery, bile salts**

1. INTRODUCTION

Oral immunization has been offering several advantages over the other routes, such as ease and convenience of administration, patient compliance, minimal side effects and thus, a greater possibility of frequent boosting. At the same time, the problem associated with oral delivery is the denaturation and degradation of antigen in the harsh environment of the gastrointestinal tract, it consequently requires larger and more frequent dosing of antigen which may lead to oral tolerance. This can be overcome by using different and appropriate delivery systems [3, 6]. Recently, vesicular delivery of drugs has gained more popularity due to its stability and improved therapeutic efficacy. Vesicles being a bilayered structure, can be used to incorporate both, hydrophilic and lipophilic drugs [2]. In 2004, Mann *et al*., developed a non-ionic surfactant vesicle having a structure similar to liposome and stabilized it with bile salts for oral delivery of vaccines and these vesicles were called as "Bilosomes" [4]. They have shown effective stimulation of both systemic and mucosal immune responses that portrays bilosomes to be a promising and potential carrier system for oral immunization [2, 6]. Bile salts are mostly used to increase bioavailability, enhance antigen delivery by offering protection, retention and premature release of antigen [3, 4]. Consisting of these, bilosomes are the soft lipid vesicular nanocarriers that consist of deoxycholic acid, sodium cholate, deoxycholate, taurocholate, glycocholate or sorbitan tristearate [8]. Bilosomes being the specialized delivery vehicles carry out several functions in order to give the best results with oral vaccine administration, such as:

- Protects vaccines from being broken down in the stomach.
- Prevents antigen degradation.
- Enhances mucosal penetration.
- Produces systemic and mucosal immune responses [4].

- Promotes good absorption of drugs in order to increase bioavailability [8].
- Effective tool in drug targeting to the liver [2].

2. METHOD OF PREPARATION

Different methods are approved for the preparation of bile salts-containing vesicles.

1. Thin-film hydration method

Bilosomes can be prepared using the thin-film hydration method where surfactant acts as a key ingredient. Span 80, methylidyne (CH) and sodium-dissolved organic carbon (Na-DOC) were dissolved, in a ratio of 2:1:0.1 along with stearyl amine (2% w/w of lipidic content) in chloroform and methanol (8:2 v/v) in a round bottom flask. Solvent removal resulted in the formation of thin film at the wall of the round bottom flask. Subsequent hydration of the film with 10% w/v drug solution (10 mL) in water for 90 min at 37°C resulted in the formation of large multilamellar vesicles (LMVs). Sonication i.e. 3 cycles of 15 sec with 1 min pulse off at 50 amplitude further converted LMVs to small unilamellar vesicles (SUVs) [1, 6].

2. Hot homogenization method

Bilosomes were prepared using hot homogenization method, where a hot paraffin oil bath was set up at 120°C alongside a water bath at 50°C. Sodium bicarbonate buffers one at a pH of 7.6 and the other with a pH of 9.7 were prepared. Then a 100 mM bile salt solution was prepared using 25 mM sodium bicarbonate buffer of pH 9.7. In a 25 mL glass beaker the correct molar ratio of the lipids MPG (Monopropylene Glycol), Chol (bile) and DCP (Dicalcium phosphate) were taken. By heating at 120°C for 10 minutes with occasional swirling the lipids were melted. The emulsion was prepared by adding 5.2 ml of the 25 mM sodium bicarbonate of pH 7.6 buffer and homogenized immediately by using an emulsion head for 2 minutes at 8000 rpm. After 3 minutes of homogenizing, 0.25 mL (10 mg/mL) of the pre-heated (50°C) antigen solution was added to the beaker containing the suspension and homogenized for further 5 minutes. To minimize exposure of the antigen to homogenization, the antigen was added at the final stage. Once homogenization had finished, the bilosome suspension was allowed to cool down to 30°C, and left for 2 hours in an incubator/shaker at 220 rpm [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 stored 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 [9].

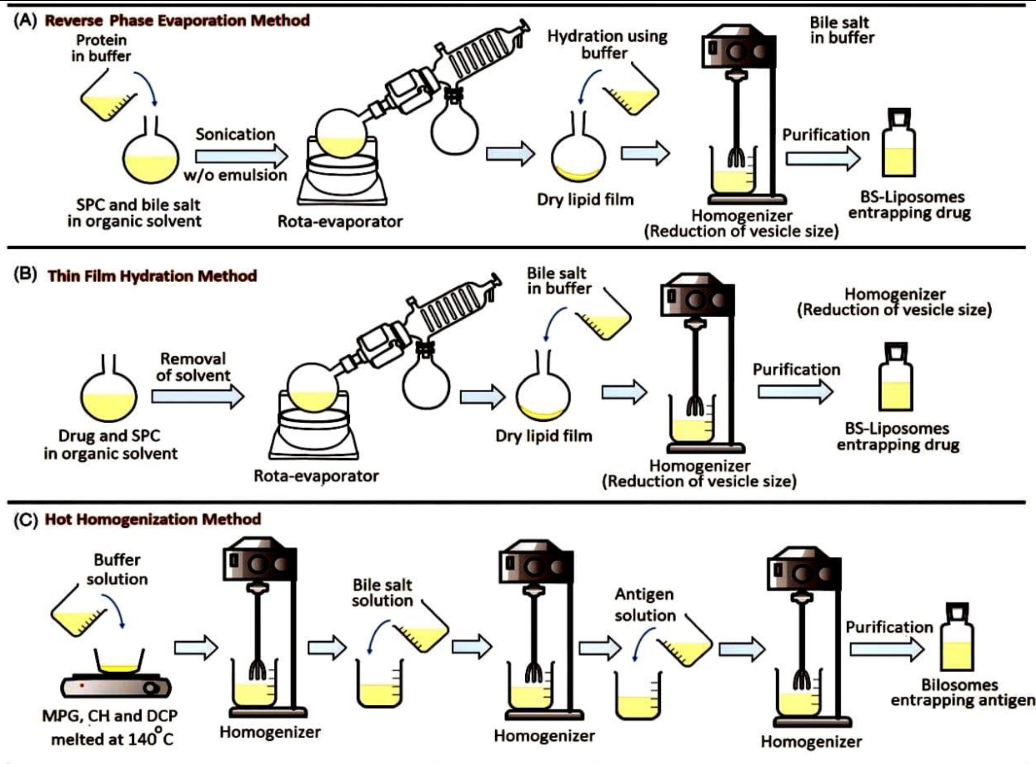

Figure 1: Representation of different methods approved for the preparation of bile salts-containing vesicles. (A) Reverse phase evaporation method (B) Thin film hydration method and (C) Hot homogenization method [8].

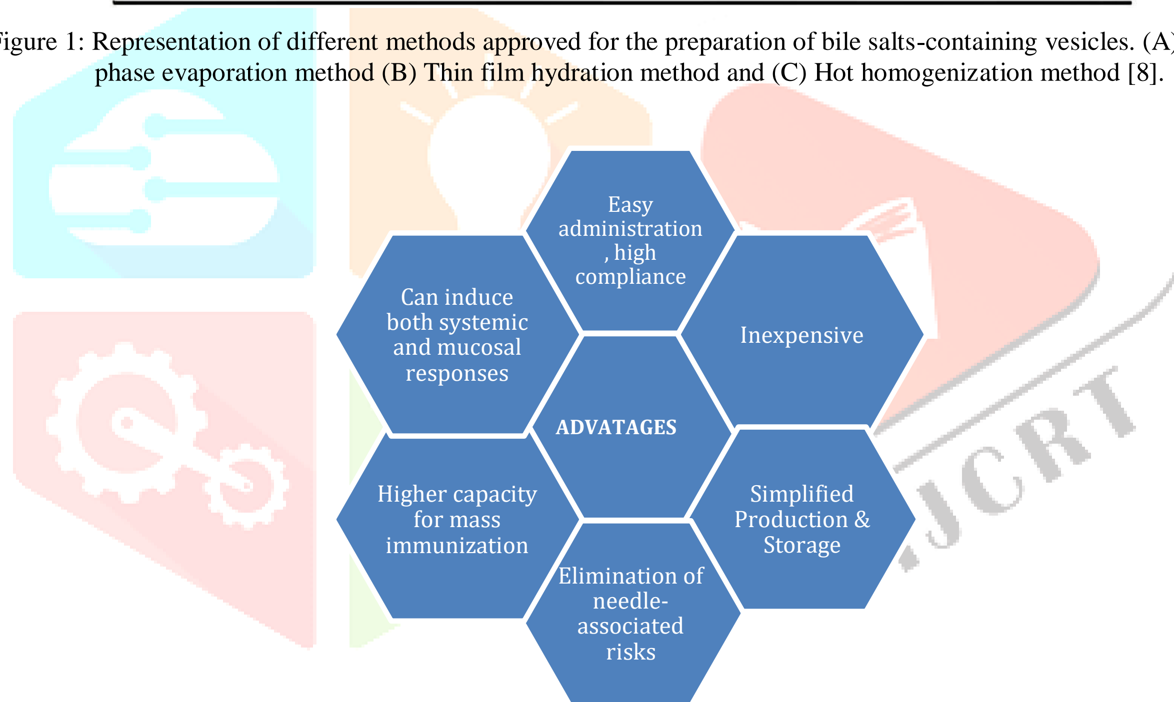

Figure 2: Advantages of bilosomes in drug delivery systems [1, 5]

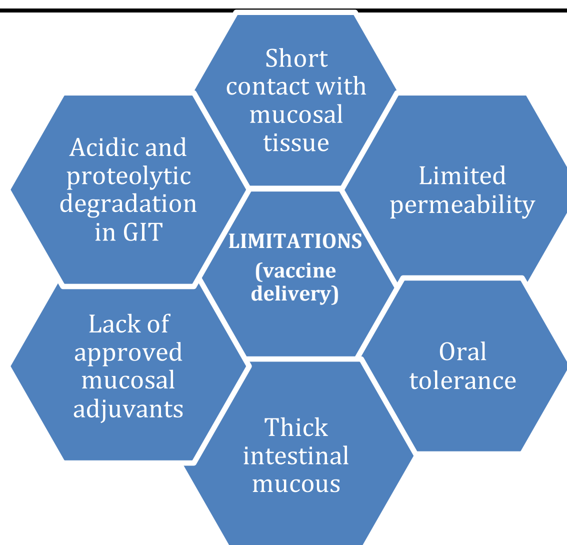

Figure 3: Limitations of bilosomes in drug delivery systems [1, 5]

3. COMPOSITION OF BILOSOMES

Generally bilosomes are composed of two layers:
• Innermost layer of hydrophilic drugs and/or antigens
• Outermost layer of bile salts and/or hydrophobic drugs
Materials used in the preparation of bilosomes comprises of lipids, nonionic surfactants and bile salts.

1. Lipids

A. Phospholipids

Phospholipids have excellent biocompatibility with cellular membrane. They are amphiphilic in nature, has self-assembling property which bring about wetting and emulsification. Due to the amphiphilic nature of the phospholipids they can form closed concentric bilayers in the presence of water. Commonly used phospholipids in bilosomes are dicetyl phosphate, soybean phosphatidylcholine, mono palmitoyl glycerol, dimyristoyl phosphatidylcholine, dilauroyl phosphatidylcholine, dioleoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine, distearoyl phosphatidylcholine, dimyristoyl phosphatidylethanolamine, dilauroyl phosphatidylethanolamine, dioleoyl phosphatidylethanolamine, dipalmitoyl phosphatidylglycerol, distearoyl phosphatidylglycerol, dimyristoyl phosphatidylglycerol, dilauroyl phosphatidylglycerol, dioleoyl phosphatidylglycerol, dipalmitoyl phosphatidylglycerol distearoyl phosphatidylglycerol [9, 10].

B. Cholesterol

Cholesterol, an amphiphilic molecule is inserted into the cellular membrane, where the hydroxyl groups orient towards the aqueous surface and the aliphatic chains align parallel to the acyl chains in the center of the bilayer. This increases the rigidity of bilosomes [11].

2. Nonionic Surfactants

The stability and compatibility properties of nonionic surfactants are much better compared to the anionic, cationic or amphoteric forms, so nonionic surfactants are widely used in the preparation of bilosomes. On the cellular surface, they cause less irritation and they are less hemolytic. They fulfill the purpose of solubilizers, wetting agents, emulsifiers, permeability enhancers and strong P-glycoprotein inhibitors. It contains high interfacial activity because of the polar and non-polar segments, and overall entrapment efficacy of the drug is affected by the chain length and size of hydrophilic head groups of nonionic surfactants. For higher entrapment efficiency of water soluble drugs, combination of tweens with long alkyl chains and cholesterol was used in a ratio 1:1. Highest entrapment efficacy can be obtained with a non-ionic surfactant of 8.6 HLB value, and if the HLB value lowers from 8.6 to 1.7 then it reduces the entrapment efficiency. The frequently used nonionic surfactants for vesicles formation are as follows:
• Alkyl esters and alkyl glyceryl ethers
• Polyoxyethylene 4 lauryl ethers
• Polyoxyethylenecetyl ethers and stearyl ethers

• Sorbitan fatty acid esters – Span 40, Span 60, Span 80 [12, 13].

3. Bile salts

Bile salts form mixed micelles when combined with polar lipids, conventional surfactants or amphiphilic drugs. This is due to the lower CMC and better solubilization capacity than what is offered by the individual components due to synergistic interactions [7].

Bile salts are commonly known as the natural biosurfactants. These are present in the gut lumen and play a crucial role in digestion and absorption of lipids. The stimulation of bile secretion causes increase in the absorption of biologically active molecule. Bile salts are also known to enhance stability of bilosomes in simulated fluids, by causing repulsion between bile salts that are found in bilosomes and external bile salts in gut lumen.

A few bile salts used in bilosomes are:

• Sodium deoxycholate (SDC)
• Sodium glycocholate (SGC)
• Sodium taurocholate (STC)
• Sodium taurodeoxycholate (STDC) [12].

Table 1: Patents on Bilosomes

Sr. no	Patent no	Title	Inventors	Applicant	Claims	Composition	Method of preparation	Ref
1	11167033	Compositions and methods for treating viral infections	Anderson; David E	Variation Biotechnologies Inc.	Preparation of a thermostable lyophilized composition comprising an inactivated viral antigen and lipid vesicles (1-monopalmitoyl glycerol). A homogenized mixture of molten lipids and an aqueous solution of volume 6.25 mg/ml-25 mg/ml is obtained then lyophilized composition is thermostable when stored for a period of up to nine months at a temperature of 8. ° C to 40° C.	Non-ionic surfactant and inactivated hepatitis A virus	Lyophilisation and rehydration method	[14]
2	US9603920B2	Compositions and methods for treating influenza	Francisco Diaz-Mitoma Andrei Ogrel Jose V. Torres David E. Anderson	Variation Biotechnologies Inc	The vesicle comprises a transport enhancer which facilitates the transport of lipid-like molecules across mucosal membranes. The vesicle comprises a non-ionic surfactant.	Lipopolysaccharides, Orthomyxoviridae, CpG (5'—C—phosphate—G—3') containing adjuvants, Gastrins and Somatostatins.	Hydration method	[15]
3	US20210316009A1	Methods for the preparation of a pharmaceutical-vesicle formulation and associated products and uses	Ethel Diane Williamson Riccardo Vincenzo D'elia Craig William Roberts Stuart Woods	University of Strathclyde UK Secretary of State for Defence	The method for the preparation of a pharmaceutical-vesicle formulation, the method comprising the heating vesicle components comprising monopalmitoyl glycerol, cholesterol and dicetyl phosphate in a 5:4:1 molar ratio respectively at a temperature in the range of 50° C to 150° C.	Esters of carboxylic acids, Togaviridae, steroids and proteins	Lyophilisation method	[16]
4	11,167,032	Methods and compositions for therapeutic agents	Kirchmeier; Marc J. Anderson	Variation Biotechnologies Inc.	A method comprising: preparing pre-formed vesicles comprising a lipid component that comprises a non-ionic surfactant, a steroid and an ionic amphiphile;	Therapeutic agents (e.g., live attenuated viral antigens, therapeutic proteins, etc.) and a lipid	Spray injection method used	[17]

					admixing the pre-formed vesicles with an aqueous solution that includes a thermolabile therapeutic agent, wherein pre-formed vesicles are prepared in the absence of thermolabile therapeutic agent	component.		
5	US 20110305 754 A1	Controlled release micro-capsule for osteogenic action	Mishra, Prabhat Ranjan, Trivedi, Ritu Gupta, Girish Kumar, Avinash Gupta, et al.	Council of Scientific & Industrial Research	A microcapsule for controlled release of a flavonoid compound comprising: a core particle consisting of (i) a calcium salt and (ii) a [poly(ethylene oxide-c-polypropylene oxide), block polyoxyethylene-polypropylene block copolymer], loaded with a flavonoid compound, said core particle having a plurality of alternate layers of cationic and anionic polyelectrolytes adsorbed thereon and an outer layer formed by a bile salt, wherein the flavonoid is present in an amount ranging between 10 to 96% by weight of said microcapsule.	Calcium salt, Pluronic F68 [poly (ethylene oxide-co-polypropylene oxide), block poly oxyethylene-polypropylene block copolymer], loaded with a flavanoid compound, cationic and anionic polyelectrolytes	-	[18]

4. EVALUATION PARAMETERS OF BILOSOMES

In vitro evaluation

1. Particle Size

Particle size can be determined using dynamic light scattering and laser diffraction particle size analyzer [7]. Optically homogeneous square polystyrene cells are used, to analyze the particle size with dynamic light scattering instrument [9]. *In vitro* and *in vivo* performances are greatly affected by the particle size of bilosomes. The vesicle size of bilosomes ranges from 90nm-3µm [12].

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 [7]. Due to electric repulsion between the particles, the zeta is around +30 mV and that system is considered to be the stable one [12].

3. % Efficiency

Entrapment efficiency percent (EE %) plays an important role to assess the use of vesicles in pharmaceutical applications. An increase in the bile salt content in the vesicles causes the leakage of an entrapped drug due to the fluidizing effect of bile salts on the lipid bilayers. The EE% also affects the hydration pH as it affects the bile salt dissociation [7]. By using spectroscopic or chromatographic methods such as high-performance liquid chromatography and UV spectrophotometry, EE% can be analyzed [12].

4. Polydispersity Index (PDI)

PDI can be measured using dynamic light scattering method [1]. 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 [7]. 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 [12].

5. Morphology Characterization using TEM

In transmission electron microscopy (TEM), the morphology of the obtained bilosomes was examined. Before the examination, a drop of the diluted sample was placed on a perforated carbon film-coated copper grid and allowed to dry for 1 h. FEI Tecnai G2 20 X-TWIN (FEI, Hillsboro, OR, USA) transmission electron microscope can be used to observe and image the sample [8].

6. Ultracentrifugation

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 [12].

7. *In vitro* Release

Dynamic dialysis is one of the most effective methods for measuring release kinetics from vesicular systems. As a result of the drug being released from the vesicle into the dialysis chamber, then diffusing across the membrane, the drug appears in the sink receiver compartment [12].

In vivo evaluation

1. 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 [20].

2. Evaluation of antinociceptive activity

The evaluation of antinociceptive is examined through abdominal contribution induced by acetic acid as follows:

40 mice (25±5g) are divided into three groups with four mice per group. One of the group is given no treatment (negative control group). One of the group is given a tablet and one of them may have a formulation applied on their shaved back. Each mouse is given a single dose of 10 mL/kg of 0.7% aqueous solution of acetic acid via intraperitoneal route half an hour following the drug application. Each mouse is located in an observation cage and abdominal constriction i.e. stretching of hind limbs within 5-15 minutes after injecting is considered. The result is given as percent reduction of nociception among three groups [20].

3. Skin Irritability Test

Skin irritability test is performed to evaluate and observe any kind of skin irritation. The formulation is applied on the wistar rats [20, 21]. The skin irritancy test is carried out as follows:

The back of healthy rats are shaved in order to avoid peripheral damage 24 hours prior to the test [21]. 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 [21]. 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: [20, 21]

1- slight erythema (light pink)
2- moderate erythema (dark pink)
3- moderate to severe erythema (light red)
4- severe erythema (extreme redness)

4. Enhancement of vaccine immunogenicity

It is known that orally administrated bilosomes induced cell-mediated responses against synthetic peptides and high antibody titers against protein antigens comparable to those engendered succeeding

systemic immunization. The potential of utilizing SDC-bilosomes loaded with either HBsAg or diphtheria toxoid in providing transmucosal immunization is investigated. In both studies, orally administrated bilosomes loaded with high dose of antigen produces systemic immunoglobulin G (IgG) response in mice comparable to those induced by intramuscular administrated antigens. In addition, bilosomes elicited measurable secretory IgA in mucosal secretions that are not induced by intramuscular administrated antigens. The effect produced by the combination of bilosomes formulation containing high dose of HBsAg and tetanus toxoid is anti-HBsAg-IgG and anti-Tetanus toxoid-IgG levels mice serum which is similar to intramuscularly administered HBsAg and Tetanus toxoid [7].

5. Transportation of bilosomes to peyer's patches

Peyer's patches contain M cells that transport macromolecules, particles, and microorganisms directly into the intestine. M cells differ from intestinal absorptive cells in their apical surface. There are also many endocytic vesicles in M cells that take up and transport food. A characteristic of M cells is that they contain an intraepithelial pocket in which macromolecules and transcytosed particles are delivered. Lymphocytes and a few macrophages are found in the pocket and interact with each other. Also, the M cells' cellular processes are extended into provides potential contact with resident lymphocytes and dendritic cells. M cells can also secrete IL-1, suggesting that they could provide co-stimulatory signals, such as cytokines and cell surface molecules, to T cells and B cells. Peyer's patches have a microenvironment of cells [7].

6. 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 relative 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 [7].

5. STABILITY CONSIDERTION OF BILOSOMES

5.1. Processing Stability

Sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is utilized to examine the chemical stability of entrapped peptides or antigens in bilosomes. According to their electrophoretic mobility, SDS-PAGE is a widely used procedure to isolate proteins [7]. In order to separate proteins using electrophoresis according to their estimated size, an anionic surfactant is added to protein substance in order to linearize its structure and impart a negative charge. It confirms that the method of preparation and the number of bile salts did not cause any reversible aggregation or decomposition of the entrapped agents when the symmetrical position of bands between the pure and extracted antigen of bilosomes is compared and if it shows the absence of additional bands [12].

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 [7, 12].

5.3. Stability in stimulated biological milieu

Since the intact fraction of vesicles dominates the *in vivo* response induced by gastric pH, bile salts, and GI enzymes, it is vital to interpret the stability of vesicular carriers and encapsulated peptides and proteins. Due to their repulsion with the external bile salts present in the solution, bisomes are superior to niosomes in protecting loaded antigens in simulated biological fluidsFor example, Conacher *et al.*, reported that niosomes lost all of the initially entrapped BSA content at bile salt

concentration of 20 mM, whereas bilosomes retained 85% of the initial BSA content, confirming bile salts' stabilizing role in vesicle construction. In agreement with this, Shukla *et al.,* reported that instability studies conducted with simulated GIF and bile salt solutions (5.0mM and 20mM concentrations), bilosomes loaded with either HBsAg or diphtheria toxoid retained significant amounts of entrapped antigen 40,41. An incubated GIF of bilosomes was used by Wilkhu et al., to determine antigen load..As a result of the degradation of antigen present on the surface of the bilosomes, the initial antigen load (32%) was retained in gastric media; however, when the bilosomes were simultaneously transferred to intestinal medium, this amount decreased to around 8.5–9.5%. Several studies have compared the stability of BS-liposomes in simulated GI media with conventional liposomes. In either simulated GI fluids (including pancreatin or pepsin) or in *ex vivo* GI fluids collected from rats, SG liposomes retained most of the insulin load. The protective effect was attributed to SGC's ability to inhibit enzymes and its ability to stabilize membranes. As a result of incorporating different bile salts (SGC, STC, SDC) into BS-liposomes, Niu *et al.,* also tested their integrity against three protease enzymes (pepsin, trypsin, and amyochymotrypsin). SGC had a superior ability to inhibit enzymes than the other investigated bile salts in formulating BS-liposomes [7].

5.4. Kinetic Stability

The liposomal kinetic stability is affected by the stability of active substances in the formulation along with the adverse phenomenon taking place during the storage time. The instabilities in liposomes may be aggregation, flocculation and coalescence which may lead to changes in the vesicle size and leakage of the encapsulated drug. The causes of the instabilities are interactions, forces between colloidal nanostructures in suspension, for example, short range van der waals interactions, etc. These instabilities can be prevented by steric or electrostatic stabilization process. Thus, stability evaluation is necessity while designing bilosomes.

The kinetic stability measurement is carried out for the optimised bilosome formulation. The Turbiscan lab xxpert optical analyzer (based on multiple light scattering technologies) is used to perform the detection of formulation instability (flocculation, sedimentation, coalescence or creaming). The analysis is carried out in a cylindrical glass cell at 25°C. The stability is measured to detect any kind of destabilization phenomenon of polymeric colloidal systems. The dynamics occurring in the formulation is investigated on Day 0 and after 7 to 14 days of storage at 4°C.

A representative turbiscan graph is plotted with levels of backscattering (in percentage) are marked on the Y axis and sample height (in mm) is on the X axis [8].

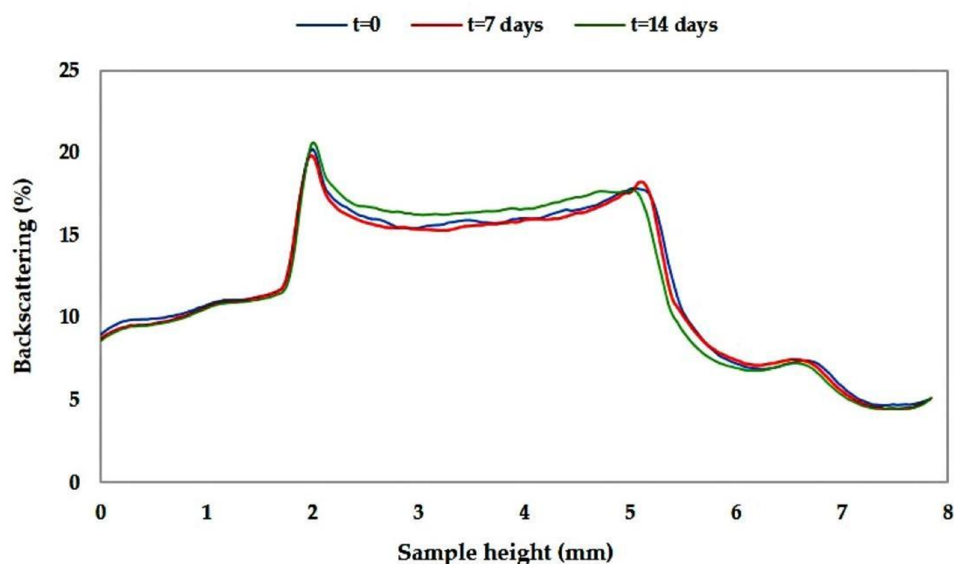

Figure 4: Exemplary backscattering (%) profiles of the obtained functionalized bilosomes as a function of sample height (mm) analyzed over 14 days of storage. Blue line represents measurement at 0 day (freshly prepared bilosomes); red line was recorded after 7 days of their storage, while green line indicates data obtained after 14 days of storage [8].

6. APPLICATION OF BILOSOMES

1) **Bilosomes in oral immunization against hepatitis B**
Using mannosylated bilosomes; Arora *et al.,* reported oral immunization against the Hepatitis B virus alongwith enhanced sIgA level at all local and distal mucosal sites, the immune response was found to be notably higher [7].
2) **Bilosomes in oral immunization with a model antigen**
Using BSA 37; Conacher *et al.,* reported oral immunization. The orally administered bilosomal formulation containing BSA brings out high antibody titers against it, which were found to be similar to those generated after systemic immunization [7].
3) **Bilosomes as Oral drug candidates**
To determine relative bioavailability of insulin, 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 [12].
4) **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 [12].
5) **Bilosomes in Oral immunization against tetanus**
Mann *et al.,* reported significant systemic and mucosal immunity with Tetanus toxoid-loaded bilosomes on oral immunization. Tetanus toxoid was entrapped in bilosomes resulted in inducing Th2 response characterized with systemic IgG1, it also resulted in SIgA antibodies whereas Th2 and IgA responses were induced with orally entrapped antigen. The end point of oral administration of unentrapped antigen was high yet comparable to parenterally delivered tetanus toxoid [12].
6) **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 [12].

7. MARKET POTENTIAL

i. The intranasal delivery of mucoadhesive *in situ* gel containing zolmitriptan-loaded bilosomes offered direct nose-to-brain drug targeting with enhanced brain bioavailability.
ii. A study reported that bilosomes can be used for the delivery of vaccines (currently under license) and can also be used for the delivery of small molecules and biological therapeutics.
iii. A study reported that the bilosomes system is a promising technique to enhance the transdermal drug delivery system with respect to good skin permeation and less skin irritation.

8. FUTURE PROSPECTS

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. It provides better immunization against the fatal diseases. It has significant potential properties like biocompatibility, stability and specificity as carriers for targeted drug delivery in vaccination too. Bilosomes will soon contribute as major counteraction for dreadful and infectious diseases [12].

9. CONCLUSION

Based on the reviewed literature, bilosomes have proved to be one of the very effective methods of enhancing the bioavailability and efficacy of drugs. The ability to entrap various proteins, antigens and peptides is exceptional. As we know, development of an effective delivery system has always been a challenge. But, various lipid based delivery systems along with bilosomes are being studied vastly and are proving to be the most effective.

10. CONFLICT OF INTEREST

The authors declare no conflict of interest.

11. ACKNOWLEDGEMENT

We would like to acknowledge Bharati Vidyapeeth's college of Pharmacy, sector – 8, C.B.D. Belapur, Navi Mumbai for providing the necessary library facilities for literature review.

12. REFERENCES

1) Jain, S, Harde, H, Indulkar, A and Agrawal, A. 2014. Improved stability and immunological potential of tetanus toxoid containing surface engineered bilosomes following oral administration. Nanomedicine: Nanotechnology, Biology and Medicine, 10(2): 431-40. Doi: 10.1016/j.nano.2013.08.012.
2) Bhairy, S, Pitchika, S, Maurya, S and Patil J. 2020. Stability and *in-vivo* efficacy of bile salts containing vesicles (Bilosomes) for oral delivery of vaccines and poorly soluble active drug molecules. Indo American Journal of Pharmaceutical Research, 10(10):1236-1334. Doi no: 10.5281/zenodo.4066496.
3) Wilkhu, J, McNeil, S, Anderson, D and Perrie, Y. 2013. Characterization and optimization of Bilosomes for oral vaccine delivery. Journal of Drug Targeting. 21(3): 291-299.
4) Chilkwar, R, Nanjwade, B, Nwaji, M, Idris, S and Mohamied, A. 2015. Bilosomes based drug delivery system. Journal of chemistry and applications, 2(1): 5.
5) Skwarczynski, M and Toth, I. 2020. Non-invasive mucosal vaccine delivery: advantages, challenges and the future. Expert Opinion on Drug Delivery, 17(4); 435-437. Doi no: 10.1080/17425247.2020.1731468.
6) Shukla, A, Khatri, K, Gupta, P, Goyal, A, Mehta, A and Vyas, S. 2008. Oral immunization against hepatitis B using bile salt stabilized vesicles (bilosomes). Journal of Pharmaceutical Sciences, 11 (1): 59-66. Doi: 10.18433/j3k01m.
7) Rajput, T and Chauhan, M. 2017. Bilosome: A bile salt-based novel carrier system gaining interest in pharmaceutical research. Journal of Drug Delivery and Therapeutics, 7(5): 4-16.
8) Waglewska, E, Pucek-Kaczmarek, A and Bazylínska, U. 2020. Novel surface-modified Bilosomes as functional and biocompatible nanocarriers of hybrid compounds. Nanomaterials, 10(2472): 2-14.
9) Ahmad, J, Singhal, M and Amin, S. 2017. Bile salt stabilized vesicles Bilosomes: A novel nano-pharmaceutical design for oral delivery of proteins and peptides. Current Pharmaceutical Design, 23(11); 1575-88.
10) Samad, A, Sultana, Y and Aqil, M. 2007. Liposomal drug delivery systems: an update review. Current Drug Delivery, 4(4): 297-305.
11) Vyas and Khar. 2007. Targeted And Controlled Drug Delivery System. CBS Publishers & Distributors, New Delhi, India.
12) Palekar-Shanbhag, P, Lande, S, Chandra, R and Rane, D. 2020. Bilosomes: superior vesicular carriers. Current Drug Therapy. 15(4): 1-9.
13) Gannu, P and Pogaku, R. 2011. Nonionic surfactant vesicular systems for effective drug delivery— an overview. Acta Pharm Sin B, 1(4): 208-19.
14) Anderson and David, E. Compositions and methods for treating viral infections. United States Patent, 2021.11167033
15) Francisco Diaz, Mitoma Andrei, Ogrel Jose, Torres David and Anderson. Compositions and methods for treating influenza. United States Patent.US9603920B2
16) Ethel Diane Williamson, Riccardo Vincenzo D'elia,Craig William Roberts and Stuart Woods. Methods for the preparation of a pharmaceutical-vesicle formulation and associated products and uses. United States Patent. US20210316009A1
17) https://patft.uspto.gov/netacgi/nphParser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearchbool.html&r=6&f=G&l=50&co1=AND&d=PTXT&s1=BILOSOMES&OS=BILOSOMES&RS=BILOSOMES (accessed on 21/1/22)

18) Mishra, P, Trivedi, R, Gupta, R, Kumar, G and Gupta, A. Controlled release micro-capsule for osteogenic action. US 20110305754 A1.
19) Chang, CJ, Liao, YC, Chou, Wei-I, Chang, HK. Baculovirus display vectors and uses thereof. US 20160331826 A1
20) Ahmed, S, Mohamed, AK, Sinar Sayed S. 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, 15; 9783–9798. Doi: https://doi.org/10.2147/IJN.S278688
21) Khalil, R, Ahmed, A, El Arini, SK, Basha, M, El-Ha-shemy, HA, Farouk, F. 2018. Development of tizanidine loaded aspasomes as transdermal delivery system: *ex-vivo* and *in-vivo* evaluation. Journal of Liposome Research. 31(1): 1-17. Doi: 10.1080/08982104.2019.1684940.