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# An Updated Review On: Liposomal Drug Delivery System

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***Abstract – Liposomes are one of the highly placed acquisitions of the pharmaceutical industry and also help multiple delivery system target a drug to a significant tissue. Owing to the similarity in structure of both the cell membrane and the lipid bilayer, liposomes are easily unfused in the drug delivery to prevent penetration of a free drug. Liposomes further have the ability to encapsulate in hydrophobic and hydrophilic materials, which is then used in drug delivery carriers. Such a technology is highly useful to treat certain diseases. As of now, huge traction is driven towards using Liposomes. The crucial object of the said technology's review is the fact that they are effective in treating certain diseases, can be easily prepared and has more benefits as compared to others. Liposomes as seen are biocompatible, with several applications starting from delivering antiviral, antibacterial drugs, enzymes, fungicides, antiparasite drugs, diagnostic tools, transdermal transporters, and adjuvant for varied vaccines. The given paper is seen to focus mainly on scalable techniques and strength, with limitations in regards to its application in industry, and the regulatory requirements relating to the drug formations liposomal on the basis of EMEA and FDA documents.***

***Keywords: Drug Delivery System, Liposomes, Components of Liposome, Phospholipids***

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## I. INTRODUCTION

Advancement in the field of combinatorial chemistry opened doors for an array of NICE or new chemical entities which has a considerable therapeutic action on the system of biology. However, majority of the discovered NCEs laid a challenge to the scientists of formulation owing to the properties that are physicochemical such as poor permeability and solubility. Although, the said problems are addressed, yet majority of the molecules are seen lacking in the reflection of potential therapeutic action in the vivo that leads to absence of correlation between in vitro and in vivo [1].

Most of the anti-neoplastic agents that seen to be cytotoxic to the cells of tumor in vitro, lay an impact on the normal cells too. The above is because of poor TI or the therapeutic index meaning that the minimal dose to infuse anti-tumor effect show toxicity towards normal cells. These kinds of drugs are targeted to a particular diseased site which helps lower the effects toxic to normal tissues [2]. So, the presence of an efficient system for drug delivery is imperative as it shows the administered sites maximum fraction on the site targeted. Several carriers such as micro-particles, nanoparticles, lectins, polysaccharides, and liposomes have been used for targeting the said drug to particular site [3].

Liposome comprise of vesicles having either bilayers or multilayers which further has cholesterol and phospholipids surrounding the aqueous compartment. Drug gets encapsulated in the liposome which then gets released to be absorbed at the surface of intestinal membrane. Such form of dosage attains considerable and then relate to their ability of enhancing absorption, their usage feasibility for promotion of drug absorption is them chemical entity or uncertain drugs. Advancement in the field of combinatorial chemistry opened doors for an array of NICE or new chemical entities which has a considerable therapeutic action on the system of biology. However, majority of the discovered NCEs laid a challenge to the scientists of formulation owing to the properties that are physicochemical such as poor permeability and solubility. Although, the said problems are addressed, yet majority of the molecules are seen lacking in the reflection of potential therapeutic action in the vivo, which leads to absence of correlation between in vitro and in vivo. Most of the anti-neoplastic agents that seen to be cytotoxic to

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Several carriers such as micro-particles, nanoparticles, lectins, polysaccharides, and liposomes have been used for targeting the said drug to particular site. The interest received by the drug delivery system of liposomes are due to the fusion of the same of different areas such as delivery of drug, cosmetic and the biological membrane structure [5]. Liposomes are the carrier for multiple drugs, and have a tremendous therapeutic action potential. Also referred to as the colloidal carriers, Liposomes have size ranging between 0.01 – 5.0 µm in diameter. Though the above are bilayered vesicles formed on excessive hydration of phospholipids in aqueous medium [6]. They have the advantage of hydrophobic and hydrophilic encapsulation of drugs and target them to a specific site diseased in ones [7]. The below given figure shows the liposome structure (bilayered vesicle) and the phospholipid.

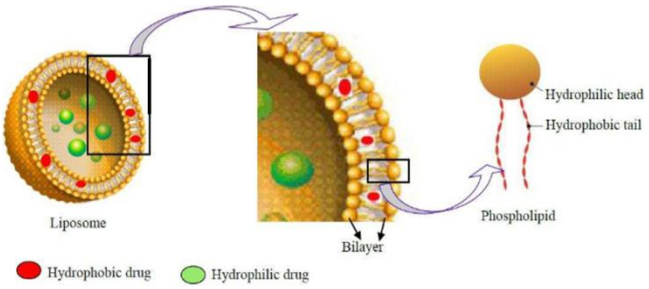


**Figure 1: Liposome and phospholipid structure**

Several therapeutic agents such as vaccines, anticancer drugs, genetic items, antimicrobials, proteins, macromolecules etc are capable of encapsulation in the bilayered vesicles [8]. The technology of Liposome has been used to encapsulate several molecules of drugs successfully. They include paclitaxel, tropicamaide, acyclovir, arteether, cyclosporine, chloroquine diphosphate, and dithranol [9]. The below given table shows the list of liposomal products approved for human usage.

**Table 1: Liposomal products list for commercial use**

| Drug | Product | Indication |
|---|---|---|
| Ambisome™ | Amphoteracin B | Fungal infection |
| DaounoXome™ | Daunorubicin | Kaposi's sarcoma |
| Doxil™ | Doxorubicin | Refractory Kaposi's sarcoma, recurrent breast cancer and ovarian cancer |
| Visudyne® | Verteporfin | Age-related macular degeneration, pathologic myopia and ocular histoplasmosis |
| Myocet® | Doxorubicin | Recurrent breast cancer |
| DepoCyt® | Cytarabine | Neoplastic meningitis and lymphomatous meningitis |
| Lipoplatin® | Cisplatin | Epithelial malignancies |
| DepoDur® | Morphine sulfate | Postoperative pain following major surgery |

## II. MECHANISM OF LIPOSOME FORMATION

The important and the most part of the liposomes are made by phospholipids, those amphiphilic molecules (ones that have both a hydrophobic tail and a hydrophilic head). It is important to have the hydrophilic part since the phosphoric acid bounds to molecules that are water soluble, and, the importance of hydrophobic part lies in the two chains of fatty acid having a tools of 10-24 carbon atoms and each chain has 0-6 double bonds [10].

On dissemination of such phospholipids or on dispersion in any aqueous medium, it leads to the formation of lamellar sheets when organized in a manner where the group of polar head is outward to the region of aqueous and the group of fatty acids is facing each other. They then form a structure similar to spherical or vesicle and so liposomes. The remains or the residual part of polar portion stays in touch or in contact with the aqueous region and is kept safe or shielded from the part non polar.

On water hydration of phospholipids with added energy input such as shaking, Sonication, homogenization, heating, etc. There occur interactions hydrophilic or hydrophobic between lipid water and lipid–lipid, molecules which then accounts for the bilayer vesicles formation so as to attain a form of thermodynamic equilibrium in watery phase. The prime reason behind formation of bilayer includes:

- The creation of unfavorable interactions between hydrophobic and hydrophilic are to be minimized by folding them to vesicles that are closed concentric.
- Formation of huge bilayered vesicles promotes the lowering of the energy differences between the hydrophobic and the hydrophilic environment.
- Highest stability to the self-assembled structure of supra molecular is attained by vesicles formation.

## III. CLASSIFICATION OF LIPOSOMES

Several liposomal classes are reported in literature. Classification is done one the basis of size, composition, bilayers numbers, and preparatory methods. On the basis of bilayer number and size, liposomes are regarded as multi lamellar vesicles (MLV), small unilamellar vesicles (SUV), and large unilamellar vesicles (LUV) and are shown below. On the basis of composition, liposomes are pH sensitive, conventional liposomes (CL), cationic liposomes, immuno-liposome and long circulating liposomes (LCL). On the basis of preparatory methods, liposomes are classified as French press vesicles (FPV), reverse phase evaporation vesicles (REV), and EIV (ether injection vesicles). Here, we describe the classification on the basis of bilayer number and size.

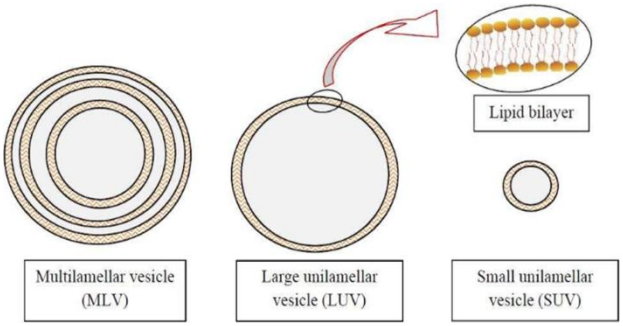


**Figure 2: Liposomal classification on the basis of size and bilayer number**

### *A. Multilamellar vesicles (MLV)*

MLV are of size more than 0.1 µm with more than two bilayers. Their preparatory method is simple, and consist of a thin – film method of hydration or lipid hydration in excess organic solvent. These are stabilized mechanically on huge storage. Owing to the greater size, they get rapidly cleared by the system cells of reticulo-endithelial and so are used to target the RES organs [11]. MLV is seen to have a considerable trapped volume, which the ratio of aqueous volume amount to lipid. The entrapment of drug into vesicles is improved by the lesser hydration rate and mixing gently. Thin film hydration of dry lipids further improves the efficiency of encapsulation [12]. Subsequent rehydration and lyophilization post fusing it with aqueous phase lead to an encapsulation efficiency of MLV of around 40%.

### *B. Large unilamellar vesicles (LUV)*

This liposomes class having a single bilayer is of size more than 0.1 µm. These are found to have greater efficiency of encapsulation as they can store greater solution volume in one's cavity. Also, their trapped volume is higher and used to encapsulate hydrophilic drugs. LUV benefit is the lesser lipid amount needed for drug encapsulation in large amounts. Much like MLV, these get cleared rapidly by the cells of RES because of large size [13]. Several methods have been used for preparation of LUV as detergent dialysis, ether injection, and evaporation techniques in reverse phase. Added to the above methods, liposomes freeze-thawing [14], SUVs dehydration or rehydration [15] and lipids slow swelling in a solution non-electrolyte are used for LUV preparation.

### *C. Small unilamellar vesicles (SUV)*

The SUV are smaller somewhere less than 0.1 µm in comparison to the LUV and MLV with single bilayer. The entrapped volume to lipid ratio is low and gets defined by greater circulation half-life. The preparation of SUV is done by the method of solvent injection (ether or ethanol) [16] or by size reduction of LUV OR MLV through extrusion or sonication process under the presence of Argon or nitrogen as inert atmosphere. The sonication is done using either a probe type or bath sonicator. SUV is also attained by MLV passing across the narrow orifice and exposed to greater pressure. Such SUV are aggregation susceptible with lower charge fusion [17].

## IV. METHODS OF PREPARATION

Liposomes preparation conventional methods are lipid solubilization in any organic solvent, drying lipids from the organic solution, lipid dispersion in the aqueous medium, resultant Liposomes purification and final product analysis [18].

Of the said preparatory methods, the simplest and the vividly used one is the method of thin-film hydration. Produced MLV has a size between 1 – 5 µm. In case the drug turns to be hydrophilic, the same is added to the aqueous buffer and if it is hydrophobic, it is added to lipid film. However, the methods drawback is poor efficiency of encapsulation (5 – 15% only) with respect to the hydrophobic drugs. By lipid hydration in organic solvents, MLVs encapsulation efficiency leverages [19]. Preparation of LUV is done through detergent analysis, ether injection, and evaporation techniques in reverse phase. The preparation of SUV is done by the method of solvent injection (ether or ethanol) [20] or by size reduction of LUV OR MLV through extrusion or sonication process under the presence of Argon or nitrogen as inert atmosphere. Such methods involves agents that are toxic and so to avoid them, bubble method, polyol dilution, and heating method are used that do not have any detergent or solvent. Liposomes preparation detailed can be found from literature [21].

## V. CHARATERIZATION OF LIPOSOMES

The production of Liposomes done using varied methods has a range of physicochemical characteristics that accounts for differences in performances in vivo and in vitro [22]. Precise,

rapid, and reproducible control quality tests are used to characterize the liposomes post formulation and then after being stored with respect to the behavior either in vivo or in vitro of products of liposomal drug [23]. A product of liposomal drug gets characterized for few discussed parameters.

### *A. Size and size distribution*

At the time of intending the liposomes for parenteral administration or inhalation, the distribution size has primary consideration, as it lays an influence on the liposomes fate of vivo with the molecules of the encapsulated drug [24]. Several techniques as used for determination of the vesicles size include microscopy (negative stain transmission electron microscopy, optical microscopy, cryo-transmission electron microscopy, scanning electron macroscopy and freeze fracture electron microscopy, scattering and diffraction techniques (photon correlation spectroscopy and laser light scattering) and the hydrodynamic techniques.

### *B. Percent drug encapsulation*

The total quantity of the entrapped or the encapsulated drug in the vesicles of liposome is noted as the percent drug encapsulation. The liposomes drug encapsulation percent has been estimated by the method of column chromatography [25]. Such a formulation comprise of encapsulated and free drug. Hence to know the idea quantity of the encapsulated drug, we need to separate the encapsulated one from the free drug. Then the liposome fraction having encapsulated drug gets treated on exposure to a detergent, for attaining lysis that accounts for drug discharge in the surrounding from the vesicles. Such an exposed drug is then assayed using a suitable technique that lays down the encapsulated percent drug and now the calculation done for encapsulation efficiency [26].

The volume trapped per lipid weight is also used to find the liposome vesicles percent drug encapsulated. This is primarily expressed as the entrapped aqueous volume per lipid per unit quantity, µl/µml and/pr µg/mg [27]. For determination of the volume trapped, several materials such as the fluorescent markets, radioactive markers, and the spectroscopically inert fluid are used. The mostly used method for trapped volume determination is Radioactive. This is found by lipid dispersal in the aqueous medium having a radioactive solute that is non-permeable such as [22Na] and [14C] inulin. On the other hand, the markets that are water soluble such as 6-carboxyfluorescein, sucrose 3-H or 14C-glucose are used for trapped volume determination.

### *C. Surface charge*

As the liposomal charge appearing on the surface is vital when disposing the vivo, it becomes important to know the vesicles surface charge. There are two methods as zeta potential measurement and free-flow electrophoresis that helps estimate the vesicles surface charge. Then calculation of surface charge is done by mobility estimation of the dispersion of liposome in the buffer appropriate (determined with the help of Helmholtz–Smolochowski equation).

### *D. Vesicle shape and lamellarity*

Several techniques of electron microscopic have been used for assessment of the vesicles shape. The bilayer number in liposomes, As., lamellarity is determined using the method of microscopy of freeze-fracture electron and the analysis of 31P-Nuclear magnetic resonance. Along with the knowledge of Lamellarity and shape, the liposomes surface morphology are assessed using freeze-etch amf freeze-fracture electron microscopy [28].

### *E. Phospholipid identification and assay*

The liposomal chemical components need to be analysed both before and after preparation [29]. Stewart Assay Barlett assay and Chromatography of thin layer are used for estimation of the liposomal formula's phospholipid concentration. The method of spectrophotometry has been used for quantification of total phosphorous in the literary sample that will measure the developed blue color intensity against water at 825 nm. Ferric Perchlorate or Cholesterol oxidase assay method and the techniques of Gas liquid chromatography are used for cholesterol concentrate determination [30].

## VI. STABILITY OF LIPOSOMES

At the time of development of products of liposomal drug, the developed formation stability holds consideration. The drug's therapeutic activity is governed by the liposomla stability right from the steps of manufacturing, then to storage and finally delivery. Forms of stable dosage are one that keeps up the chemical integrity and physical stability of the molecule active at the time of procedures development and storage. The stability study well designed has evaluation of the parameters chemical, physical, and microbial with product integrity assurance across the storage period. So, there is need of a stability protocol for studying the chemical and physical integrity of the product of drug in storage.

### *A. Physical stability*

Liposomes as the bilayered vesicles are formed on water hydration of phospholipids. These vesicles are obtained in different sizes. When storing, these aggregate and also increase in their size so as to reach the state of thermodynamic. At the time of storing, leakage of drug from vesicles might occur because of vesicles break or fusion, deteriorating

the liposomal drug physical stability. So morphology, distribution size and size of the vesicles are parameters important when the physical stability is assessed. To monitor the above, several techniques such as electron microscopy and light scattering [31] are used for estimating the vesicles size and visual appearance (morphology).

### *B. Chemical stability*

Phospholipids also referred to as the fatty acids that are chemically unsaturated are prone to hydrolysis and oxidation that have an impact on the drug product stability. In addition to the above, ionic strength, pH, buffered species, solvent system play a crucial role when maintaining the formulation of liposomes. Indeed several chemical reactions are induced even by oxygen, light, heavy metal ion, and temperature. The Oxidation deterioration consists of cyclic peroxide formation along with the hydroxyperoxidases owing to the radical generation in process of oxidation. Liposomes are prevented from degrading oxidative through light protection, by addition of anti-oxidants like alpha – tocopherol or the butylated hydroxyl toluene (BHT), with the product produced in the inert environment [32].

Ester bond Hydrolysis of the phospholipid glycerol moiety at carbon position account for lyso-phosphatidylcholine (lysoPC) formation, and this can enhances the liposomal permeability. So, it is important to control the lysoPC limit in the product of liposomal drug. The above can be attained by liposomal formulation with phosphatidylcholine and are lysoPC free [33].

## VII. IN VIVO BEHAVIOR OF LIPOSOMES

At the time of liposomal formulation optimization, several physico-chemical elements get altered for attaining the drugs cellular uptake and desired bio-distribution. Such parameters that lay an impact on performance of in vivo (biological) are given below [34].

### *A. Liposome size*

The vesicles size determines the liposomal vivo fate as; it finds the RES cleared fraction [35]. The liposomal uptake rate by RES is seen to leverage the size of vesicle. Liposomes having size more than 0.1 µm are uprorted easily and quickly by RES as compared to ones with size less than 0.1 µm.

The vesicles size further determines the liposomal extravasationl. The capillaries of tumor are far more permeable in comparison to the normal capillaries. Owing to the leaky vasculature, small size liposomes and the fluids easily pass across the gaps thereby leading to accumulation of liposomes loaded with drug in tumor tissue. The interstitial pressure and the intravascular hydrostatic difference are the forces that drive small size liposomes extravasation [36].

### *B. Surface charge*

The interaction between lipid – cell are governed by the charge density and the nature of liposome surface. The lipid composition charging alters the liposomal charge and nature. Absence of charge in the liposome of SUV account for aggregation which then reduces the liposomal stability whereas, the cell interaction with the liposomes neutrally charged are negligible [37]. The liposomal surface high electrostatic charge render results useful to promote the interaction between lipids – cell. The density that is negatively charged has an impact on the lipid – cell interactions which further increase the liposomal intracellular uptake by targeted cells [38]. However, liposomes that are positively charged are rapidly cleared post systemic administration.

### *C. Surface hydration*

Liposomes having surface coatings that are hydrophilic have less possibility of opsonization, so the uptake is reduced by cells of RES. The above is attributed to the surface coating that is hydrophilic and lowers the liposomal interaction with blood and cell components. Such liposomes that are sterically stabilized are more like to be stable in the environment of biology further exhibiting the half-lives of high circulation in comparison to the hydrophobic coated liposomes. The hydrogenated phospotidyl Inositol, Monogangliosides, polyethylene glycol are few hydrophilic groups held responsible for liposomes steric stabilization. Cholestrol incorporation in bilayer can reduce the fluidity of membrane at temperature higher than that of phase transition, stabilizing liposomes.

## VIII. THERAPEUTIC APPLICATIONS OF LIPOSOMES

When the form of conventional dosage cannot lay the expected therapeutic effect, development of a newer system of drug delivery occurs. One such system is Liposomes and provide greater therapeutic efficacy along with safety as compared to formulation that exist. Few of these are:

### *A. Site-avoidance delivery*

The anti-cancer drugs cytotoxicity to the normal tissues gets attributed to the TI narrowed. Given these circumstances, TI is improved by reducing the drug delivery to normal cells by liposomal encapsulation. Free doxorubicin is seen to have fatal side effect related to cardiac toxicity, however on formulation with liposomes, there occurs reduction in toxicity without therapeutic activity alterations [39].

### *B. Site specific targeting*

Delivering drugs in larger fraction to site desired (diseased), by lowering the exposure of drug to the tissues normal are attained by targeting specific site. Liposomal drug encapsulation is used for passive and active drug targeting to infuse therapies that are safer and efficacious. On the basis of systemic administration, huge immunoliposomes circulating can bind and recognize target cells with higher specificity. For patients having recurrent osteosarcoma, monoctyes enhanced tumoricidal activity occurs, when derivatives of muramyl peptide are made as liposomes and systematically administered.

### *C. Intracellular drug delivery*

Increased potent drug delivery to cytosol is accomplished using the delivery system of liposomal drug. The compound taken up in cel normally is N-(phosphonacetyl)-L-aspartate (PALA). These drugs at the time of liposomal encapsulation reveal higher activity against the tumor cells ovarian as compared to the drug free.

### *D. Sustained release drug delivery*

Liposomes are used to provide drugs sustained release that needs the concentration of prolonged plasma at levels of therapeutic for attaining the efficacy of optimum therapeutic. Drugs such as cytosine Arabinoside has been encapsulated for the optimized release rate and sustained release in vivo.

### *E. Intraperitoneal administration*

Tumors which are seen to develop in the cavity of intra-peritoneal (i.p.) are treated by drug administration. However, the drug rapid clearance from the cavity of i.p. leads to lowered drugs concentration at the site of diseased. But, liposomal drugs that are encapsulated attain lower rate of clearance, in comparison to the free drug that provides highest drug fraction in a manner prolonged to the site of target.

### *F. Immunological adjuvants in vaccines*

Immune responses are seen to be enhanced by antigens delivered and liposomes encapsulated. On the basis of antigens lipophilicity, the liposomes are seen to accommodate antigens in cavity that are aqueous or bilayers incorporation. For the enhancement of the response of immune to the diphtheria toxoid, the first adjuvants used are liposomes [40].

## IX. CONCLUSION

Variety of candidate of drug being highly potent and having lower indication of therapeutics is targeted to the site of the required diseased taking help of the delivery system of liposomal drug. Liposomes encapsulated drugs show significant alterations in pharmacokinetics. The liposomal formulation efficacy relies on the ability of drug molecule delivery at the site targeted over huge time and the time of reducing the effects toxic. Encapsulation of drugs in the bilayers of phospholipid is expected of diffusing out slowly. Several factors such as drug to lipid ratio, drug concentration, efficiency of encapsulation and release of vivo drug has to be considered when formulating of delivery system of liposomal drug. The deformable liposomes development along with of ethosomes under the liposomal administration of loaded drug via inhalation and the ocular route are little technological advancement. So the liposomal approach can be utilized successfully for betterment of pharmacokinetics and efficacy of therapeutic, simultaneously lowering the potential drugs toxicity.

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