# Bilosomes: A Bile Salt based Novel drug delivery system

**[1]Dr. P.Tripura sundari[*], [2]G.Akhila**

[1]Associate Professor, [2]M.Pharmacy
RBVRR Women's college of pharmacy,
Osmania University, Hyderabad-500027.

***Abstract*: Bilosomes are specialised form of delivery system which protects vaccines from being broken down in stomach, thereby enabling the oral delivery of vaccines as an alternative to administering treatment by injection. Bilosomes are formed by incorporating deoxy cholic acid into the membrane of niosomes. It prevents from harsh GI environment by use of bile salts which usually has emulsifying and solubilising properties. It improves hydrophilicity for water insoluble drugs and thus enhances its bioavailability. Tremendous research in last decade has made bilosomes a potential carrier system. Bile acid based bilosomes are now a days most growing research area, hence multiple applications in the field of pharmaceutical and biomedical in related to bile salts are expected in near future.**

***Keywords*: Bilosomes, bilesalts, deoxycholic acid, vaccines.**

**Introduction:-**

Bilosomes are first described by Conacher et.al stated that these are bilayer structure of non-ionic amphiphiles that closely correlates to non-ionic surfactant vesicles i.e., niosomes but incorporating bile salts in it. For years, bile acids are recognised as biosurfactants with major roles in endogenous organotropism. Bile salts commonly used as penetration enhancers by pharmaceutical industry and promote its bioavailability. In the past years larger and more frequent oral antigen doses were given so as to overcome the digestive barrier. This in turn led to problems such as induction of tolerance. Another advantage of bilosomes is as they are produced from naturally occurring lipids making them incompatible.[1]

What are bilosomes?

Bilosomes are specialised form of delivery vehicles which protects vaccine from being broken down in the stomach thereby it enables the oral delivery of vaccines as an alternative for administering treatment by injection.

In 2004, Mann et al., developed non ionic surfactant vesicle having liposomes like structure and stabilise them with bile salts for oral delivery of vaccines[2]. These are called bilosomes and differ from liposomes, niosomes in terms of their composition, chemical stability and their storage conditions (Table-1). In order to avoid problems during GI transit, bilosomes were developed which is not only prevented antigens from degradation, but also enhanced mucosal penetration. Bilosomes based vaccine produced both systemic as well as mucosal immune response which was equvivalent to immune response produced by the sub cutaneous route[3].

Table-1: - Comparative overview of liposome, niosomes, bilosomes.[4]

| **Parameter** | **Liposomes** | **Niosomes** | **Bilosomes** |
|---|---|---|---|
| Composition | Natural phospholipids, cholesterol | Non-ionic surfactant with cholesterol | Non-ionic surfactant and bile salt |
| Chemical stability | Phospholipids undergo the oxidative degradation | Stable | Stable |
| Antigen dose | Comparatively high | Comparatively high | Comparatively low |
| GIT stability | Unstable | Unstable | Stable |
| Storage and handling condition | Required special conditions. | Do not required special condition | Do not required special condition |

Bilosomes are highly biocompatible and improves the therapeutic efficacy of drugs because of their stability in gastro intestinal tract. They increase the bioavailability of drugs as they can be readily absorbed through small intestine to the portal circulation (hepato circulation). By this circulation they approach to liver and releases the drug and shows that it is an effective tool for drug targeting in liver. To stabilise the vesicles optimum mannan coating was used in gastri intestinal environment and acts as a targeting ligand for mannose receptors expressed on macrophages and dendritic cells.[5]

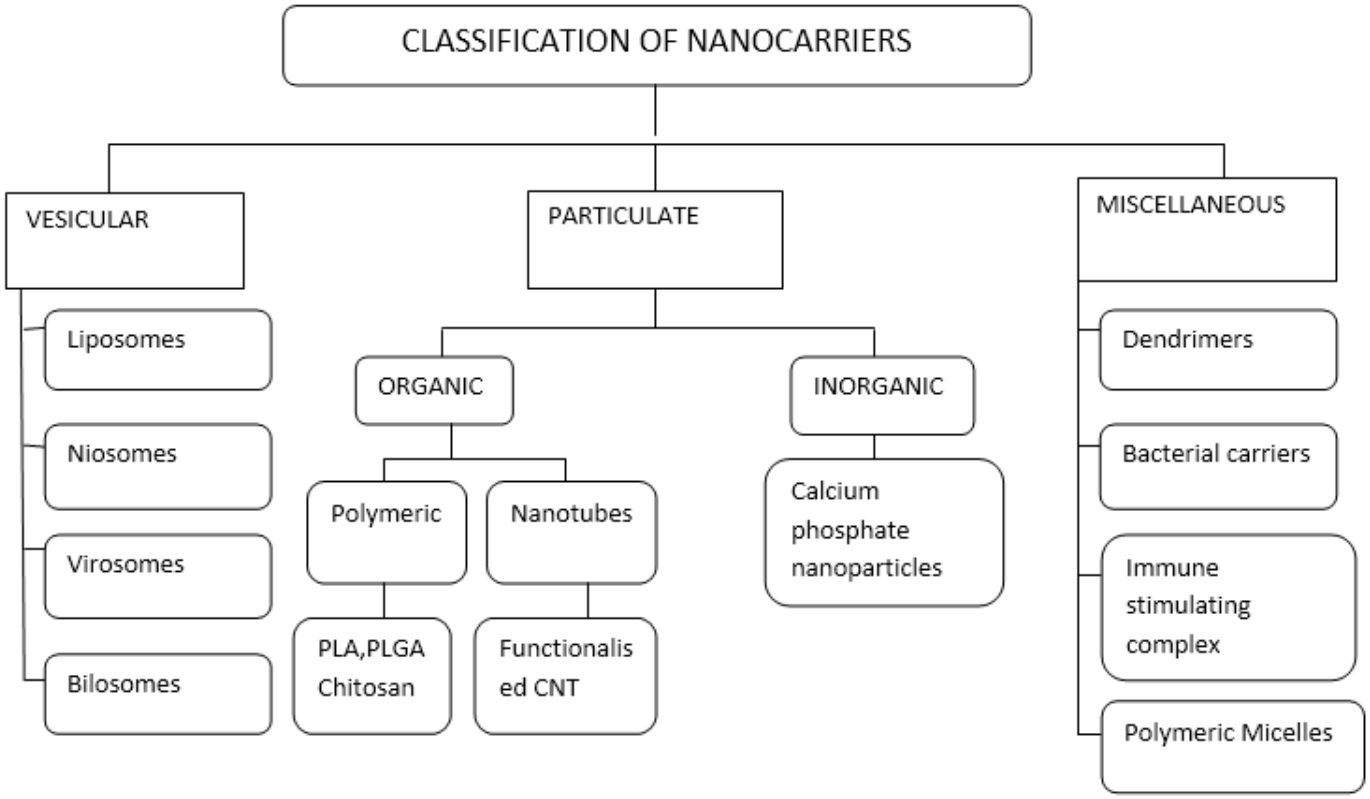
**Benefits:-**

- Bilosomes allow small quantities of antigen to be effective and also increase its efficacy of antigen which are weak when injected.
- They do not require the use of live pathogens, making them safe and effective alternative for traditional vaccines.
- This non invasive system offers advantages in terms of patient acceptance and compliance.
- Compared to bilosomes, conventional injection method suffers from high relative costs and required trained persons to administer.
- Bilosomes has less toxicity envelope suitable for a wide range of therapeutic agents.

- Immune response can be manipulated by controlling the size of carrying vesicles.
- Bilosomes provides new delivery system improving patient compliance, its ease of administration and potentially providing extended patent life.[6]

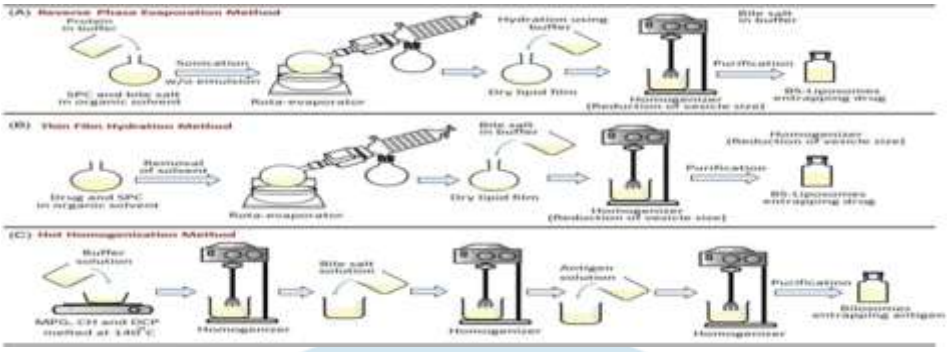
Bilosomes used oral immunization against hepatitis b virus: -
Hepatitis B is a type of deadly viral disease caused by hepatitis B virus (HBV). It is a partially double-stranded DNA virus of the Hepadnaviridae family which includes some viruses that are responsible for liver injury in animals. HBV is carried in blood and in other body fluids which includes tears, saliva, semen, and vaginal secretions and it can be transmitted from person to person by a various means.[7]
In some studies it was concluded that after its acute infection, between 1 and 10% of healthy adults and 30-90% of infected babies become chronic carriers and on later stages they become high risk of life threatening diseases such as cirrhosis and primary hepatocellular carcinoma(HCC).[8] Globally approximately 4.5million new HBV infections occur each year progresses to liver disease. Immunization is the most successful measure to reduce the global prevalence of hepatitis B[9]. Vaccination is the most important and economically attractive option for healthcare intervention in terms of cost-effective and benefit-cost ratios. The present concept of vaccination is based on invasive parenteral method which was not completely effective against number of diseases [6-9]. These traditional needle-based vaccination methods suffer from various drawbacks, like the need for trained personnel for the administration of vaccines, patient inconvenience and the risk of needle-borne infections(AIDS, hepatitis, etc)due to use of contaminated needles.[10]
One of the needle free mode of immunization is the oral mucosal immunization that can be commonly and successfully exploited. Oral route is also associated with some major problems including low oral bioavailability of protein related formulations, mainly vaccines. So, to improve the response and to deliver the vaccine via oral route, suitable delivery carrier is needed.[11] Incorporation of bile salts in niosomal formulation could stabilize the membrane against harmful effects of bile acids in GI tract. These bile salt stabilised vesicles known as "Bilosomes". These allow small quantities of antigen to be effective and induces both cellular and humoral immune responses.[12]

**Characterization of Bilosomes: -**
Bilosomes are characterised for their shape, size and morphological examination which are determined by using transmission electron microscope. For the determination of mean particle size a photon correlation spectroscopy is used. To carryout vesicle size and size distribution studies are done by particle size analyser. The pH of the vesicle suspension was determined using a pH meter where the tip was placed into the bilosome suspension and left for a few minutes. The particle sizes and surface charge determination through zeta potential measurement, phase transitions through differential scanning calorimetry. [1,13]




Based on the reviewed literature, bilosomes were prepared by using different methods: -
Preparation of BS-vesicles:-
A) Reverse phase evaporation method,
B) Thin film hydration method and

C) Hot homogenization method.



[1] Reverse-phase evaporation method:- Reverse-phase evaporation method was used to formulate BS-liposomes loaded with insulin or hexamethyl melamine. Soya bean phosphatidyl choline and bile salts were added to the ethyl ether and to this mixture buffer solution containing insulin is added and ultra-sonicated to form reverse w/o emulsion. The solvent was removed by using rotary evaporator under reduced pressure. The formed dried lipids are hydrated by buffer to get homogenous aqueous vesicles that enters through a high pressure homogeniser to obtain BS- liposomes entrapping protein. If poorly water-soluble drug is used then hexamethyl melamine was dissolved with lipid component in the organic solvent.

[2] Thin film hydration method:- This method was used to prepare both BS-liposomes and bilosomes. To prepare antigen loaded bilosomes thin film hydration technique was used. In this the lipid components, surfactants, cholesterol, and diacetyl phosphate were dissolved in a suitable organic solvent in a round bottomed flask and kept under rota-evaporator for solvent evaporation. A thin film is formed on the glass wall and it was hydrated by using buffer containing bile salt and antigen to form a large multilamellar vesicles and it was reduced by small lamellar by ultra-sonication.

[3] Hot homogenization method:- Bilosomes are prepared by using lipid components such as monopalmitoyl glycerol, cholesterol and DCP are melted at 140oC for 5min and hydrated by buffer. This lipid mixture was homogenised followed by adding bile salt solution that forms dispersion of empty vesicles and homogenised. Then the antigen buffered solution was homogenised and further homogenisation leads to protein entrapment. This method was used to entrap influenza A antigen and gonadotropin-releasing hormone (GnRH) to bilosomes.[14]

Applications: -

- Bilosomes are very effective in the vaccine delivery.
- These can be applied to the delivery of biological therapeutics and traditional small molecular drugs.
- Formulation and characterization of vesicular drug delivery system for Anti-HIV drug.[3]

- **Bile Acids role in the Central Nervous System: -**

Bile acids are also known as detergents whose main function is to breakdown of dietary lipids. This is done by the formation of mixed micelles with cholesterol and other phospholipids. In the past years bile acid synthesis occurs solely in the liver, as Cyp27a1 is a liver specific enzyme and an alternative or “acidic pathway” was discovered which starts with the side chain 27-hydroxylation catalysed by Cyp27a1. It is expressed in an array of extra hepatic tissues such as kidney, immune cells and brain and mutations occurs in Cyp27a1 gene has shown to underlie the sterol storage disorder cerebro tendinous xanthomatosis that leads to cholesterol accumulation in the brain and neurological dysfunction.

If bile acids acts in a physiological manner in the brain then bile acids to exert their actions signalling machinery is necessary and must be present in the brain. As with all signalling pathways, during disease states and bile acids there is a potential for dysregulation. By examining the dysregulation of the bile acid signalling system in the CNS during different pathologies are lacking, hence bile acids as a potential therapeutic agent for various neurological disorders. In this study it was known that bile acids have neuroprotective effects in models of Huntington’s disease and Alzheimer’s disease.[15]

- **Bile in digestion and absorption: -**

Endogenous bile plays an important role in the digestion of lipids and its absorption process. The process of digestion of lipids takes place to a small extent in the stomach (10%-30%) via gastric lipase and more significantly in the small intestine (70%-90%) via pancreatic lipase. The digested food upon reaching the small intestine, it is mixed with bile and pancreatic secretions in the duodenum and forms an emulsion which gets stabilized by endogenous surfactants.[16]

- **Oral route of drug administration:-**

High oral bioavailability is an important factor for the development of bioactive molecules as therapeutic agents. The oral route of administration is the most convenient and acceptable for the patients. The gastrointestinal mucosa represents a physiological and biochemical barrier to the systemic availability of orally ingested, pharmacologically active molecules.[17] Bilosomes has significantly higher bioavailability than that from liposomes and the micronized form. In gastrointestinal physiology, the exact mechanism of enhanced bioavailability of oral bilosomes has not been explained fully. One can be assumed that it is a consequence of the interplay between many factors which include protective effect against GI harsh environment, ability of membrane fluid and physicochemical properties of incorporated bile salts.[18]

**CONCLUSION: -**

Vesicular drug delivery system has made a diverse application in pharmaceuticals, cosmetics and cosmeceuticals and also in food industries. The delivery of drug directly to the site of infection by reducing its drug toxicity with no adverse effects. It also reduces the cost related to therapy by showing better biopharmaceutical properties of a drug, which results in improved bioavailability especially in case of hydrophobic drugs. Oral administration became most favourable method of drug delivery by patients and drugs absorption by the body is improved. An active molecule is required to transit through the stomach which upon transfers across the intestinal wall then to the blood stream. Approximately 1/3 of small molecular drugs face challenges related to oral absorption which mainly focus on central nervous system targets.

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