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An Integrated Review on Liposomes: A Potential Novel Drug Delivery System

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Abstract
Liposomes are a novel drug delivery (NDDS), which are vesicular constructions consisting of hydrated bilayers which form spontaneously when phospholipids are dispersed in water. They are used to deliver enzymes, proteins, and drugs to target cells or tissue (mostly cancer). Liposomes have been viewed as one of the most outstanding, versatile, flexible carrier systems, which provide an extensive chance for the delivery of multifarious drug molecules and applications. A liposome carrier can overcome the limitation and drawbacks of conventional therapies. Due to new traits in liposome technology, countless liposome-based drug formulations are presented in the clinical trial, and currently, some of them have been accepted for clinical use. Reformulation of drugs in liposomal form has provided the possibility to improve the therapeutic index of a variety of drugs and medicinal agents and in most cases through alteration in their *in-vivo* biodistribution.

Keywords
Novel drug delivery system, Liposomes, Phospholipids, Vesicular carriers

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INTRODUCTION:

This century has witnessed excellent advances in the area of medicine. Pharmaceuticals have principally consisted of simple, fast-acting chemical compounds that are administered orally, parenterally, and externally. During the previous three decades, formulations that manipulate the price and length of drug delivery (i.e., time-release medications) and target particular areas of the body for the cure of disease are emerging frequently [1].

However, the present-day strategies of drug transport show off unique problems. For example, many drug's potencies and therapeutic activities are restricted or in any other case decreased due to the fact of the partial degradation in achieving the favored target site. The purpose of all state-of-the-art drug target systems, therefore, is to install drugs intact to mainly centered components of the body through a medium that can manipulate the therapy's administration through both a physiological or chemical trigger. To attain this goal, researchers are turning to advances in the worlds of micro and nanotechnology [1].

To decrease drug degradation, to stop dangerous side-effects and to enlarge drug bioavailability with the aid of bettering drug concentration on specific site, several drug transport carriers are presently beneath development. Colloidal particulates result from bodily incorporation of the drug into a carrier system such as liposomes, niosomes, micro and nanospheres, erythrocytes, polymeric and reverse micelles. Different drug carrier systems such as micellar solutions, liposomes, liquid crystal dispersions, as well as nanoparticle dispersions consisting of small particles of 10 – 400 nm diameter exhibit exquisite promise as drug transport systems [2].

Among these carriers, liposomes have been most studied. Liposomes are one of the most convenient drug delivery systems to deliver the drug to the target organ and minimize the distribution of the drug to non-target tissues [3]. Liposomes have been receiving a lot of attraction as a carrier for advanced drug delivery. Their enhancement lies in their composition, which makes them biocompatible and biodegradable. They consist of an aqueous core entrapped within the useful resource of one or more bilayers composed of natural or synthetic phospholipids.

The liposome is derived from two Greek words: 'Lipos' which means 'fat' and 'Soma' which means 'body'[4]. Liposomes are nano-sized to micro sized vesicles comprising a phospholipid bilayer that surrounds an aqueous core [5]. The Liposome is a drug carrier loaded with a huge variety of molecules such as small drug molecules, proteins nucleotides and even plasmids [6].

STEPS CONCERNED IN LIPOSOME DRUG DELIVERY:

Adsorption: Adsorption of liposomes to cell membranes motives its contact with the cell membrane.

Endocytosis: Adsorption of liposomes on the cell surface membrane accompanied by engulfment and internalization of the liposomes.

Fusion: Fusion of lipid bilayers of liposomes with the lipoidal cell membrane via lateral diffusion and intermingling of lipids outcomes in direct delivery of liposomal contents in the cytoplasm.

Lipid exchange: Due to the similarity of liposomal lipid membrane with cell membrane phospholipids, lipid switch proteins in the cell membrane without problems recognize liposomes and reason lipid exchange.

For example, in the case of most cancer cells; they consume giant quantities of fat to fill the requirement of fast growth, they recognize the liposomes (loaded with anti-cancer drug) as a manageable supply of nutrition. When they are centered by using liposomes, they get absorbed. Once the anti-cancer drugs are released from the liposome into the site, most cancer cells are killed by way of the drug [7].

ADVANTAGES:

- Liposomes accelerate the efficacy and therapeutic index of the drug (Actinomycin-D).
- Liposomes minimize the toxicity of the encapsulated agent (Amphotericin B, Taxol).
- Stability is enhanced if liposome prepared by encapsulation.
- Flexibility to couple with site-specific ligands to acquire active targeting.
- Suitable for the transport of hydrophobic, amphipathic and hydrophilic drugs.
- Improved solubility of lipophilic and amphiphilic drugs. The hydrophobic anti-cancer drugs can encapsulate in the lipid membrane of the liposome that will improve the solubility of poorly soluble anti-cancer drugs.
- Stabilization of entrapped drug from hostile surroundings [8-11].

DISADVANATAGES:

- There may be possibilities of leakage and fusion of encapsulated drug/molecules.
- Allergic reactions may occur due to liposome constituents.
- The phospholipids may undergo oxidation and hydrolysis.
- The possibility of dose dumping due to misguided administration.
- Can modulate the distribution of drug [9, 12].

PROPERTIES OF LIPOSOMES:

- Overcoming the rapid clearance of drugs.
- Intracellular delivery of drugs.
- Receptor-mediated endocytosis of ligand-targeted liposomes.
- Triggered release.
- Delivery of nucleic acids and DNA.
- They are osmotically sensitive [12,13].

HANDLING OF LIPOSOMES:

The phospholipids used in the preparation of liposomes are un-saturated and due to this fact inclined to oxidation. Volatile solvents such as chloroform which are used will tend to evaporate from the container. Thus, liposomes are preserved in inert surroundings of nitrogen and in the dark glass vessels with a tightly fixed cap.

CLASSIFICATION OF LIPOSOMES:

Liposomes are classified based on size and they are:

- **SUV-small unilamellar vesicles-(<50 nm)**
- **LUV-large unilamellar vesicles-(>50 nm)**

The liposomes whose dimension varies from (2,000 - 40,000 nm) are referred to as multilamellar liposomes. Liposomes of dimension (10,000 - 10, 00,000 nm) are giant liposomes these can be both unilamellar and multilamellar liposomes. These are commonly desired carrier system in the modern trends as these formulations enhances the therapeutic efficacy at a low dose, reduces toxicity and improves pharmacokinetic profile e.g., liposomal

Doxorubicin; elevated steadiness of drugs specifically in opposition to enzymatic degradation [14,15].

METHODS OF PREPARATION:

The general method of preparation involves four steps:

- Drying down lipids from organic solvents
- Dispersion of lipids in aqueous media
- Purification of resultant liposomes
- Analysis of final product

1) Physical Dispersion Methods:

In these methods, the aqueous volume enclosed within lipid membranes is about 5-10%, which is a very small proportion of the total volume used for preparation. So, a huge amount of water-soluble drug is wasted during preparation. But the hydrophobic drug can be encapsulated to a high percentage. In these methods, MLVs are formed, and further treatment is required for the preparation of unilamellar vesicles [9].

Lipid film hydration by handshaking:

In this method, liposomes are prepared by hydrating the thin lipid film in an organic solvent, and then the organic solvent is removed under a vacuum. After completely removing the solvent, the solid lipid mixture is hydrated by the aqueous buffer. The lipids spontaneously swell and hydrate to form liposomes. This method has low encapsulation efficiency [16].

Freeze drying:

Another technique of dispersing the lipid in a sooner or later divided structure before the addition of aqueous media is to freeze-dry the lipid dissolved in an appropriate natural solvent. The solvent mostly used is tertiary butanol. This technique produces MLVs which are too giant or too heterogeneous. To regulate the dimension, the organized MLVs are in addition processed by usage of the following methods [9].

Micro emulsification:

Microfluidizer is used to formulate small vesicles from targeted lipid suspension. The lipids can be added into the fluidizer as a suspension of giant MLVs. The tools pump fluid at very excessive pressure via a 5 mm screen. Then it is compelled with long microchannels, which drive two streams of fluids, collide collectively at proper angles at very excessive velocity. The collected fluid can be recycled via the use of the pump and in the interaction chamber till vesicles of spherical dimensions are obtained [17].

Ultrasonic Method:

This method is used for the preparation of SUVs with diameters in the range of 15-25 nm. Ultrasonication of an aqueous dispersion of phospholipid is done by two types of sonicators i.e. either probe sonicator or bath sonicators. The probe sonicator is used for the small volume which requires high energy while the bath sonicators are employed for the large volume [18].

High-pressure extrusion method:

This is another method of converting MLV to SUV suspension. MLVs formulate via thin-film hydration technique rapidly passed via polycarbonate membranes at 20,000 psi at 4°C thru a small orifice lowering the liposome measurement in the high-pressure extrusion method. When the MLVs are pushed through the small orifice their layers are regularly separated and solely one of their layers remains intact. This technique causes uniform particle size distribution by lowering the size of the liposomes. The approach is simple, rapid, reproducible and involves gentle handling of unstable materials. The resulting liposomes are quite large than sonicated SUVs. The drawbacks of the technique are that the temperature is difficult to achieve and the working volumes are fairly small (about 50 ml maximum) [10].

Freeze and thaw sonication:

The freeze-thaw technique under mechanical dispersion strategies is a protocol used to create massive unilamellar vesicles from small unilamellar vesicles. By freezing the small unilamellar vesicles then thawing them countless times, the vesicle membranes are in a position to the mixture to create the giant vesicles. One of the difficulties with this strategy even though is that ice crystals can damage the lipid bilayers for the duration of freezing. Because of this, new techniques for defending the bilayers at some stage in freezing are of interest. However, it has been discovered that this approach of freeze-thawing liposomes aids in the manufacturing of liposomes when they are freeze-dried [19,20].

2) Solvent Dispersion Methods:

Ether injection (solvent vaporization):

A solution of lipids dissolved in diethyl ether or ether/methanol combination is slowly injected into an aqueous solution of the material to be encapsulated at 55-65°C or underneath decreased pressure. The subsequent elimination of ether beneath vacuum leads to the formation of liposomes. The important drawbacks of the approach are that the population is heterogeneous (70-190 nm) and the exposure of compounds to be encapsulated to solvents or excessive temperature [21].

Ethanol injection:
A lipid solution of ethanol is rapidly injected into a massive excess of the buffer. The MLVs are at as soon as formed. The hazards of the technique are that the population is heterogeneous (30 to 110 nm), liposomes are very dilute, the elimination of all ethanol is challenging due to the fact it varieties into azeotrope with water and the probability of the number of biologically active macromolecules to inactivate in the presence of even low quantities of ethanol [22].

3) Detergent Depletion Technique:
The detergent depletion approach is a method for the manufacturing of a broad range of vesicle types and highly homogeneous liposomes. The technique is primarily based on the formation of detergent-lipid micelles, observed through the elimination of the detergent to form a concentration of liposomes in the solution and entrapment of any hydrophobic compound is additionally low. The detergent additionally stays in the formulation. The size and homogeneity of liposomes produced through detergent depletion are primarily dependent on the rate at which the detergent is eliminated and the preliminary ratio of detergent to phospholipid. The technique is very time-consuming and the method of removing the detergent might also additionally eliminate any different small hydrophilic compound [23].

CHARECTERIZATION OF LIPOSOMAL FORMULATIONS:
Liposomal are characterized to make sure their predictable *in-vitro* and *in-vivo* performance. The characterization parameters for the reason of contrast should be categorized into three huge classes which encompass physical, chemical, and biological parameters.
Physical characterization consists of several parameters which include vesicle size, shape, surface characteristics, lamellarity, phase behavior and drug release profile. Chemical characterization consists of those studies which set up the purity and efficiency of a variety of lipophilic constituents. Biological characterization parameters are useful in setting up the security and suitability of the system for therapeutic utility [9]. Some of these techniques are as follows:
Optical microscopy:
The microscopic technique consists of the use of bright-field, phase contrast microscope and fluorescent microscope and is beneficial in evaluating the size of the vesicle.

Negative stain TEM:
Electron microscopic techniques which are used to determine liposome structure and dimension are typically negative- stain TEM and scanning electron microscopy. The latter method is much less preferred. Negative stain electron microscopy visualizes shiny areas towards darkish history (hence termed as negative stain).
The negative stains used in TEM evaluation are ammonium molybdate or phosphotungstic acid (PTA) or uranyl acetate. PTA and ammonium molybdate are anionic in nature and uranyl acetate is cationic.
Differential scanning calorimetry (DSC) Study:
In this technique, drug-loaded multilamellar liposomes are scanned into a DSC analyzer. This approach is used to determine the phase transition temperature of the phospholipids sample. The temperature of maximal extra heat capacity is described as the phase transition temperature. Thermograms are received at a scanning rate of 10ºC/min or 20ºC/min. Each pattern is scanned between 20ºC to 200ºC or 25ºC to 500ºC [24].
Drug entrapment studies:
The liposome preparations are a combination of encapsulated and unencapsulated drug fractions. The first step for the determination of the encapsulation efficiency is the separation between the encapsulated drug (within the carrier) and the free drug. Aliquots of liposome sample (0.5 ml) and 5 ml of 10% sodium lauryl sulfate (SLS) are mixed, and volume is made up to 50 ml with distilled water. The sample are warmed on a water bath at 70°C for 30 min. Similarly, a blank liposome (without drug) suspension (0.5 ml) and 5 ml of 10% SLS are taken in a 50 ml volumetric flask and the volume was made up with distilled water. The blank liposomes warmed on a water bath at 70°C for 30 min. The absorbance of the test solution was taken in a UV spectrophotometer against the blank solution [25].
Percentage entrapment efficiency:
It is determined by using the ratio of the entrapped drug (mg) to the total drug (mg), which may be expressed by the following formula:

Entrapment efficiency = amount of drug entrapped / total amount of drug × 100

Zeta potential measurement:
Another parameter in liposome characterization is the surface charge of the liposome measured by the way of zeta potential. The higher value of zeta potential implies greater colloidal stability and results in inhibiting the aggregation of the liposomal formulation. It is reported that microspheres with a

zeta potential above (+/-) 30 mV show stabilization in suspension. Zeta-sizer is used for measuring the zeta potential of the liposomes by applying a voltage across a pair of electrodes at either end of a cell containing the particle dispersion.

***In-vitro* drug release study:**

This can be carried by using a Franz Diffusion cell which has a diameter of 25 mm. It contains a reservoir compartment of 22 ml which was filled with a buffer that contains 20% v/v methanol to maintain sink condition. The diffused drug content is determined using UV spectroscopy [26].

Stability study:

The behavior of the liposome to retain the drug studied by storing the liposome at 4 to 8°C (refrigerator) for 1 month. The liposomal preparations are kept in a sealed vial and analyzed for drug content [27].

CLINICAL APPLICATIONS OF LIPOSOMES:

While this paper focuses on liposome use in drug delivery, liposomes may additionally be used in gene therapy [28]. Within drug delivery, these liposomes can be used as drug carriers that can target particular tissues, subcellular compartments, or malignant cells [29]. As for the targeting, liposomes have been used in treatment against inflammation, fungi and most cancers [28]. Since they can target inflammation, they ought to probably be used in the treatment of prion disease as these are regularly related to the pro-inflammatory response of microglial cells [30]. Some drug-loaded liposomes have been examined and been considered to minimize tumor measurement in cats [28]. Liposomes are additionally studied extensively because beyond targeting specific cell types, they are also in a position to target intracellular factors as well. One study uses the liposomes's capacity to go to the intracellular surroundings to target mycobacterium tuberculosis that had been engulfed through macrophages [31]. This capacity for liposomes to enter the intracellular compartment is a necessary function that makes liposomes a famous vicinity of study, particularly in targeting macrophages [19].

MARKETED FORMULATIONS OF LIPOSOMES:

In 1995, Doxil (PEGylated liposome-encapsulated Doxorubicin) became the first liposome drug delivery system approved for human use by the US FDA. There was a list of marketed formulations of liposomes. Other marketed products of liposomes are shown in Table 1.

Table-1: Marketed Product of liposomes [32]

S. No.	Product name	Drug	Company
1.	Ambisome	Amphotericin B	NeXstar pharmaceuticals Inc.CO
2.	Abelcet	Amphotericin B	The Liposome company, N.J.
3.	Amphocil	Amphotericin B	Sequus pharmaceuticals Inc.CA
4.	Doxil	Doxorubicin	Sequus pharmaceuticals Inc.CA
5.	Dounoxome	Daunorubicin	NeXstar pharmaceuticals Inc.CO
6.	Mikasome	Amikacin	NeXstar pharmaceuticals Inc.CO
7.	DC99	Doxorubicin	Liposome CO., NJ, USA
8.	Epaxel	Hepatitis A vaccine	Swiss Serum Institute, Switzerland
9.	ELA max	Lidocaine	Biozone Labs, CA, USA

ADAVANCES IN LIPOSOMES:

Ethosomes: They are efficient at delivering to the skin and are composed of soya phosphatidylcholine and 30% ethanol.

Immuno liposomes: They are modified with antibodies.

Niosomes: They are small unilamellar vesicles made from non-ionic surfactants [33].

Stealth liposome: They are a new type of liposomes which are prepared to improve stability and lengthen their half-life in circulation. Coating of liposomes should be done by polyethylene glycol (PEG) for preparing these liposomes [32, 34].

CONCLUSION:

It is concluded from the review that liposomes can be a promising carrier for providing targeted delivery of a wide variety of drugs such as antimicrobial agents, drugs against cancer, antifungal drugs, peptide hormones, enzymes, vaccines and genetic materials. Liposomes are administrated orally, parenterally, topically as well as employed in a huge range of pharmaceutical and pharmacology applications with therapeutic and diagnostic purposes and as good carriers in gene delivery of various drugs. Liposomal delivery systems have been approved as a suitable carrier for therapeutic effectiveness in terms of duration of action and decrease in dose frequency and delivering drugs at higher efficiency and lower toxicity.

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