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REVIEW ARTICLE

A REVIEW ON ENTERIC COATED LIPOSOMES

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ABSTRACT

Liposomes are novel drug delivery system which delivers the drug directly to the place of action. The liposomes are colloidal carriers with a diameter ranging from 0.01 to 5.0 µm. Liposomes can be formulated using different approaches like thin-film hydration method, reverse phase evaporation method, solvent injection method etc. However, this drug delivery mechanism fails to transport the medication to the GIT when liposomes are employed to treat diseases related to the GIT, because of the medication release into acidic environments. In this instance, the liposomes are coated using the enteric coated polymer. The word "enteric" indicates small intestine; therefore enteric coatings prevent release of medication/drug before it reaches the small intestine. At low pH levels, the enteric coated polymer continues to unionize and stays insoluble. Enteric-coated liposomes are formulated for avoiding the first pass metabolism, gastric irritation and degradation and to direct the drug to the intestine. The Polymer used for enteric coating are cellulose acetate phthalate (CAP), polyvinyl acetate phthalate (PVAP), hydroxypropyl methylcellulose phthalate (HPMCP), acrylate polymer etc. The review focuses on the general overview of enteric coated liposomes, their manufacturing method, characterization, and their application in the drug delivery system

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INTRODUCTION

Liposomes are spherical shaped concentric vesicles. The term liposome is derived from the Greek words lipos means fat and soma means body. Liposomes were made by Bangham *et al* in 1961. It was accidentally made, when he dispersed the phosphatidyl choline molecule in water, he found that the molecule was forming a closed bilayer structure having an aqueous phase were entrapped by a lipid layer. Liposome is colloidal carrier, having a size range of 0.01-5.0µm in diameter. Due to their size and amphiphilic nature, liposomes are promising system for drug delivery. Drug encapsulated by liposome achieves therapeutic level for long duration. Liposomes are novel drug delivery system which delivers the drug directly to the place of action. Liposomes encapsulate different types of drugs such as antibiotics, immunomodulator, antifungal agents, anticancer drugs, proteins and peptides etc.The source of the lipids and stability of the phospholipids, which are considered as critical excipients, play a major role in the characterization of product performance. Lipid degradants such as lysolipids can be formed during manufacturing or storage. Lysolipids have been known to be associated with

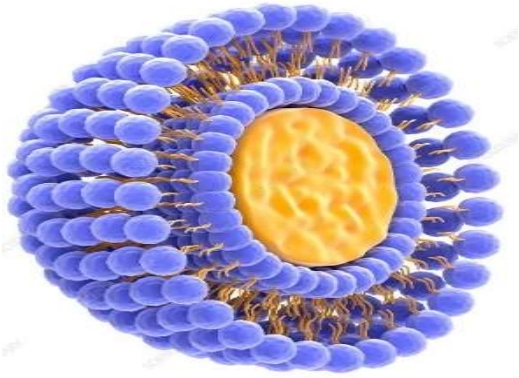

Figure 1. Structure of liposome

CLASSIFICATION OF LIPOSOMES: The size of the liposomesvary from very small (0.025µm) to large (2.5µm) vesicles. Vesicles size is the main important parameter in determining the circulation half-life of liposomes, and both size and number ofbi-layer affects the amount of drug encapsulation in the liposomes.

Depending on their size and number of bi-layer, liposomes can also be classified into one of two categories:

Multilamellar vesicles (MLV)

Unilamellar vesicles: Multilamellar vesicles **(MLV**) MLV having a size greater than 0.1μm and consists of two or more bilayer. Their formulation method is simple and easy to carry which includes thin–film hydration method or hydration of lipids in excess of organic solvent. They are mechanically stable on long storage condition. The drug entrapment or corporate into the vesicles can be improved by slower rate of hydration and gentle mixing. Thin films of dry lipids can also easily enhance encapsulation efficiency by hydration. Unilamellar vesicles in this, the vesicle having a single phospholipids bilayer sphere enclosing the aqueous solution. In multilamellar liposomes, vesicles have a structure similar to an onion. Classically, several unilamellar vesicles will form on the inside of the other with smaller size, making a multilamellar structure of concentric phospholipids spheres separated by layers of water.

Unilamellar vesicles having two categories

- Large unilamellar vesicles (LUV)
- Small unilamellar vesicles (SUV)

Large unilamellar vesicles (LUV) These classes of liposomes particularly have a large unilamellar vesicles consist of a single bilayer and have a size greater than 0.1μm. They have higher encapsulation property, since they can hold a large volume of solution in their cavity. They have high bounded volume and can be useful for encapsulating hydrophilic drugs. The most useful advantage of LUV is that less amount of lipid is required for encapsulating large quantity of drug. LUV can be formulated by using different methods like ether injection, detergent dialysis and reverse phase evaporation techniques.

Small unilamellar vesicles (SUV) SUV are smaller in size (less than 0.1 μm) when compared to MLV and LUV, and have a single bilayer. They have a low entrapped aqueous volume to lipid ratio and characterized by having long circulation halflife. SUV can be prepared by using solvent injection method or alternatively by reducing the size of MLV or LUV using sonication or extrusion process under an inert atmosphere like nitrogen or Argon. The sonication can be performed by using a bath or probe type sonicator. SUV can also be attained by passing MLV through a narrow orifice under high pressure.(6,7,8)

- Conventional liposomes
- PH sensitive liposomes
- Cationic liposomes
- Immune liposomes
- Long circulating liposomes

Conventional liposomes: Conventional liposomes, the first generation of liposomes, are a family of vesicular structurebased on lipid bilayers surrounding aqueous compartments. These particles are typically composed of only phospholipids such as egg phosphatidylcholine, 1, 2-distearoryl-sn-glycero- 3- phosphatidyl choline (DSPC) and sphingomyelin and/or cholesterol without modification. With its hydrophobic lipid bilayer and the hydrophilic aqueous space, various types of drug compounds can be incorporated accordingly.

pH-sensitive liposomes: pH-sensitive liposomes are stable at physiological pH, they destabilize under acidic conditions, leading to the release of their aqueous contents. In addition, they appear to destabilize or fuse with the membranes of endosomes in which they are internalized, enabling even macromolecular liposome contents to enter the cytoplasm. These are used to deliver content to cytosol not to lysosomes. Because lysosomes degrade the drug and decreases drug concentration. Hence the pH sensitive liposomes are used for cytosol targeting. pH-sensitive liposomes were inspired by viruses that merge with endosomal membranes and before reaching the lysosomes they deliver their genetic material to the cytosol.

Cationic liposomes: Between 40 nm and 500 nm, and they can either have one lipid bilayer (monolamellar) or multiple lipid bilayers (multilamellar).

Immune liposomes: These liposomes have wideapplications in generating immune response. The incorporation of antigens into liposomal membranes or in the aqueous core causes increased immune response by antibody production, macrophage activation subsequent antitumor activity and effective induction of cytotoxic cell. Liposomes have several advantages as immunological adjuvants, including low toxicity, low antigenicity, biodegradability, and the ability to target specific cells in vivo. Liposomes are effective adjuvants for increasing immunogenicity to proteins, pathogenic viral antigens, glycolipids (gangliosides), and other antigens.

Long circulating liposomes: Long-acting liposomes are incorporated into the mononuclear phagocytic system through intrahepatic absorption. Long circulation periods were accomplished by covalently adding polyethylene glycol to the phospholipid. The molecular mass should be between 1500 and 5000 Da(4,5)

MANUFACTURING PROCESS: Liposomes can be formulated using different approaches. The manufacturing process of liposome and the type of phospholipids critically affects the final liposomes characteristics. Liposomes production procedures can be classified into: Thin film hydration method (Bangham method): The most common method employed for liposome synthesis is thin film hydration. Using a roundbottom flask, all lipids and the hydrophobic medication are dissolved in a suitable organic solvent. The organic solvent is then slowly evaporated under decreased pressure to form a thin film layer. The thin film is then hydrated with an aqueous buffer solution at temperatures above the lipid's transition temperature (Tm). The hydration solution may comprise a hydrophilic medication or drugs that will be placed into the aqueous core of the liposomes. (9,10,11)

Thin film hydration method (Bangham method): The most common method employed for liposome synthesis is thin film hydration. Using a roundbottom flask, all lipids and the hydrophobic medication are dissolved in a suitable that will be placed into the aqueous core organic solvent. The organic solvent is then slowly evaporated under decreased pressure to form a thin film layer. The thin film is then hydrated with an aqueous buffer solution at temperatures above the lipid's

transition temperature (Tm). The hydration solution may comprise a hydrophilic medication or drugs of the liposomes.

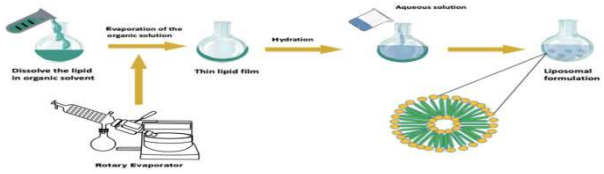

Figure 2. Thin film hydration method

Reverse-phase evaporation method: By generating a water-in-oil emulsion, reverse-phase evaporation is commonly utilised as an alternative to thin-film hydration. The lipids are first dissolved in an organic solvent before being combined with an aqueous buffer containing the hydrophilic medication. The organic solvent is then evaporated in a low-pressure rotary evaporator, resulting in lipid vesicles scattered in the aqueous solution. Extrusion can diminish the average size and polydispersity of produced vesicles.

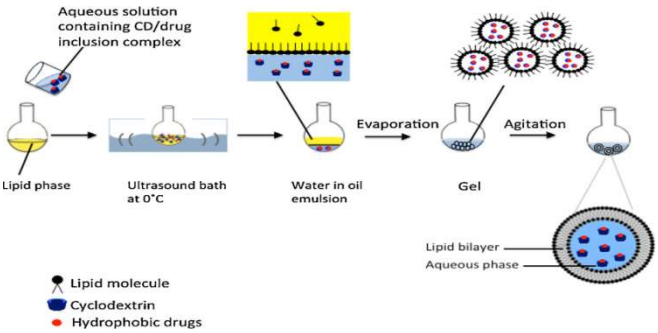

Figure 3. Reverse phase evaporation method

Solvent Injection Method: The injection techniques were categorised based on the organic solvent employed. An aqueous phase was immediately injected with an organic solvent that dissolved the lipids and hydrophobic active ingredients. Diethyl ether allows for direct solvent evaporation during the mixing procedure at temperatures above the boiling point of the solvent utilized.

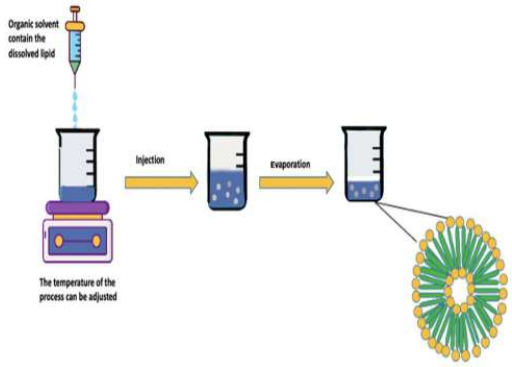

Figure 4. Solvent injection method

Detergent Removal Method: Using a round bottom flask, lipids and a high critical micelle concentration (CMC) surfactant were dissolved in a suitable organic solvent. After mild solvent evaporation, a thin coating was formed at the bottom of the flask. After soaking the lipid film in an aqueous solution containing the drug molecules, a mixed micelles solution was formed. Dialysis, size exclusion chromatography, adsorption onto hydrophobic beads or dilution is subsequently used to remove the surfactant.

Dehydration-Rehydration Method: Sonication is an organic solvent-free approach for producing LUVs. This approach involves scattering lipids at low concentrations directly into an aqueous solution containing drug molecules, followed by sonication. The first process is to evaporate water under nitrogen to generate a multi-layered film that entraps the drug molecules. The drug molecules are then encapsulated in giant vesicles formed by hydration. This approach is easy; however, the liposome sizes vary greatly.

Heating Method: It is also a solvent-free organic method. Lipids are hydrated directly with aqueous solution and heated for at least one hour above the Tm of the utilised phospholipids in the presence of a 3-5% hydration agent like glycerin or propylene glycol. When adding cholesterol to the recipe, the suspension can be heated to 100 °C. The hydrating agents operate as stabilisers and isotonizers, preventing nanoparticle coagulation and sedimentation. Moreover, the cryoprotective action of the hydration ingredients makes the heating process an efficient approach for the production of powder inhalable liposomes.

pH Jumping Method: The pH jumping approach is another solvent-free method for producing liposomes. To break down MLVs into SUVs, an aqueous solution of phosphatidic acid and phosphatidylcholine is subjected to a nearly four-fold rise in pH over a short period of time. The fraction of SUVs to LUVs formed is determined by the phosphatidic acid: phosphatidyl choline ratio.

Microfluidic Channel Method: The microfluidic channel approach has recently been presented as a revolutionary method for producing liposomes. Lipids are dissolved in ethanol or isopropanol before being injected vertically or in the opposite direction to the aqueous medium within the micro-channels. This approach requires constant axial mixing of organic and aqueous solutions, which results in the creation of liposomes. Surfactants are used as stabilizer in liposomes to prevent coagulation and separation.

Supercritical Fluidic Method: Instead of employing organic solvents, this approach uses a supercritical fluid, carbon dioxide (CO2), to dissolve lipids. A high-performance liquid pump delivers a continuous flow of the aqueous phase into a cell containing the supercritical lipid solution, allowing the dissolved phospholipids to phase transition. This approach results in 5-fold greater encapsulation efficiency. Even when employing ecologically safe and inexpensive carbon dioxide, this technology suffers from high costs, limited yield, and specialized infrastructure.

ENTERIC COATING: Coating is a process where a layer is applied to a substrate in order to achieve different benefits. Besides being used purely for cosmetic purposes to add color in order to differentiate white substrates from each other, coatings can also be designed as functional systems. Pharmaceutical coatings can be applied on multiple oral solid dosage forms (OSD), such as tablets and pellets, granules or capsules.

Figure 5. enteric coated capsules,enteric coated tablets, enteric coated pellets

Why applying an enteric coating on OSDs?: Enteric coating is a type of functional coating. It can be used to allow a release of the active pharmaceutical ingredient (API) after the stomach in the small intestine or in a lower part of the digestive tract. Another application is to use it as a protective layer of the OSD against the acidity of the stomach. Thus, avoiding the release of the drug in the stomach. This protection can be needed for different reasons, such as:

- Irritation of the stomach with prolonged contact with some APIs
- APIs that can be inactive or decomposed by the gastric fluid
- APIs that should arrive to the intestine high concentration

Figure 6. Enteric Coating of the Tablet Formulation

Once the medicine passes the pyloric valve, it reaches the first small intestine called the duodenum having a pH around 5. Depending on the pH solubility of the enteric coating, the API can already be released in this region of the digestive tract and be absorbed into the blood stream.

How to apply an enteric coating on OSDs?: During the enteric coating process a uniform polymer film layer should be applied to the surface in order to obtain the desired gastric resistance. This film should be insoluble in gastric media, but soluble in the intestine. According to the Pharmacopoeias, two hours of gastric resistance is required for an efficient enteric coating layer. Different equipment can be used depending on the OSD form. Usually when tablets and capsules are coated, a pan coater (Figure 1a) is used and in the case of pellets or granules a fluid bed (Figure 1b) is used. The figures 1a and 1b are showing typical equipment used in a laboratory scale during the development phase. The amount of polymer to be applied to achieve an optimal gastric resistance is defined by the polymer weight gain linked to the surface area of the substrate. Due to the higher surface area of pellets/granules compared to tablets/capsules, higher weight gain is needed for these oral solid dosage forms. Tablets and capsules are coated to around 8% weight gain and pellets/granules to a weight gain of 15-30%, as a reference.

POLYMER USED FOR ENTERIC COATING

- Cellulose acetate phthalate (CAP)
- Polyvinyl acetate phthalate (PVAP)
- Hydroxypropyl methylcellulose phthalate (HPMCP)
- Acrylate polymers

Cellulose acetate phthalate (CAP): Cellulose acetate phthalate, also known as cellacefate is one of the oldest and most widely used synthetic enteric coating polymer. CAP is obtained by acetate ester of cellulose with phthalic anhydride in the presence of the tertiary organic base such as pyridine, or a strong acid such as sulfuric acid.

APPLICATIONS: CAP has been used for several decades as a pharmaceutical excipient due to its solubility dependent on the pH of the aqueous media. Enteric coatings based on CAP are resistant to acidic gastric fluids, but easily soluble in mildly basic medium of the intestine. The pH sensitive solubility of CAP is mainly determined (as other properties of this mixed ester) by the degree of substitution (DS), namely the average number of substituent groups bound to an anhydroglucose unit (AGU), as well as by the molar ratio (acetyl and phthaloyl groups). These two structural characteristics of the polymer are dependent on the method employed for its synthesis.

Polyvinyl acetate phthalate (PVAP): It is a free-flowing white to off-white powder with a slight odour of acetic acid. The onset of aqueous dissolution of PVAP begins at a pH about 5.0 allowing for enteric release as well as potential for targeted drug release to the proximal small intestine.

APPLICATIONS: Poly vinyl acetate phthalate is a viscosity modifying agent that is used in pharmaceutical formulation. To produce enteric coating for products and for the core sealing of tablets prior to a sugar-coating process. PVAP does not exhibit tackiness during coating and produces strong robust films. Plasticizers are often including in PVAP coating formulation to unable a continuous, homogeneous, non cracking film to be produced. For enteric coating application, PVAP is dissolved in solvent system together with other additives such as diethyl phthalate and stearic acid. Methanol may be used as a solvent if a colour less film is required; for a coloured film ethanol (or) methanol/water may be used depending on the amount of pigment to be incorporated.

Hydroxypropyl methylcellulose phthalate (HPMCP): HPMCP is a white to slightly off-white, free-flowing flakes or granular powder with a slightly acidic odour and detectable taste. It is a derivative of hydroxypropyl methyl cellulose.

APPLICATIONS: Hypromellose phthalate (hydroxypropyl methylcellulose phthalate, or HPMCP) is a phthalic acid ester of hydroxypropyl methylcellulose. In the pharmaceutical industry, hypromellose phthalate is used as a coating agent for tablets and granules. It is a colorless, odourless, white powder. Hypromellose phthalate was introduced in 1971 as a cellulose derivative for enteric coating. An enteric coating agent is used to protect drugs from degradation by gastric acid or to present them from causing side effects in the stomach. It is widely used as an enteric coating agent by the pharmaceutical industry. HPMCP has been admitted into the U.S. National Formulary (US/NF). HPMCP is also used in sustained-release preparations, in binders and as microcapsule bases.(

Acrylate polymers: Two forms of commercially available enteric acrylic resins are Eudragit L and Eudragit S both resins produces film that are resistant to gastric fluid. Eudragit L and Eudragit S are soluble in intestinal fluid at pH 6 to 7 respectively. Eudragit L is available as an organic solution, solid, or aqueous dispersion. Eudragit S is available as an organic solution and solid.

APPLICATIONS: (15,16)

Ideal properties of Enteric coating material: Itshould release the drug in basic pH in small intestine and be resistant to gastric fluid. Susceptible/permeable to intestinal fluid. Compatible with most coating solution components and the drug substrate. Should form continuous film. Ability to be readily printed. (17,18). It will be non toxic and form a continuous film to the drug and not change due to aging. Should be of low cost.

Advantages of Enteric coating: Protect the drug from the stomach. Protect the acid liable drugs from the gastric fluid e.g. enzymes and certain antibiotics. Coatings are necessary for tablets that have an unpleasant taste, and to provide a smoother finishing. Forbid gastric distress or nausea due to irritation from a drug, e.g. sodium salicylate. Deliver drugs intended for local action in the intestines, e. g. intestinal antiseptics could be delivered to their site of action in a concentrated form.(19)

Disadvantages of Enteric Coating: Requires the expertise of highly skilled technician. This process is tedious and time-consuming.(20)

Applications of enteric coating: Reduced toxicity. To overcome the GI adverse events, an enteric-coated formulation is developed Targeting to Specific Regions of the GI Tract.(21)

Procedure for Enteric coating: Enteric coating solution is prepared by first making milky latex of eudragit-S 100 using 1M ammonia and stirring for 1hrs. The enteric coating dispersion is filtered by passing through a 0.3 mm sieve before use. The liposomes are added in the enteric coating dispersion. Throughout the coating process, the coating dispersion is stirred using a magnetic stirrer.(22)

EVALUATION PARAMETERS OF ENTERIC COATED LIPOSOMES

- Vesicle Size and Distribution:The vesicle size and size distribution (polydispersity index, PDI) are determined by dynamic light scattering method using zetasizer (Nano ZS, Malvern, UK).
- Morphological StudyThe transmission electron microscopy (TEM) is used to observe the internal morphology of liposomes. Samples are fixed using 2.5% glutaraldehyde and mounted on metal grids. Staining can be performed using uranyl acetate for one min and then the samples are rinsed by immersion in deionized water and dried with filter paper. Observations are made at high resolution (80 kV) with a electron microscope.(23)
- Entrapment Efficiency: Drug Entrapment Efficiency of liposomes is determined by centrifugation method. The liposomal suspension is subjected to centrifugation at 4,000 rpm for 20mins. The clear supernatants are removed carefully and absorbance of the supernatant is recorded. Percentage entrapment of the drug is calculated by the following formula:

$$\% \text{ drug entraptment efficiency} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug}} \times 100 \quad [24,26]$$

4. In vitro drug release in GIT fluids of different pH

In vitro drug release studies are carried out by using modified USP dissolution test apparatus. The scheme of using the simulated fluids at different pH is as follows:

- **Hour 1:** Simulated gastric fluid of pH 1.2
- **Hours 2–3:** Mixture of simulated gastric and intestinal fluid of pH 4.5
- **Hours 4–5:** Simulated intestinal fluid of pH 7.5
- **Hours 6–8:** Simulated colonic fluid of pH 7.0
- An accurately weighed amount of enteric coating.(23,25)

CONCLUSION

It is concluded from the review that liposomes can be a promising carrier for improving targeted delivery of a large number of drugs. The enteric coated liposomes overcome the drawback of plain liposomes. Entericoated liposomal formulation for avoiding the first pass metabolism, gastric irritation and degradation and to direct the drug to the target intestines. The degradation of drugs in the acidic medium is avoided by administering the enteric coated liposomal formulation.

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