LIPOSOME: A CARRIER FOR EFFECTIVE DRUG DELIVERY
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ABSTRACT
Liposomes are the spherical vesicles containing one or more phospholipid bilayer, which was first described in the middle of 60s by Bangham. The bilayer vesicles are considered as an efficient carrier for drug delivery, diagnostic agents, and also an effective tool for vaccine delivery. Liposome has been used as a potential carrier for several diseases from cardiovascular disease to bacterial infection and also it has the ability to reducing the toxicity of highly potent drugs and simultaneously utilized to improve pharmacokinetics and therapeutic efficacy. A liposome is a formulation which has the capacity to overcome with the limitation of conventional therapies. For the delivery of liposome ocular and inhalation route are some advanced technology. In poorly water soluble substance pulmonary delivery is very much useful. However liposome based vaccines have been demonstrated in clinical trials and further progress in human trails. This review discusses the mechanism of action, Method of preparation, evaluation, application of liposomal drug delivery system along with the recent developments some of the commercially available products.

INTRODUCTION
English hematologist Alec Bangham in 1961, first being described artificial lipid vesicles (also called liposomes). It has been recognized and extensively used as delivery vehicles for pharmaceuticals, as chemical microreactors, and as model biomembrane systems [1]. Liposome can be processed and formulated to differ in composition, charge, size and lamellarity [2]. Further liposome may be described as concentric bilayered vesicles within which associate degree binary compound core is entirely encapsulated by a membranous lipid bilayer principally composed of natural or artificial lipid. The size of a vesicle ranges from some 20 nm up to many micrometers. The lipid molecules square measure sometimes lipid amphipathic moieties with a hydrophobic tail. On the addition of excess water, such lipid moieties spontaneously originate to offer the most thermodynamically stable conformation. Where, polar head teams face outwards into the binary compound medium and therefore the lipid chains turns inward to avoid the water section, giving rise to double layer or atomic number 83 layer lamellar structures.

Structural components of liposomes-[3, 4, 5]
• The main components of liposomes are-
  1. Phospholipids
  2. Cholesterol

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Phospholipid

Phosphatidylcholine- The most common phospholipids used is phosphatidylcholine (PC), an amphipathic molecule, which consists of a hydrophilic polar head group, phosphocholine, a glycerol bridge and a pair of hydrophobic acyl hydrocarbon chains.

Cholesterol - Cholesterol is a waxy, fat-like substance is an essential component of our body. It helps to produce hormones, vitamin D, and substances that help to digest foods. Cholesterol itself does not form a bilayer structure. It acts as fluidity buffer. It interacts with phospholipid molecules and alters the mobility of carbon molecules in the acyl chain and Restricts the transformations of Trans to gauche conformations. Cholesterol incorporation increases the separation between choline head group & eliminates normal electrostatic & hydrogen bonding interactions.

Mechanism of liposome formation-
Liposome is composed of phospholipids. The hydrophilic part is mainly phosphoric acid bound to a water-soluble molecule, whereas the hydrophobic part consists of two fatty acid chains with carbon atoms 10-24 and double bonds 0-6 in each chain. An aqueous environment the phospholipid orient themselves to form bilayer where one layer of the phospholipids faces outside of the cells whereas another layer of the phospholipid faces inside the cell to avoid the water phase. The hydrocarbon tail of one layer faces the hydrocarbon tail of another layer and combines to form bilayer this structure is also called lamella. Upon further hydration, the lipid cake (lamella) swells eventually that curves to form closed vesicles in the form of spheres known as a liposome. [6]

Table 1: Advantages &disadvantages of liposome- [7, 8, 9]

| Advantages                                      | Disadvantages                                      |
|------------------------------------------------|---------------------------------------------------|
| Liposome increased efficacy and therapeutic index of drug | Sometimes phospholipid undergoes oxidation and hydrolysis like reaction |
| Liposome is non-toxic, flexible, biocompatible, completely biodegradable and non-immunogenic for systemic and non-systemic administration | Drug leakage/ entrapment/ drug fusion |
| Liposome increase stability via encapsulated drug. | Biological activity is short / t 1/2 |
| Liposome reduced the toxicity of the encapsulated agent. | Low solubility and oxidation off bilayer phospholipid |
| Liposome reduce the exposure of sensitive tissue to toxic drugs | Rate of release and altered biodistribution |
| Improved pharmacokinetic effects (reduced elimination increased circulation lifetimes) | Low therapeutic index and dose effectiveness |
| Suitable for controlled release                   | Repeated IV administration problems |

Classification- [7, 10]
1. Based on structural parameters
   a. MLV: Multi lamellar vesicle (0.5 μm)
b. OLV: Oligolamellar vesicle (0.1-1 μm)
c. UV: Unimamellar vesicle (All size range)
d. SUV: Small unimamellar vesicle (30-70 nm)
e. MUV: Medium sized unimamellar vesicle
f. LUV: Large unimamellar vesicle (> 100 μm)
g. GUV: Giant unimamellar vesicle (> 1 μm)

2. Based on the method of preparation
   a. REV: Reverse phase evaporation vesicles
   b. MLV-REV: Multi lamellar vesicle by REV
   c. DRV: Dehydration - rehydration method
   d. VET: Vesicle prepared by extraction method
   e. SPLV: Stable plurilamellar vesicles
   f. FATMLV: Frozen and thawed MLV

3. Based on composition of application
   a. Conventional liposome
   b. Fusogenic liposomes
   c. pH sensitive liposomes
   d. Cationic liposomes
   e. Long circulatory liposome
   f. Immuno liposomes

Method of preparation- [8, 11, 12, 13, 14]

Hand shaking MLVs
It is the most common and simple method used for the preparation of MLVs. In these processes, the lipids are dissolved in solvents (chloroform: methanol) which are then transfer to a round bottom flask. The RBF containing the mixture is then attached to rotary evaporator and then it was rotated at 60 rpm until a dry thin layer is formed after that it is dried in lyophilizer to remove the last traces of solvent and it is hydrated with phosphate buffer saline containing the material to be entrapped and then it was attached to rotary evaporator at 60 rpm or below it was rotated until the layer adhering on the wall of the RBF is removed and it was kept stand at room temperature for 2 h upon hydration milky white disperse appear.

Non-hand shaking LUVs-
In these methods lipid mixed with solvent is spread over the conical flask and the solution is evaporated at room temperature without disturbing by the flow of nitrogen. After the solution gets dried it is hydrated by water-saturated nitrogen which is passed through the conical flask until the opacity of dried lipid film disappears. After hydration, the lipid gets swelled. Then the flask is inclined to one side and 10 to 20 ml of 0.2 M sucrose in distilled water is added to the side of the flask and then the flask is slowly returned to its original position. The fluid gently runs over the lipid layer on the bottom of the flask. Then the flask is flushed with nitrogen and sealed it was then allowed to stand for 2 h at room temperature. After swelling the suspension is centrifuged at 12000 g for 10 min at room temperature. The remaining fluid add iso-osmolar glucose solution then LUVs formed

Freeze drying method -
In these method lipid and solvent are mixed and evaporate at room temperature by flow of nitrogen for drying. Then add some water saturated nitrogen until opacity disappears. Add water fluid and 10-20 ml of 0.2 M sucrose solution to swell. After that stand for 2 hour at 37 c then centrifuged at 12000 rpm for 10 min at room temperature and reaming fluid are add to iso-osmolar glucose solution to formed LUVs.

Membrane extrusion-

In this technique vesicles contents are exchanged with dispersion medium during breaking and resealing of phosphate lipid bilayer as they pass through polycarbonate membrane and less pressure is required here as compare to French pressure cell then use to process MLVs and LUVs. At last tortuous and nucleation trach membrane are formed.

Dehydration rehydration method

In these technique, liposomal suspension was prepared by THF are frozen in liquid nitrogen then freeze-dried overnight. After hydration with water, the liposome is prepared.

Sonication method

In this method surfactant and cholesterol are mixed in 2 ml of the aqueous phase in a vial and then the mixture is sonicated for 3 min at 60 c using titanium probe sonicator after that unilamellar vesicle is formed (Fig. 6).

Reverse phase evaporation method (REV)

The lipid mixture is taken in a round bottom flask followed by removal of the solvent under reduced pressure by a rotary evaporator. The system is purged with nitrogen and the lipids are re-dissolved in the organic phase. The reverse-phase vesicles will form in this phase. The usual solvents used are diethyl ether and isopropyl ether. An aqueous phase which contains the drug to be encapsulated is added after the lipids are re-dispersed in this phase. The system is kept under continuous nitrogen and the two-phase system is sonicated until the mixture becomes clear one-phase dispersion. The mixture is then placed on the rotary evaporator and the removal of organic solvent is done until a gel is formed followed by the removal of non-encapsulated material. The resulting liposomes are called reverse-phase evaporation vesicles (Fig. 7).

Dried reconstitute vesicle

Here the preformed liposomes are rehydrated to an aqueous fluid containing an active ingredient which is followed by dehydration of the mixture (Fig 8)

French pressure cell

The liposomes prepared by this technique are less likely to suffer from the structural defect and instability as observed in the sonicated vesicle. Leakage of contents from liposome preparing using French press is slower and slower than sonicated liposome. It has used to reduce the heterogeneity of populations of proteoliposomes obtained by detergent dialysis technique. The method has several advantages over the sonication method. The method is simple rapid, reproducible and involving gentle handling of unstable materials. The resulting liposome is somewhat larger than sonicated SUVs. The main drawback of
the method is that the temperature is difficult to achieve and the working volume is relatively small.

**Fig. 7 Method of reverse phase evaporation**

**Evaluation of liposome [12, 15]**

The characterization parameters for purpose of evaluation could be classified into three categories which are physical, chemical and biological parameters.

**Table no 2 Physical characterization**

| Characterization parameter                      | Analytical method/ instrument                                      |
|-------------------------------------------------|---------------------------------------------------------------------|
| Vesicle shape and surface morphology            | Transmission electron microscopy, freeze-fracture electron microscopy |
| Surface charge                                  | Free flow electrophoresis                                           |
| Mean vesicle size and size distribution         | Photon correlation spectroscopy, laser light scattering, gel permeation and gel exclusion |
| Lamellarity                                     | Small angle X-ray scattering, 31 P-NMR., Freeze fracture electron microscopy |
| Electrical surface potential and surface pH     | Zeta potential measurement                                          |
| Phase behavior                                  | Freeze fracture electron microscopy, Differential scanning calorimetry |
| Percent of free drug/ percent capture           | Minicolumn centrifugation, ion-exchange chromatography.             |

**Table no 3 Chemical characterization**

| Characterization parameter                      | Analytical method/ instrument                                      |
|-------------------------------------------------|---------------------------------------------------------------------|
| Phospholipid concentration                      | Lipid phosphorous content using barlet assay, HPLC                 |

**Table no 4 Biological characterization**

| Parameters      | Analytical method/ instrument |
|-----------------|------------------------------|
| Sterility       | Aerobic or anaerobic cultures |
| Pyrogenicity    | Limulus Amebocyte Lysate (LAL) test |
| Animal toxicity | Monitoring survival rates, histology and pathology                  |

**Application of liposome [16, 18 – 20]**

Application of liposome in pharmacology and medicine can be distinguished between diagnostic and therapeutic application of liposome. That containing various drug or markers, and their use as a tool, a reagent or a model in the basic studies of interaction of cell, recognition processes, and mode of action of certain substances. Unfortunately, many drugs have a very narrow therapeutic window, meaning is to the therapeutic concentration is not much lower than the toxic one. In various cases, the efficacy can be enhanced or the toxicity can be reduced by the use of a suitable drug carrier which alters the spatial and temporal delivery of the drug, i.e., its pharmacokinetics and bio distribution. It is clear from many pre-clinical and clinical studies that drugs, for instance antitumor drugs, parceled in liposome demonstrated reduced toxicities, while retentive enhanced efficacy.

**Liposomes in parasitic diseases and infections**

From the time when conventional liposomes are digested by phagocytic cells in the body after intravenous management, they are ideal vehicles for the targeting drug molecules into these macrophages. The best known instances of this ‘Trojan horse like mechanism are several parasitic diseases which
normally exist in the cell of MPS. They comprise leishmaniasis and several fungal infections.

Cancer therapy
Liposomes are successfully used to entrap anticancer drugs. This increases circulation life time, protect from metabolic degradation

Liposome as carrier of drug in oral treatment
- Steroids used for arthritis can be incorporated into large MLVs.
- Alteration in blood glucose levels in diabetic animals was obtained by oral administration of liposome-encapsulated insulin.

Liposome for topical application
- Drugs like triamcinolone, benzocaine, corticosteroids, etc. can be successfully incorporated as a topical liposome.

Liposome for pulmonary delivery-
- Inhalation devices like nebulizer are used to produce an aerosol of droplets containing liposomes.

Enhanced antimicrobial efficacy or safety
Antimicrobial agents have been encapsulated in liposomes for two reasons.
- First, they protect the entrapped drug against enzymatic degradation. For instance, the penicillin and cephalosporin are sensitive to the derivative action of J-lactamase, which is produced by certain microorganisms.
- Secondly, the lipid nature of the vesicles promotes enhanced cellular uptake of the antibiotics into the microorganisms, thus reducing the effective dose and the incidence of toxicity as exemplified by the liposomal formulation of amphotericin B.

CONCLUSION
Several drug candidates which are highly potent and have low therapeutic indication can be targeted to the required diseased site using the liposomal drug delivery system. Drugs encapsulated in liposomes can have a significantly altered pharmacokinetics. Liposomes have been used in a broad range of pharmaceutical applications. Liposomes are showing particular promise as intracellular delivery systems for antisense molecules, ribosomes, proteins/peptides, and DNA. Liposomes with enhanced drug delivery to disease locations, by the ability of long circulation residence times, are now achieving clinical acceptance. Also, liposomes promote targeting of particular diseased cells within the disease site. Finally, liposomal drugs exhibit reduced toxicities and retain enhanced efficacy compared with free complements. The efficacy of the liposomal formulation depends on its ability to deliver the drug molecule to the targeted site over a prolonged period, only time will tell which of the above applications and speculations will prove to be successful. However, based on the pharmaceutical applications and available products, we can say that liposomes have established their position in the modern drug delivery system.

Table 5: FDA approved Marketed product [12, 17-22]

| Drug                        | Disease                                   | liposomal delivery system |
|-----------------------------|-------------------------------------------|----------------------------|
| Amphotericin B              | Anti-fungal prophylaxis                    | Conventional              |
| Daunorubicin                | Leukemia and solid tumors                  | Conventional              |
| Cytarabine or cytosine arabinoside | Neoplastic meningitis and lymphomatous meningitis | Conventional              |
| Morphine sulphate           | Pain management                            | Conventional              |
| Verteporfin                 | Molecular degeneration                     | Conventional              |
| Vincristine                 | Non – Hodgkin lymphoma                     | Conventional              |
| Doxorubicin and bortezomib  | Relapsed or refractory multiple myeloma    | PEGylated                 |

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Nil

CONFLICT OF INTEREST
The authors declare no conflict of interest

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REFERENCES
[1] Zylberberg C, Matosevic S. Pharmaceutical Liposomal Drug Delivery: A Review Of New Delivery Systems And
A Look At The Regulatory Landscape. Drug Delivery, 23, 3319-3329 (2016)

[2] Torchilin VP. Liposomes As Pharmaceutical Carriers. Nature Reviews/Drug Discovery, 4, 145-160(2005)

[3] Maheswaran A, Brindha P, Mullaicharam AR, Masilamani K. Liposomal Drug Delivery Systems – A Review. International Journal Of Pharmaceutical Sciences Review And Research, 55, 295-301 (2013)

[4] Roy AS, Das SU, Samanta AR. Design Formulation And Evaluation Of Liposome Containing Isoniazid. Int. J. App Pharm, 10, 52-56 (2018)

[5] Li J, Wang X, Zhang T, Wang C, Huang Z, Luo X, Deng Y. A Review On Phospholipids And Their Main Applications In Drug Delivery Systems. Asian Journal Of Pharmaceutical Sciences, 10, 81-98 (2015)

[6] Kalepu S, Sunilkumar KT, Betha S, Mohanvarma M. Liposomal Drug Delivery System A Comprehensive Review. Int J Drug Dev Res, 5, 62-75 (2013)

[7] Kant S, Kumar S, Prashar B. A Complete Review On Liposome. International Research Journal Of Pharmacy, 3, 2230-8407 (2012)

[8] Goswami P, Changmai A, Barakoti H, Choudhury A, Kumar D B. A Brief Review On Liposomal Drug Delivery System. Journal Of Pharmaceutical Advanced Research, 1, 362-368 (2018)

[9] Laouini A, Jaafar-Maalej C, Limayem-Blouza I, Sfar S, Charcosset C, Fessi H. Preparation Characterization And Applications Of Liposomes: State Of The Art. Journal Of Colloid Science And Biotechnology, 1, 147-168 (2012)

[10] Mansoori MA, Agrawal S, Jawade S, Khan MI. A Review On Liposome. IJARPB, 2, 453-464 (2012)

[11] Samad A, Sultana Y, Aqil M. Liposomal Drug Delivery Systems: An Update Review. Current Drug Delivery, 4, 297-305 (2007)

[12] Akbarzadeh A, Sadabady RR, Duvaran S, Joo WS, Zarghami N, Hanifehpour Y, Samiei M, Kouhi M, Koshki NK. Liposome: Classification, Preparation, And Applications. Nanoscale Research Letters, 8, 102 (2013)

[13] Dua JS, Rana AC, Bhandari AK. Liposome: Methods Of Preparation And Applications. Int J Pharm Stud Res, 3, 14-20 (2012)

[14] Andhale VA, Patil PR, Dhas AU, Chauhan PD, Desai SV. Liposome: An Emerging Tool In Drug Carrier System. Int J Pharmacy Technol, 8, 10982-11011 (2016)

[15] Patel RP, Patel H, Baria AH. Formulation And Evaluation Of Liposomes Of Ketoconazole. Int J Drug Deliv Technol, 21, 16-23 (2009)

[16] Sercombe L, Veerati T, Moheimani F, Sherry YW, Sood AK, Hual S. Advances And Challenges Of Liposome Assisted Drug Delivery. Front Pharmacol, 6, 1-13 (2015)

[17] Kulkarni PR, Yadav JD, Vaidya KA. Liposomes: A Novel Drug Delivery System. International Journal Of Current Pharmaceutical Research, 3, 10-18 (2011)

[18] Giuseppina B, Agnese M. Liposomes As Nanomedical Devices. International Journal Of Nanomedicine, 10, 975-999 (2015)

[19] Saha S, Roy A, Bahadur S, Choudhury A. Fabrication and in-vitro evaluation of liposomal quercetin and its optimization. Research Journal of Pharmacy and Technology, 11(1), 61 – 64 (2018)

[20] Roy A, Saha S, Choudhury A, Bahadur S. Bioenhancement of curcumin by combined approaches of adjuvants and liposomal fabrication. Asian Journal of Pharmaceutics, 10(4), S688 – S692 (2016)

[21] Saini A. A Platform For Liposomal Drug Delivery. Int J Pharm Drug Anal, 3, 6-11 (2015)

[22] Daraee H, Etemadi A, Kouhi M, Alimirzalu S, Akbarzadeh A. Application Of Liposomes In Medicine And Drug Delivery. Artificial Cells, Nanomedicine, And Biotechnology, 44, 381-91 (2016)