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REVIEW ON BILOSOMES: SUPERIOR VESICULAR CARRIERS

Charamcharla Bhanu Chandu*, Bethapudi Tharun Teja, Datla Y. N. V. Pavan Kumar Varma, Gudibandi Hari Nadh Reddy and Innamuri Karthikeya

Department of Pharmaceutics, Shri Vishnu College of Pharmacy (Autonomous), Vishnupur, Bhimavaram-534202, Andhra Pradesh, India.

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***Corresponding Author**
Charamcharla Bhanu Chandu
Department of Pharmaceutics, Shri Vishnu College of Pharmacy (Autonomous), Vishnupur, Bhimavaram-534202, Andhra Pradesh, India.

ABSTRACT

In the current era, many formulations have been designed in the form of vesicular carriers like liposomes and niosomes which have been proved to be one of the potential candidates fordrug delivery by the oral route but due to the gastrointestinal environment i.e. pH, presence of enzymes, and bile salts, their use is limited. Because of these difficulties, research is being done toincrease the stability and efficacy of the drug. Thus bilosomes have been developed as a potentialvesicular carrier system for oral vaccine delivery, transdermal and parenteral targeted drug delivery. The present article covers various aspects related to the novel vesicular system that is based on bilesalts called bilosomes, for targetted drug delivery systems. It includes information related to bilosome composition, formulation techniques, characterization methods, applications in oral immunization as vaccine delivery approach and advantages over conventional nanocarriers such as liposomes and niosomes. It also focuses on the stability and applications of bilosomes along with scalability and potentiality in biomedical field of oral immunization against various dreadful diseases.

KEYWORDS: Bilosomes, Vesicular carriers, Targeted drug delivery.

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 described by Conacher et al. from the

University of Glasgow in 2001.[2] 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. Most of the protein/ peptides or vaccines usually given parenterally, were seen to be showing only systemic immunity but when these vaccines were encapsulated in bilosomes and administered, they have shown systemic as well as mucosal immunity with no interaction between the pathogens and host at mucosal surfaces.[3] The aim of the present review article is to provide a comprehensive overview of the fundamentals of bilosomes that are based on the use of bile salts for targeted drug delivery. The fundamentals of bilosome as vesicular systems that are put forth in this article are bilosome composition, formulationtechniques, characterization methods, applications in oral immunization as vaccine delivery approach and advantages over conventional nanocarriers such as liposomes and niosomes. Along with these it covers the stability and applications of bilosomes in oral immunization as vaccine therapy against various dreadful diseases.

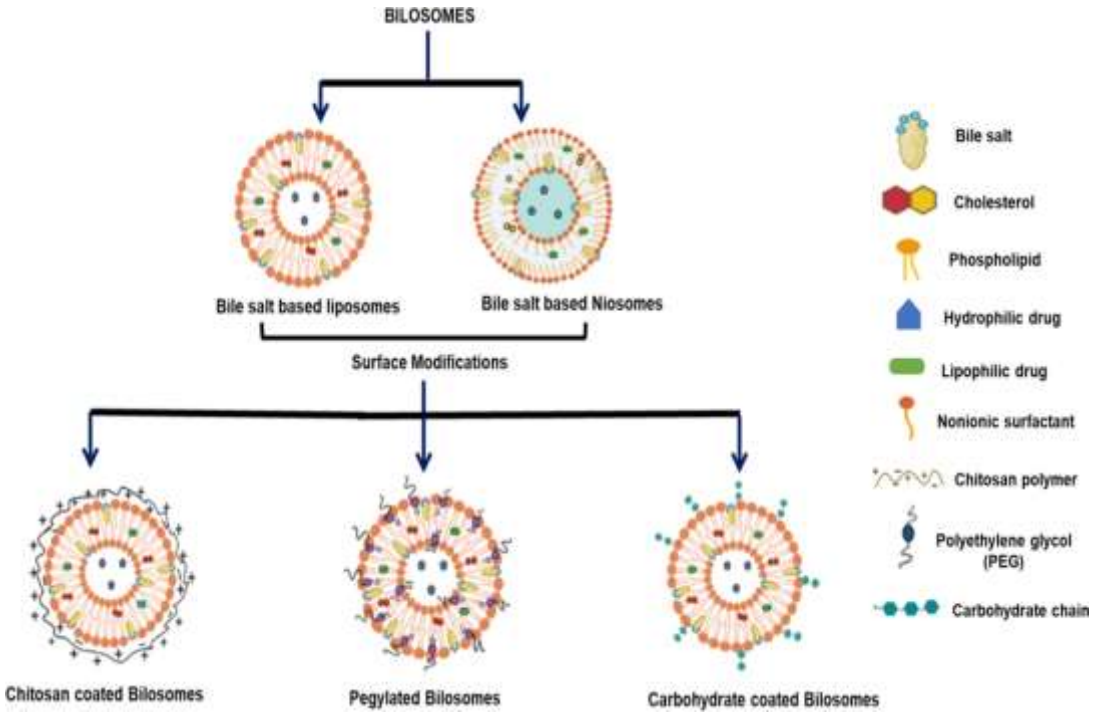

Fig. 1: Strcture of bilosomes.

2. Composition of bilosomes

Generallybiosomes are composed of two layers:

- Innermost layer of hydrophilic drugs and / or antigens
- Outermost layer of bile salts and / or hydrophobic

2.1 Materials used in the preparation of bilosomes

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

2.1.1 Phospholipids

Phospholipids have excellent biocompatibility with cellular membrane. They are amphiphilic in nature that confers them self assembling property thus bring about wetting and emulsification. Due to the amphiphilic nature of the phospholipids they can form closed concentric bilayers in the presence of water. Phospholipids have good emulsifying property therefore can stabilize emulsions.[4,5] Commonly used phospholipids that are used in bilosomes are as follows.[6,7]

- Dicetyl phosphate
- Soybean phosphatidylcholine
- Mono palmitoyl glycerol
- Dimyristoyl phosphatidylcholine
- Dilauroyl phosphatidylcholine
- Dioleoyl phosphatidylcholine
- Dipalmitoyl phosphatidylcholine
- Distearoyl phosphatidylcholine
- Dimyristoyl phosphatidylethanolamine

2.1.2 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.[8]

2.1.3 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.[9-11] They are less haemolytic and cause less irritation to cellular surfaces and help to maintain the nearest physiological pH in solution. They function as solubilizers, wetting agents,

emulsifiers, and permeability enhancers. They also function as strong P-glycoprotein inhibitors. They enhance drug absorption and drug targeting to specific tissues. Nonionic surfactants are made of polar and non-polar segments and contain high interfacial activity. The chain length and size of hydrophilic head groups of nonionic surfactants affect the overall entrapment efficacy of the drug. Nonionic surfactants which include stearyl (C18) chains show higher entrapment efficacy than the lauryl (C12) chains.[9] For entrapment of water soluble drugs, a combination of Tweens with long alkyl chains and large hydrophilic moieties, and cholesterol in the ratio 1:1 is used to obtain higher entrapment efficiency.[9, 12-14] Also HLB value of surfactants plays an important role in controlling drug entrapment in the vesicles. The surfactants with HLB value ranging between 14 -17 are said to be not suitable to produce bilosome vesicles, while an HLB value of 8.6 gives highest efficacy for entrapment. The entrapment efficiency reduces as theHLB value lowers from 8.6 to 1.7[9,15,16] The commonly used nonionic surfactants for vesicles formation are as follows[9]:

- 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
- Polyoxyethylene fatty acid esters - Tween 20

2.1.4 Bile salts

Bile salts are natural biosurfactants present in the gut lumen and play a very important role in both digestion and absorption of lipids. Absorption of biologically active molecules increases due to the stimulation of bile secretion. Taking advantage of this various mixed micelle systems were successfully employed for improved solubility of highly lipophilic drugs. 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. Bile salts used in bilosomes are as follows.[3,17-19]

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

3. METHODS OF PREPARATION

3.1 Reverse phase evaporation method

Reverse phase evaporation method produces water-in-oil emulsions in which the water phase contains the drug and the organic phase consists of lipids to form bilosomal bilayer.[20] In this method, soybean phosphatidylcholine and bile salts are dissolved in the organic solvent such as absolute ether, into which buffer solution with protein is added drop by drop. The mixture is sonicated for 5 minutes in a water bath until w/o emulsion is formed. The emulsion is rotaevaporated with a rotating speed of 50 rpm to remove the organic solvent. A buffer is then added to hydrate the dry lipids until a homogeneous dispersion is formed. Finally, this dispersion is extruded through a high pressure homogenizer which is further purified by ultracentrifugation to obtain bilosomes loaded with the drug.[21] This method is used to prepare bilosomes loaded with protein drugs like porcine insulin, recombinant human insulin etc.[1]

3.2 Thin film hydration method

For preparing drug loaded bilosomes by thin film hydration method, the lipid component; soybean phosphatidylcholine and the drug is dissolved in an organic solvent followed by solvent evaporation under reduced pressure using a rotary vacuum evaporator. The thin film thus formed is then hydrated with buffer containing the bile salt to form large multilamellar vesicles that are transformed into small unilamellar vesicles using high pressure homogenization These vesicles are then purified to obtain bilosomes loaded with drug.[3,18,21,22] This method is used to prepare bilosomes loaded with tacrolimus, fenofibrate, cyclosporine A, diphtheria toxoid, hepatitis B Antigen, tetanus toxoid etc.[1]

3.3 Hot homogenization method

For preparing bilosomes using hot homogenization method, the lipid components such as mono palmitoyl glycerol, cholesterol and dicetyl phosphate, are melted at 140°C for 5 min and subsequently hydrated with buffer solution. This mixture is then homogenized followed by addition of bile salt solution to form dispersion containing empty vesicles and then homogenized again. Thereafter the antigen buffered solution is added to the homogenate, and the protein entrapment is achieved by several Freeze Thaw cycles. Antigen is added at the final stage to minimize long exposure to homogenization.[3,18,21,22] This method is used to entrap influenza A antigen, recombinant influenza antigen (rHA or H3N2 subunit protein) and TTx (Tetrodotoxin).[3]

Table 1: Comparison between three vesicular systems.

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 inducer
Chemical stability	Undergo Oxidative degradation	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

4. Characterization 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.[1,31] The vesicle size of bilosomes ranges from 90nm-3µm.[2,32] 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] Analytical instruments constructed on dynamic light scattering principle, i.e. photon correlation spectroscopy, are used to estimate particle size of bilosome vesicle dispersion.[32,33] This technique measures time- depend-ent fluctuations in the intensity of scattered light which occurs because of the particles that undergo Brownian motion. Analysis of these intensity fluctuations enables the determination of diffusion coefficient of the particles and is reported in terms of size distribution.

4.1.2 Polydispersity Index (PDI)

The term polydispersity is used to describe degree of non-uniformity of particle size distribution.[34-36] In drug delivery applications using lipid-based carriers, such as liposome and nanoliposome formulations, a PDI of 0.3 and below is considered to be acceptable and indicates a homogenous population of phospholipid vesicles.[34,37-39]

4.1.3 Zeta Potential (ZP)

Zeta potential means overall charge acquired by the particles in a particular medium. Vesicles with surface charge are more stable against accumulation than uncharged ones. Bilosomes

acquire negative charge due to the presence of bile salts that stimulate zeta potential and avoid aggregation of the vesicles.[1,32] Negatively charged vesicles are favorably taken up by the Peyer's patches.[1,19] In general, the system is considered stable when the zeta potential is around +30 mV due to electric repulsion between the particles.[40,41] Description on various methods/ techniques used to study morphological characteristics of bilosomes as vesicular systems.

4.1.4 Ultracentrifugation

Ultracentrifugation is a separation technique in which a high speed centrifuge is optimized for spinning a rotor at very high speed and is capable of generating acceleration as high as 10,00,000 g (approx. 9800 km/s²). It is able to separate out non encapsulated drug from the drug loaded bilosomes.

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.[1,50] Also EE% increases with increase in the lipid content.[1,51]

EE% = (Total amount of drug – Total amount of free drug) X 100 Total amount of drug

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

4.1.6 In vitro release

Dynamic dialysis method is one of the most common methods for determination of release kinetics from vesicular systems. Drug appearance in the sink receiver compartment is a consequence of release from the vesicle into the dialysis chamber followed by diffusion across the dialysis membrane.[52]

4.2 In vivo performance of bilosomes

4.2.1 Improvement in oral drug bioavailability

Based on the published literature, incorporation of drugs or proteins into bilosomes substantially enhances the bioavailability and in vivo efficacy.[1] When fluorolabeled bilosomes were administered orally into the mice, fluorescence was observed in the gut associated lymphoid tissue (GALT) region with the help of Confocal Laser Scanning

Microscopy (CLSM) while untrapped fluorophore administered orally did not show any fluorescence in the GALT region**.**

4.2.2 Enhancement of vaccine immunogenicity

Orally administered bilosomes loaded with high dose of antigen produced systemic immunoglobulin G (IgG) response in mice comparable to those induced by intramuscular administered antigens.[1,2,53] In addition, bilosomes elicited measurable secretory IgA in mucosal secretions that were not induced by IM administered antigens.

4.2.3 Transportation of Bilosomes to Peyer's Patches(M Cells)

Microfold cells (or M cells) are found in the Gut Associated Lymphoid Tissue (GALT) of the Peyer's patches in the small intestine, and in the Mucosa Associated Lymphoid Tissue (MALT) of other parts of the gastrointestinal tract. M cells are known to initiate mucosal immunity responses on the apical membrane of the M cells and allow transport of microbes and particles across the epithelial cell layer from the gut lumen to the lamina propria.[1,54, 55] The apicalsurface of M cells do not contain brush border. M cells contain an intraepithelial pocket in which transcellularly transported particles and macromolecules are delivered. The pocket contains lymphocytes and a few macrophages which interact with the transported antigen or microorganisms.[1] Bilosomes containing antigens are transported into the pocket where lymphocytes and macrophages interact with antigens and produce systemic and mucosal immunity. The M cells can also secrete IL-1, which indicates that M cells could provide co- stimulatory signals such as cytokines and cell surface molecules to the T cells and the B cells in the microenvironment of Peyer's patches.

5. Advantages of bilosomes

- 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.

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.[57,58] 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.[29,57]

6.2 Improved hypoglycemic activity

Subcutaneous administration of insulin leads to hypoglycemia while oral administration of insulin is rendered safe.[29,57] The comparison of bilosomes and conventional liposomes (with cholesterol) emphasized on the advantages of Sodium Glycocholate (SGC) in improving hypoglycemic effects of rhINS in non diabetic or diabetic rats. Sodium glycocholate being a potent permeation enhancer and GI enzyme-inhibitor, failed to show any enhancing effect on free rhINS absorption due to the deleterious situations in the GI tract. If rhINS was released and exposed to the GI environment, it would have been digested completely and resulted in no hypoglycemic effect in spite of the presence of SGC.[57,58] The results indicated that SGC containing bilosomes, maintained the integrity of the vesicles and the bioactivity of the encapsulated rhINS.[57,59] Ayogu et al. used male Wistar rats to demonstrate that bilosomal insulin formulation could prove to be a good oral delivery system for insulin that would affect the enteroinsular axis similar to that of endogenous insulin.[59] Another example is the in vivo activity of eprosartan mesylate loaded nanobilosome formulation that induced a nephroprotecting outcome with substantial decrease in serum creatinine, urea, lactate

dehydrogenase, total albumin, and malondialdehyde. It was also seen that oral administration of eprosartan mesylate loaded nanobilosomes decreased the raised expressions of Angiotensin II Type 1 receptor, inducible nitric oxide synthase, and transforming growth factor-β1 in Wistar rats. It has also been established that the formulation also showed nephroprotective effect which was studied by histopathological examination.[60]

6.3 Oral immunization

6.3.1 Bilosomes in oral immunization with model antigen

Conacher et al. reported oral immunization using Bovine Serum Albumin (BSA).[1,2] The orally administered bilosomal formulation containing BSA induced high antibody concentration against it, which was found to be equivalent to that generated after systemic immunization. Singh et al. formulated BSA-loaded and CTB-conjugated bilosomes to enhance their affinity toward M cells of Peyer's patches.[1,24]

6.3.2 Bilosomes in oral immunization against hepatitis b

Arora et al. reported oral immunization against Hepatitis B virus using mannosylated bilosomes. 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.[1,61]

6.3.3 Bilosomes in oral immunization against tetanus

Mann et al. reported significant systemic and mucosal immunity with Tetanus toxoid-loaded bilosomes on oral immunization.[62] Tetanus toxoid entrapped in bilosomes was capable of inducing Th2 response characterized by systemic IgG1. A clear dose-dependency was observed withspecific Tetanus toxoid IgG1 antibody titers, which was induced only with a higher concentration of Tetanus toxoid (200 mg/dose), not with the lower one (40 mg/dose). In addition to antibody production, only the Tetanus toxoid entrapped in bilosomes resulted in SIgA antibodies. The endpoint antibody titers were superior to oral administration of the un-entrapped antigen, but comparable to parenterally delivered Tetanus toxoid. Only Th2 and IgA responses were induced with orally delivered entrapped antigen.[23]

6.3.4 Bilosomes in ocular drug delivery

A previous study indicated that liposomes loaded with tacrolimus can facilitate penetration of the drug across the cornea. However, transcorneal permeation from liposomal suspension was

too small to achieve any therapeutic effect. Dai et al. observed significant corneal permeation of tacrolimus bilosomes containing different types of bile salts as permeation enhancers.[1,32]

6.3.5 Bilosomes in transdermal drug delivery system

Tenoxicam (TX) a long-acting NSAID is used for the management of rheumatic diseases. TX has the side effect of epigastric pain, indigestion, dyspepsia, vomiting and GI ulceration. TX showed poor transdermal penetration .On the basis of investigation carried out by Al-mahallawi et al., bilosomes showed the ability to increase transdermal transport of TX, thereby avoiding unnecessary GI side effects associated with oral administration.[63]

7. 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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