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A REVIEW ON BILOSOMES: ADVANCED DRUG DELIVERY SYSTEM

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ABSTRACT

Bilosomes are bilayer vesicles that transport lipids incorporating non-ionic surfactants and bile salts. The current review was based on the properties, materials used in the preparation, various methods of formulation, characterization parameters and various pharmaceutical and clinical applications. They are spherical, uni-lamellar, and 5-200 nm-sized and vesicles with many membranes. Under normal conditions, bile acids exist as ionised bile salts after being synthesised in the liver and stored in the gall bladder. They have a steroid nucleus that is both hydrophilic. Materials used in bilosomes comprise of lipids, non-ionic surfactants and bile salts. Bilosomes are prepared by various methods including Reverse phase evaporation, Thin Film Hydration and Hot Homogenization. Bilosomes are characterized in terms of particle size, polydispersity index (PDI), zeta potential, ultracentrifugation, entrapment efficiency, in-vitro drug release and stability. Bilosomes have wide range of applications i.e., oral drug candidates, improved hypoglycaemic activity, oral immunization against tetanus, in ocular and transdermal drug delivery. For immunologists, the formulation of a reliable oral delivery mechanism for mucosal vaccines presents a considerable problem. In this regard, bilosomes and other lipid-based delivery methods have been investigated and developed for oral immunisation. Due to the tremendous potential of bilosomes, which include biocompatibility, stability, and specificity as carriers for targeted administration in immunisation. Currently, it is crucial for clinical researchers to use their knowledge of bilosomes for safe and effective trials in human patients and to identify the precise immunological mechanism triggered by bilosome oral administration.

Keywords: Bilosomes, formulation, characterization parameters, advantages, applications.

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INTRODUCTION

Incorporating nonionic surfactants and bile salts, bilosomes are bilayer vesicles that transport lipids. They are spherical, unilamellar, and 5-200 nm-sized and vesicles with many membranes [1]. Conacher et al. [2] originally described bilosomes in 2001. Under normal conditions, bile acids exist as ionised bile salts after being synthesised in the liver and stored in the gall bladder. They have a steroid nucleus that is both hydrophilic. Through the creation of mixed micelles, they aid in the emulsification and solubilization of dietary lipids. Therefore, many physiologically active compounds are more readily absorbed when taken orally because of the effect of bile salts on the permeability of lipophilic drug molecules across the plasma membrane. Vaccines consisting of proteins or peptides are typically administered intramuscularly; however, when these vaccines were encapsulated in bilosomes and administered, they elicited both systemic and mucosal immunity, with no pathogen-host interaction occurring at mucosal surfaces [3].

They 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 Preparations

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

1. Phospholipids

The cellular membrane is very compatible with phospholipids. Wetting and emulsification are facilitated by their amphiphilic properties, which allow them to self-assemble. Phospholipids, according to their amphiphilic properties, can arrange themselves in water to create closed concentric bilayers. Phospholipids are able to stabilise emulsions because of their high emulsifying capacity [4,5]. The following phospholipids [6,7] are frequently found in bilosomes.

2. Cholesterol

Cholesterol is introduced into the cellular membrane as an amphiphilic molecule, with the hydroxyl groups facing the aqueous surface and the aliphatic chains aligning parallel to the acyl chains in the bilayer's central region. This makes bilosomes stiffer [8].

3. Sodium-Free Detergents

The stability and compatibility qualities of nonionic surfactants make them preferable to anionic, cationic, or amphoteric forms in the formation of bilosomes [9-11]. They are less haemolytic and irritant to cellular surfaces and aid in keeping the solution pH as close to physiological as possible. They help break down molecules and act as solvents, wetting agents, emulsifiers, and permeability boosters.

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They are effective P-glycoprotein inhibitors as well. They improve both medication absorption and tissue targeting. High interfacial activity and polar and non-polar components characterise nonionic surfactants. The drug's entrapment efficiency is affected by the chain length and size of the hydrophilic head groups of the nonionic surfactant.

Compared to lauryl chains, the entrapment efficiency of nonionic surfactants containing stearyl (C18) chains is greater [9]. Combining Tweens with long alkyl chains and large hydrophilic moieties with cholesterol in a ratio of 1:1 increases entrapment efficiency for water-soluble medicines [9,12–14]. The HLB value of surfactants is also crucial in regulating vesicular drug entrapment. Surfactants with an HLB value between 14 and 17 are not optimal for bilosome vesicle production, while those with an HLB value of 8.6 are the most effective. Common nonionic surfactants employed for vesicle production include the following [9]; however, the entrapment efficiency decreases when the HLB value decreases from 8.6 to 1.7.

4. Bile salts

Bile salts are naturally occurring biosurfactants in the gastrointestinal lumen that facilitate the breakdown and absorption of fats. Stimulating bile secretion improves absorption of bioactive compounds. This was used by several mixed micelle systems to increase the solubility of extremely lipophilic medicines. By increasing the repulsion between the bile salts inside the bilosomes and the external bile salts in the gut lumen, bile salts contribute to the stability of bilosomes in artificial fluids. The following bile salts are found in bilosomes [17–19].

Advantages

- Small amounts of antigens can be effective when packaged in bilosomes, and bilosomes can boost the potency of weak antigens that are injected.
- With its low toxicity and broad therapeutic utility, the non-invasive nature of this device is a major selling point.
- The size of the carrier vesicles can be adjusted to alter the immune response.
- The cold chain, necessary for products like vaccinations, is broken by bilosomes.
- Antigens can be shielded from the digestive system by being encased in a polymerized liposome, microsphere, nanoparticle, or bilosome.
- After lyophilization (for both microspheres and nanoparticles), they can be stored without the need for cold storage for an extended period of time.
- Many vaccination combinations involving many substances can be administered with relative ease.
- Administration requires no specialised staff members.
- With oral dose, there is no need for constant dosing.
- Antigens can be delivered more slowly and precisely if they are encapsulated in biodegradable microspheres or microcapsules.

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Limitations

Due to the lack of an in vitro approach that can accurately reproduce the condition, bilosome formulations exhibit poor in vitro/in vivo correlation, which is a significant problem. Traditional in vitro release techniques are devoid of biological elements that greatly influence vesicular digestion. When employed to assess vesicular permeability in cell lines and ex vivo permeation models, it also has a disadvantage. Despite being great transporters for cationic substances, bilosomes fail to effectively entrap anionic active molecules, resulting in low entrapment efficiency. This is due to the fact that bile salts have a negative charge and are hydrophilic, making it feasible to incorporate or trap cationic actives with a membrane-stabilizing effect.

Preparation Methods

- **Reverse Phase Evaporation Method**

  The drug-containing water phase of the emulsion is separated from the organic phase of lipids used to create the bilosomal bilayer through the reverse phase evaporation process. This technique involves dissolving soybean phosphatidylcholine and bile salts in an organic solvent such absolute ether, then slowly mixing in a buffer solution containing the protein. For 5 minutes, the mixture is sonicated in a water bath until a non-emulsified state is reached. Rotaevaporation at 50 rpm is used to extract the organic solvent from the emulsion. The dried lipids are then rehydrated with a buffer until a uniform suspension is created. Finally, the suspension is extruded through a high pressure homogenizer, and the drug-loaded bilosomes are obtained through ultracentrifugation [20]. Porcine insulin, recombinant human insulin, and other protein medicines are prepared in this way as bilosomes.

- **Thin Film Hydration Method**

  Using a rotating vacuum evaporator set to a decreased pressure, the organic solvent containing the lipid component (soybean phosphatidylcholine) and the drug is evaporated, leaving behind the drug-loaded bilosomes. After forming a thin layer, the bile salt-containing buffer is added, resulting in big multilamellar vesicles, which are then homogenised under high pressure to create small unilamellar vesicles. After being isolated, these vesicles are purified to yield drug-loaded bilosomes [21,22]. Toxoid, hepatitis B antigen, tetanus toxoid, diphtheria toxoid, and cyclosporine A bilosomes are all prepared using this technique.

- **Hot Homogenization Method**

  Lipid components, including mono palmitoyl glycerol, cholesterol, and dicetyl phosphate, are melted at 140°C for 5 minutes before being hydrated with buffer solution to prepare bilosomes by the hot homogenization process.

  After this mixture is homogenised, bile salt solution is added to create a dispersion that includes empty vesicles, and the process is repeated. After mixing in the antigen buffered

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solution, the homogenate undergoes repeated Freeze Thaw cycles to entrap the proteins. To reduce prolonged homogenization, antigen is introduced at the very end [21].

Characterization Parameters

Particle Size

The in vitro and in vivo activities of bilosomes are significantly affected by their particle size [22]. Bilosome vesicle sizes vary from 90nm to 3m [23]. In a trial including an influenza challenge, vesicles having a diameter of 6m (as opposed to 2m) were more efficiently taken up by the Peyer's patches, leading to a lower median temperature differential shift and a decrease in viral cell burden. Photon correlation spectroscopy, an analytical tool based on the idea of dynamic light scattering, is used to assess the particle size distribution of bilosome vesicles [24]. Time-dependence is measured with this method.

Polydispersity Index

Polydispersity refers to the degree to which the distribution of particle sizes is not continuous [25]. A PDI of 0.3 or less is deemed acceptable and suggests a homogenous population of phospholipid vesicles for use in drug delivery applications using lipid-based carriers, such as liposome and nanoliposome formulations [26-28].

Zeta Potential

The term "zeta potential" refers to the total charge that the particles in a given medium have. Surface-charged vesicles are more resistant to aggregation than their uncharged counterparts. Due to the presence of bile salts, bilosomes acquire a negative charge, which stimulates zeta potential and prevents the vesicles from aggregating [1, 32]. Peyer's patches readily absorb negatively charged vesicles. Due to electrostatic repulsion between the particles, the system is often considered stable with a zeta potential of roughly +30 mV [29-30]. Explanation of the many approaches and methodologies for investigating the vesicular structure of bilosomes.

Ultracentrifugation

For ultracentrifugation, a high-velocity centrifuge that can spin a rotor at speeds of up to 10,00,000 g (about 9800 km/s2) is used as a separation technique. The drug-loaded bilosomes can be isolated from the unencapsulated drug.

Entrapment Efficiency (%)

The effectiveness of entrapping a medicine into vesicles is measured in terms of the percentage of the drug that is successfully encapsulated.

Drug entrapment efficiency and solubility in the dispersion media are both improved by an increase in bile salt concentration [31]. The EE% also rises as lipid levels rise.

$$\text{Effectiveness Equivalent Percentage} = \frac{(\text{Total Amount of Drug} - \text{Free Drug Amount})}{\text{Total amount of Drugs}} \times 100$$

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In vitro Release

Determining release kinetics from vesicular systems is commonly done using the dynamic dialysis approach. Diffusion over the dialysis membrane is responsible for the drug's presence in the washbasin receiver compartment after it has been released from the vesicle [32].

Stability

Stability tests investigate the release of the encapsulated substance from the vesicles over time. Shukla et al. compared the amount of Diphtheria toxoid still present in the bilosomes after storage at 53°C and 252°C with 70% relative humidity. After a month, the antigen content of bilosomes kept at ambient temperature was only around 94%, while the antigen content of samples kept in the fridge was over 98%. Because of the electrostatic repulsion between bilosomes due to the negative charge created by dicetylphosphate, the formulations were shown to be stable [18].

Applications

Oral Drug Candidates

Recombinant human insulin (rhINS) loaded bilosomes containing various bile salts (sodium glycocholate, sodium taurocholate, and sodium deoxycholate) were administered to male Wistar rats in order to ascertain the relative bioavailability of insulin based on blood levels. Bilosomes containing sodium glycocholate were found to have oral bioavailability of 8.5% and 11%, respectively, in non-diabetic and diabetic rats [33-34]. These insulin oral bioavailability levels were discovered to be greater than the outcomes previously reported. This demonstrates how encapsulated rhINS has a better protective effect against enzymatic degradation [35].

Improved Hypoglycaemic Activity

Insulin administered subcutaneously causes hypoglycemia, whereas insulin administered orally is safe [36]. The advantages of Sodium Glycocholate (SGC) in enhancing the hypoglycemic effects of rhINS in non-diabetic or diabetic rats were highlighted in the comparison of bilosomes and traditional liposomes (with cholesterol). Due to the harmful conditions in the GI tract, sodium glycocholate, a powerful permeability enhancer and GI enzyme-inhibitor, failed to demonstrate any boosting effect on free rhINS absorption. Despite the presence of SGC, if rhINS had been released and exposed to the GI environment, it would have been entirely digested and had no hypoglycemic impact [37]. The findings showed that SGC containing bilosomes preserved both the vesicle's integrity and the encapsulated rhINS's bioactivity [38]. Male Wistar rats were used by Ayogu et al. to show how a bilosomal insulin formulation might work well as an oral insulin delivery method and have effects on the enteroinsular axis that are comparable to those of endogenous insulin [39].

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Bilosomes in Oral Immunization against Tetanus

With Tetanus toxoid-loaded bilosomes on oral immunisation, Mann et al. reported strong systemic and mucosal immunity [40]. Bilosome-entrapped tetanus toxoid was able to trigger a Th2 response that was characterised by systemic IgG1. Specific Tetanus toxoid IgG1 antibody titers showed a clear dose-dependency and were only elicited by the higher Tetanus toxoid concentration (200 mg/dose), not the lower one (40 mg/dose). Only the Tetanus toxoid trapped in bilosomes produced SIgA antibodies in addition to antibody production. The endpoint antibody titers were superior to parenterally administered Tetanus toxoid but comparable to oral delivery of the unentrapped antigen. With orally administered entrapped antigen, only Th2 and IgA responses were elicited [41,43].

In Ocular Drug Delivery

Tacrolimus-loaded liposomes may make it easier for the medication to pass the cornea, according to a prior study. To obtain any therapeutic impact, transcorneal penetration from liposomal suspension was, however, insufficient [44-46].

In Transdermal Drug Delivery System

A long-acting NSAID called tenoxicam (TX) is used to treat rheumatic conditions. Epigastric discomfort, gastrointestinal ulceration, dyspepsia, indigestion, and vomiting are all adverse effects of TX. Poor transdermal penetration was seen in TX. According to research done by Al-mahallawi et al., bilosomes have the ability to boost TX transdermal transport, preventing the needless GI adverse effects of oral delivery [47-49].

CONCLUSION

According to the literature study, bilosomes increase a drug's bioavailability as well as its effectiveness and capacity to entrap proteins, peptides, and antigens [50]. For immunologists, the formulation of a reliable oral delivery mechanism for mucosal vaccines presents a considerable problem. In this regard, bilosomes and other lipid-based delivery methods have been investigated and developed for oral immunisation.

Future Aspects

Due to the tremendous potential of bilosomes, which include biocompatibility, stability, and specificity as carriers for targeted administration in immunisation, bilosomes will likely soon play a large role in the treatment of terrifying and infectious diseases, leading to their eventual eradication.

Currently, it is crucial for clinical researchers to use their knowledge of bilosomes for safe and effective trials in human patients and to identify the precise immunological mechanism triggered by bilosome oral administration.

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CONFLICT OF INTEREST
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