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Liposome Drug delivery system: a Critic Review

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ABSTRACT:

Amongst various carrier systems, liposomes have generated a great interest because of their versatility. Liposomes not only deliver both hydrophilic and lipophilic medicaments for cancer, immunomodulation, diagnostics, antibiotics, antifungal, opthalmics, aniashamatic, vaccines, enzyme and genetic modification, but also gives wide choice of delivery like pulmonary, oral, vaginal, brain, transdermal, systemic, vaccine and antigen delivery with advantage of low cost, greater stability, purity of raw material, ease of storage. Although there are certain factors and hurdles that affect the development of liposome drug delivery system. About 40 years has been passed but still the delivery system is on R & D scale and only few countable liposome products have been reached to market. Here in this review some of these possible reasons and hurdles in the liposome drug delivery system are being discussed.

KEYWORDS: Liposome, Phospholipid, Hurdles in liposome development

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INTRODUCTION:

When phospholipids are dispersed in water, they spontaneously form closed structure with internal aqueous environment bounded by phospholipid bilayer membranes, thus forms vesicular system called as Liposomes. About 40 years ago Bangham and co-workers defined Liposomes as the small vesicle of spherical shape that can be produced from cholesterols, non toxic surfactants, sphingolipids, glycolipids, long chain fatty acids and even membrane protein.[1], which has become the versatile tool in biology, biochemistry and medicine today. In 1960s, liposome has been used as a carrier to deliver a wide variety of compounds in its aqueous compartment. They can encapsulate and effectively deliver both hydrophilic and lipophilic substances,[2, 3]and may be used as a non-toxic vehicle for insoluble drugs.[4]

Liposome can be formulated and processed to differ in size, composition, charge and lamellarity. The most important use of liposomes expected to be in biotechnology, medicine and pharmacology, where they serves as vehicles for controlling delivery of entrapped medicament viz. immunomodulator, cancer chemotherapeutics, diagnostics, antibiotics, antifungal, opthalmics, aniashamatic, vaccines, enzyme and genetic material. Till the date liposomal formulations of anti-tumor drugs and antifungal agents have been commercialized on large scale. In coming years one sees an enormous potential in liposome manufacturing as more and more industrial manufacturing methods are developed. Though there are many hurdles in their formulation and developments, which are not negligible. The source of the lipids and stability of the phospholipids, which are considered critical excipients, plays a key role in the characterization of product performance. Moreover, its clinical use has found limited application due to the remarkable barrier properties of the stratum corneum, the outermost layer of skin [5]. Further the individual delivery system of liposoms having their own limitations which are discussed in later part of the review.

LIPOSOME RAW MATERIALS [5, 6]

Various lipids and amphiphiles are available as liposome raw materials or additives that are required for the formation of lipid bilayers.

Phospholipids:

Natural Phospholipids:
Phosphotidylcholine,
Phosphotidylserine,
Phosphotidylethanolamine,
Phosphotidylinositol

Synthetic Phospholipids:
1, 2-Dilauroyl-sn-Glycero-3-Phosphocholine (DLPC);
1, 2-Dioleoyl-sn-Glycero-3-[Phospho-L-Serine] (Sodium Salt) (DOPS);
Dipalmitoylphosphotidylcholine,
Distearoylphosphotidylcholine;
Dipalmitoylphosphotidylserine,
Dipalmitoylphosphotidylglycerol;
1,2-Dilauroyl-sn-Glycero-3-Phosphocholine (DLPC)

Unsaturated:
1-Stearoyl-2-Linoleoyl-sn-Glycero-3-[Phospho-L-Serine] (Sodium Salt)**;**
Dioleaylphosphotidylcholine

Sphingolipids: Shingomyelin

Glycosphingolipids: Gangliosides

Steroids: Cholesterol

Polymeric material: Lipids conjugated to diene, methacrylate,& thiol group

Charge-inducing lipids: Dioctadecyldimethyl ammonium bromide/chloride (DODAB/C)**;** Dioleoyl trimethylammonium propane (DOTAP)

Other Substances: Stearylamine & Dicetylphosphates, Polyglycerol & polyethoxylated mono & dialkyl amphiphiles

PREPARATION OF LIPOSOMES:
There are mainly two mechanisms of vesicle formation.

a) The budding theory.

- Stress induced hydration of phospholipids.
- Organization in to lamellar arrays.
- Results in to budding of lipid bilayer leading to down sizing. (Figure 1)

b) The bilayer phospholipids theory.

- Liposomes (lipid vesicles) are formed when thin lipid films or lipid cakes are hydrated and stacks of liquid crystalline bilayers become fluid and swell.
- The hydrated lipid sheets detach during agitation and self-close to form large, multilamellar vesicles (LMV).

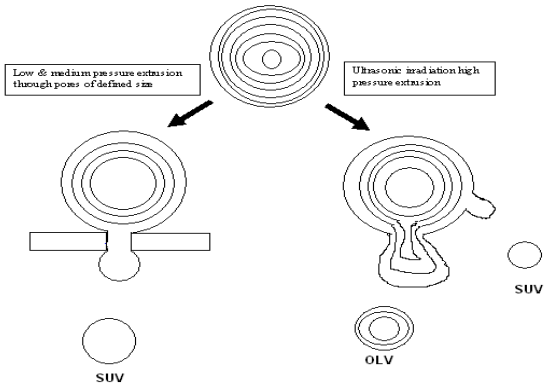

Figure 1 the budding theory

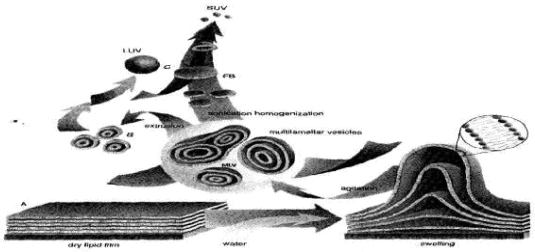

Figure 2 Mechanism of vesicle formation.

- Once these particles have formed, reducing the size of the particle requires energy input in the form of sonic energy (sonication) or mechanical energy (extrusion).(figure 2)

In the early 1980s the quality of lipids of several suppliers could vary considerably; both in quantitative and qualitative terms. Nowadays, a few suppliers provide the global market with high-quality products. Quality is ensured by improved purification schemes, the introduction of validated analytical techniques and a better insight into lipid degradation mechanisms. But still selection of proper lipid depends on few factors.

FACTORS AFFECTING SELECTION LIPID:

I. **<u>Phase transition temperature</u>:** When developing a new product, procedure, or method, controlling the transition temperature of the lipid could be useful. The phase transition temperature is defined as the temperature required to induce a change in the lipid physical state from the ordered gel phase, where the hydrocarbon chains are fully extended and closely packed, to the disordered liquid crystalline phase, where the hydrocarbon chains are randomly oriented and fluid.[7] Choosing a high transition lipid where the lipid vesicle would always be in the gel phase would provide a non-leaky packaging system. Alternatively, a lipid with a transition temperature between the starting temperature and the ending temperature of the system would provide a means of releasing packaged material as the lipid passes through its phase transition temperature and the vesicle becomes leaky. Also, one should consider how the transition temperature of the lipid could impact the

processing steps. Using a high transition lipid when filtration is necessary could present some technical problems.

II. **Charge:** The charge may provide a special function for the membrane. Several steps of the blood coagulation cascade require a lipid membrane. The assembling of protein aggregates on the surface of platelets requires a negatively charged surface. For the conversion of prothrombin to thrombin, not only does it require a negative surface, the requirement is somewhat specific, limited to phosphatidylserine (PS) and phosphatidic acid (PA).[8] Coagulation proteins bind as tightly to negatively charged surfaces containing phosphatidylglycerol and phosphatidylinositol as they do to PS or PA membranes, however, the activity is only a fraction of that obtained with PS or PA membrane. Therefore, in some systems, not only must the charge requirement be satisfied, the system specificity for a particular species must be satisfied.

III. **Lipid mixture:** In many case, a single lipid species is not sufficient to yield the exact physical properties needed for a particular system, or does not adequately mimic the natural system for which it is intended to reproduce. For these issues, consider a complex lipid mixture composed of two or more individual lipid species, the composition designed to create or reproduce a particular charge ratio, unsaturation ratio, phase transition temperature, or biological function. To reproduce the function of native brain tissue extracts, a blend of synthetic lipids (dioleoyl acyl composition) in the ratio 5:3:2 (wt %), PE: PS: PC, has been found to be satisfactory.[9] This represents the general phospholipid composition of most brain tissues.

STABILITY CONSIDERATION OF LIPOSOMES

The stability of liposomes is of major concern in their development of pharmaceutical applications, includes physical, chemical and biological stability of liposomes.

Physical stability: The aggregation of liposomes may lead to fusion. This property depends on the bilayer constituents, particle size, ionic strength of medium, encapsulated drug and temperature. One can monitor it by visual inspection, light scattering and probe fluorescence technique. To induce optimum stability Lipid composition of the bilayer & pH of aqueous solvent can be adjusted Lyopilization and Proliposomes can also improve physical stability.

Chemical stability: This mainly includes about the stability of phospholipids. Major factors affecting the stability of phospholipids include hydrolytic and peroxidation reactions. Temperature, bilayer rigidity and pH are parameters that strongly influence hydrolysis kinetic of phospholipids. Eg. For phosphatidylcholine liposomes an optimum stability was found at pH 6.5. Peroxidation reactions can be avoided by selecting lipids with only saturated bonds, storage under inner environment, addition of antioxidants and chelating agents.

Biological stability: Major events responsible for destabilization of liposomes include protein binding and membrane fusion or simply aggregation and fusion. Liposome stability in blood and plasma- Destabilization is due to interaction with plasma proteins like albumin, globulin, and lipoproteins. The pH of the blood also affects the stability of pH sensitive liposomes. Stability of liposomes in GIT- affected by-Low pH of gastric environment, Degradation by phospholipases, surfactants, and bile salts present in intestinal environment.

SHELF- LIFE

In Liposome shelf-life may be limited as it depends on the lipid species used in the formulation. This is because of two factors. First is physical instability where drug leakage from or through the bilayer and liposome aggregation or fusion. Second is chemical instability in which oxidation of unsaturated acyl groups or hydrolysis of the ester bonds.

Lipids from biological sources (e.g., egg, bovine, or soybean) typically contain significant levels of polyunsaturated fatty acids and therefore are inherently less stable than their synthetic counterparts.

Stability issues due to hydrolytic degradation are a general problem with lipid products. Aqueous formulations of drug products tend to be less stable since the presence of excess or bulk water leads to rapid hydrolytic degradation in lipid preparations. Oxidation can be prevented by excluding oxygen from the injection vial, by addition of an anti-oxidant (e.g. vitamin E) or by selection of saturated acyl-chains in the phospholipid.

Minimizing hydrolysis is possible by selecting an environmental pH of 6.5 and low temperatures. If those conditions cannot be met, (freeze) drying may be considered. Liposomes can be successfully freeze dried if the proper lyoprotectant is used and proper freeze-drying conditions are chosen. Disaccharides are excellent lyoprotectants. They prevent aggregation and fusion upon reconstituting the cake.

SCALE-UP:

Most of methods are useful for only lab-scale liposome preparation. If possible, the use of a high shear homogenizer for the production of small vesicles is a first choice. No organic solvents are required to dissolve the lipids first, nor are detergents necessary to hydrate the lipids, and there is easy access to the appropriate (commercially available) equipment.

The leakage **problem** is one of the limiting factors in commercial development of liposome products. Nevertheless, limited success was attained to improve the long-term retention of encapsulated solutes by freeze-drying which make product further costlier [11].

Further issues that are related to the parenteral administration of liposomes are the product sterility and the absence of pyrogens. The preferred way of sterilizing liposomes is by autoclaving. This is a realistic option; if the pH conditions are optimal, the drug is heat stable and lipophilic. Otherwise, reliance on filtration through membranes with 0.2

mm pores or aseptic production procedures are necessary. Standard procedures for pyrogen-free production of parenterals can be utilized.Limulus amoebocyte lysate (LAL) tests on pharmaceutical liposomes should be thoroughly validated.

Safety data:

For the present generation of pharmaceutical liposomes containing highly potent drugs with a narrow therapeutic window (e.g. cytostatics and fungicides) no safety problems directly related to the liposomes have been observed.However, changes in their side-effect profile may occur. For example, the 'hand-and-foot' syndrome (see below) observed after administration of long-circulating doxorubicin liposomes is not found after administration of free doxorubicin in standard protocols [12].

Physicochemical properties:

Liposome behavior *in vitro* and *in vivo* strongly depends on their size, bilayer rigidity, charge and morphology (i.e. unilamellar, multilamellar, multivesicul[10].Therefore, a full physicochemical characterization of pharmaceutical liposomes is required in early stages of research. In a later development stage, the outcomes of the listed quality control assays can be used to obtain regulatory approval for the liposome product. A selection can then be used to ensure batch-to-batch consistency.

Pay load:

After finishing the hydration stage of the liposome preparation process, non- liposome-associated drug is removed. Polar drugs and drugs that don't have an electric charge opposite to the (usually negatively charged) bilayer show poor encapsulation after hydrating the lipids: there 'a pay load' is problematic.

LIPOSOME CHARACTERIZATION AND CONTROL

- The quality control parameters for pharmaceutical liposomes include chemical characterization, physical characterization and biological characterization.
- Chemical characterization focuses on the quality of raw materials and prepared liposomes.
- The following chart shows the parameters to be characterized and the factors that influence the characteristics. (figure 3)

LIPOSOMAL DRUG DELIVERY SYSTEM

Liposomes have shown great potential as versatile drug delivery systems to deliver numerous bioactives. These include proteins, peptides, antineoplastic agents, antibiotics, and antiviral drugs

Oral delivery: Oral liposome drug delivery has been the subject of much suspicion. The use of liposomes as oral drug delivery system is difficult due to the poor stability of the vesicles under the physiological conditions typically found in the GI tract and binding to specific sites. Transport via

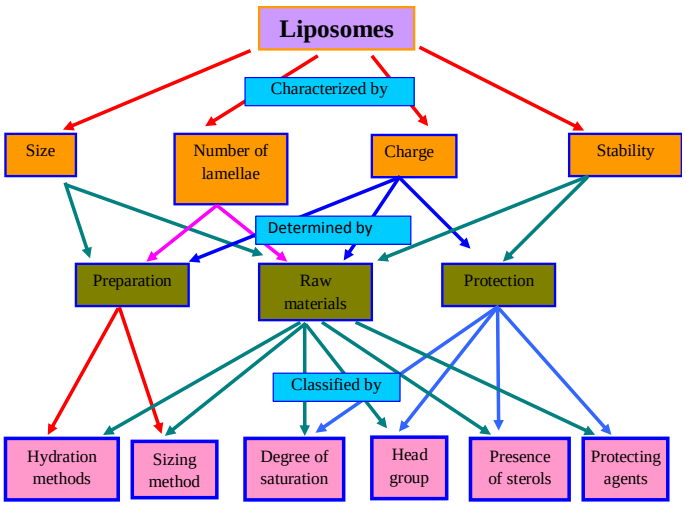

Figure3 liposome characterization and control

paracellular and transcellular routes from normal epithelial tissue or Peyer's patches leads to different outcomes of drug delivery and immunization, respectively. Nevertheless, there are manifold studies and recent publications that indicate the potential of phospholipid-based liposomes to enhance the bioavailability of poorly soluble and low-bioavailability drugs, including peptides and proteins.[13] Polymerized, microencapsulated, and polymer-coated liposomes have all increased the potential of oral liposomes.

Rogers et al [14] explains the potential of liposomes in oral drug delivery. Polymerized, microencapsulated, and polymer-coated liposomes have all increased the potential of oral liposomes. Using targeted liposomes and a greater understanding of their cellular processing will ultimately lead to effective therapies from oral liposomes.

Guo et al.[15] formulate Cyclosporin A-lecithin vesicles with an incorporation of >98%, and did a comparative study with the marketed CyA-formulation Sandimmun Neoral® in rabbits proved both formulations to be equivalent after oral administration.

Transdermal delivery

One of the most powerful approaches for transdermal drug delivery system is to encapsulate drugs in liposome to enhance delivery efficiency. This liposome-based drug delivery can essentially be applied to any drugs. Many liposomes have been formulated to deliver a variety of drugs into the body through diffusion across the skin layers. Though, application had been limited due to the barrier properties of the stratum corneum, the outermost layer of skin. The interest in designing transdermal delivery systems was relaunched after the discovery of elastic vesicles: transferosomes and ethosomes.

Ethosomes are noninvasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation. Although ethosomal systems are conceptually sophisticated, they are characterized by simplicity in their preparation, safety, and efficacy a combination that can highly expand their

application. Ethosomes are soft, malleable vesicles tailored for enhanced delivery of active agents Because of their unique structure, ethosomes are able to encapsulate and deliver through the skin highly lipophilic molecules such as cannabinoids, testosterone, and minoxidil, as well as cationic drugs such as propranolol and trihexyphenidil. Ethosomes are provides a number of important benefits including improving the drug's efficacy, enhancing patient compliance and comfort and reducing the total cost of treatment.[16]

In 1992 Ceve and coworkers developed vesicles composed of phospholipids as their main ingredient with 10-25% surfactant (sodium cholate) and 3-10% ethanol with high penetration efficiency because of deformability of those vesicles known as transfersomes. The surfactant molecules act as "edge activators", conferring ultradeformability on the transfersomes, which reportedly allows them to squeeze through channels in the stratum corneum that are less than one-tenth the diameter of the transfersome. where liposomes are too large to pass through pores of less than 50 nm in size, transfersomes up to 500 nm can squeeze through to penetrate the stratum corneum barrier spontaneously.

Systemic delivery

Intramuscular and subcutaneous Injection of a drug at an extravascular site leads depot formation to initiate a number of events that collectively make up the absorption process. **Jain** *et al* [17] prepared a multivesicular liposomal (MVL) drug delivery system comprising of Acyclovir Sodium, which forms a depot on intramuscular injection. The MVL provides control release of acyclovir for prolong period of timeand is advantagenious over oral route as the absorption is dose dependent and highly variable with a bioavailability ranging from 10% to 30%.

Liu *et al* [18] prepared Cationic liposomes to introduce gene into cells using a transfection reagent. The transfection efficiency of cationic liposomes depended upon the cationic lipid to DNA ratio, structure of cationic lipids, liposome composition, and particle size of lipid particles. The major advantage of systemic gene delivery over local injection is that many more sites and a greater number of cells in the body can be targeted. Thus, the successful development of lipid-based carriers for systemic transfection has a great potential for increasing the overall usefulness of lipid carriers in gene therapy.

Vaginal delivery

Kang *et al* [19] present a dispersion of cationic liposomes in a thermosensitive vaginal gel delivery system of amphotericin B. Amphotericin B (AmB) is used in the treatment of fungal infections; however, its clinical use is limited by its toxic side effects. AmB-loaded cationic liposome gels were formulated with 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), and cholesterol (CH) at a molar ratio of DOPE:DOTAP:CH = 4:5:1 in thermosensitive gel composed of poloxamer 407 (P407) and poloxamer 188 (P188). The thermosensitive gel at the ratio of P407:P188 = 15:15 (w/w) gelled at 37°C, approximating body temperature.

Pulmonary delivery

Targeted drug delivery to the lungs has evolved to be one of the most widely investigated systemic or local drug delivery approaches. This route also makes it possible to deposit drugs more site-specific at high concentrations within the diseased lung thereby reducing the overall amount of drug given to patients as well as increasing local drug activity while reducing systemic side effects and first-pass metabolism.

Lu *et al* [20] develop liposomal dry powder aerosols for protein delivery. In this study β-Glucuronidase (GUS) was used as a model protein to evaluate dry powder liposomes as inhaled delivery vehicles. Dimyristoyl phosphatylcholine:cholesterol (7:3) was selected as the liposome composition. The lyophilization of liposomes, micronization of the powders, aerosolization using a dry powder inhaler (DPI), and in vitro aerodynamic fine particle fraction upon collection in a twin-stage liquid impinger were evaluated. The results demonstrate that milled liposome powders containing protein molecules can be aerosolized effectively at a fixed flow rate

Brain delivery

The delivery of therapeutic agents into the parenchyma of the brain is problematic because it has been virtually impossible to know with any certainty where infused material is going, and how much to infuse. Krauze *et al* [21] use liposomes loaded with Gadoteridol (GDL) as a tracer that allows us to follow infusions in real-time on magnetic resonance imaging (MRI). MRI allows precise tracking and measurement of liposomes loaded with markers and therapeutics. The review provides an overview of real-time delivery of liposomes to the central nervous system (CNS), and discusses the technical aspects of delivery, liposomes as colloidal systems of delivery, real-time distribution of liposomes in CNS, and quantification of liposome distribution. This data suggests that real-time monitoring of liposomal drug infusion is likely to improve outcomes of clinical trials where convection-enhanced delivery (CED) is being used to target drugs to specific brain structures through limitation of systemic toxicity and reduction of side effects.

Chen *et al* [22] developed a new drug carrier for brain delivery, lactoferrin-modified procationic liposome, and evaluated in vitro and in vivo. The procationic liposomes (PCLs) were neutral or negatively charged at physiological pH, and when they touched brain capillary endothelial cells with the help of a brain-targeting ligand, lactoferrin (Lf), they were changed into cationic liposomes (CL). The primary brain capillary endothelial cells (BCECs) were cultured to investigate the potential cytotoxicity and uptake of liposomes in vitro. An in vitro model of the blood-brain barrier (BBB), developed by the co-culture of BCECs and astrocytes (ACs), was employed to evaluate the ability and mechanisms of liposomes to cross endothelial cells. The liposome uptake by the mouse brain in vivo was detected by HPLC-fluorescence analysis. The results

indicated that compared with the conventional liposomes and CLs, PCL and Lf-PCLs showed an improved performance in the uptake efficiency and cytotoxicity.

Vaccine and antigen delivery

Oral vaccination requires an antigen delivery vehicle to protect the antigen and to enhance translocation of the antigen to the mucosa-associated lymphoid tissue. Liposomes have been widely exploited as antigen delivery systems for a variety of diseases

Nonionic surfactant vesicles formed from the self assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer. Shilpa *et* al [23] describes the protein or vaccine loaded niosomes using various combination of lipids, Surfactants, and solvents for Pulmonary delivery, Oral delivery, Vaginal delivery, Brain delivery, Transdermal delivery, Systemic delivery, Vaccine and antigen delivery with advantage of Low cost, Greater stability, Purity of raw material, Ease of storage.

Payne *et* at [24] reduces the problem associated with aqueous liposome dispersions by preparation of a dry product which is hydrated immediately before use, called as 'Proloposomes'. A dry, free-flowing granular product of amphotericine B was prepared which, on addition of water, disperses/dissolves to form an isotonic multilamellar liposomal suspension suitable for administration either intravenously or by other routes, resulted in a decrease in drug associated toxic side effects and often in an increase in efficacy and shelf life.

OTHER HURDLES IN DEVELOPMENT

Hurdles for in vivo application of virosomes [25] as for recombinant viruses, a possible drawback for the application of virosomes for in vivo delivery of nucleic acids is their immunogenicity. Depending on the route of administration, virosomes are expected to induce the generation of HA-specific and Neuraminidase-specific antibodies, which could interfere with their efficacy upon repeated administrations. Moreover, the presence of antibodies induced by prior influenza infections or vaccination against influenza may hamper successful application of virosomes.

Regulatory science of liposome drug products

A safety concern in liposome formulation has draw serious attention in scientists**.** Lipid toxicity for blood component particularly RBC lysis, Presence of protein and lipoprotein for natural lipids, Residual solvent, Overload of RES, critical particle size as tail above 1 um may block capillaries also size affects RES uptake and tissue targeting, Stability problems during shelf-live and *in vivo*, Dose dumping via protein binding, sterility of final dosage form.

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