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Review Article

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Liposome – A Novel Drug Delivery

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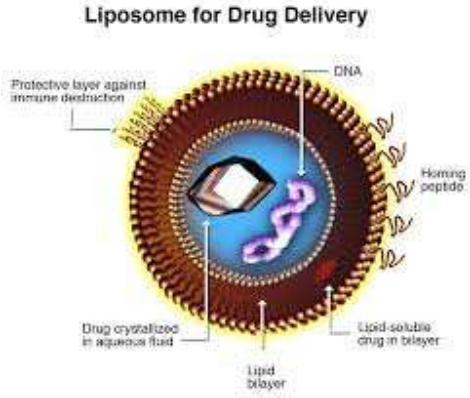
ABSTRACT

Liposomes, consist of lipid vesicles which aqeous layer enclosed by phospholipid layer . Liposomes are one of unique drug delivery system which can be use in controlling and targeting drug delivery system. Liposomes, which are biodegradable and essentially non-toxic vehicles, can encapsulate both hydrophilic and hydrophobic materials, and are utilized as drug carriers in drug delivery systems which protwcts drug from degradation. Liposomes can be filled with drugs, and used to deliver drugs for cancer and other diseases Among several new drug delivery systems, liposomes characterize an advanced technology to deliver active molecules to the site of action. Research on liposome technology has progressed from conventional vesicles to 'second-generation liposomes', in which long-circulating liposomes are obtained by changing the lipid composition, size, and charge of the vesicle. Liposomes with modified surfaces have also been developed using several molecules, such as glycolipids or sialic acid. This review discusses the potential applications of liposomes in drug delivery with examples of formulations approved for clinical use,their preparation method, targeting, mechanism of formation, liposome component and the problems associated with further exploitation of this drug delivery system.

Keywords: *Lipsome,phospholipid,glycolipids.*

INTRODUCTION

When phospholipids are dispersed in water, they spontaneously form closed structure with internal aqueous environment bounded by phospholipids bilayer membranes, this vesicular system is called as liposome. Liposomes are concentric bilayered vesicle in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic phospholipids. Liposomes can target a drug to the intended site of action in the body thus enhancing its efficacy. Liposomes can act as a depot from which the entrapped compound is slowly released over time; such a sustained release process can be exploited to maintain therapeutic drug levels in blood stream. Thus liposome surfaces can be readily modified by attaching polyethylene glycol (PEG) units to the bilayer, the circulation time of liposomes. Liposomes are extensively used as carriers for numerous molecules in cosmetic and pharmaceutical industries. Additionally, food and farming industries have extensively studied the use of liposome encapsulation to grow delivery systems that can entrap unstable compounds (for example, antimicrobials, antioxidants, flavors and bioactive elements) and shield their functionality. Liposomes can trap both hydrophobic and hydrophilic compounds, avoid decomposition of the entrapped combinations, and release the entrapped at designated targets bloodstream is increased dramatically.



Liposomes made up of:

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

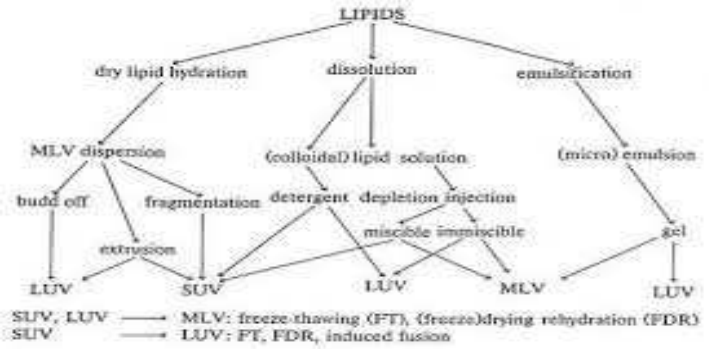
Glycosphingolipids: Gangliosides

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



Why only liposome for targeted drug delivery:

Liposomes increased efficacy and therapeutic index of drug (actinomycin-D) Low solubilityLiposome increased stability via encapsulation Short half-life.Liposomes are non-toxic, flexible, biocompatible, completely biodegradable, and nonimmunogenic for systemic and non-systemic administrations.Sometimes phospholipid undergoes oxidation and hydrolysis-like reaction. Liposomes reduce the toxicity of the encapsulated agent (amphotericin B, Taxol) Leakage and fusion of encapsulated drug molecules. Liposomes help reduce the exposure of sensitive tissues to toxic drugs Production cost is high. Flexibility to couple with site-specific ligands to achieve active targeting.

PREPARATION OF LIPOSOMES:

There are mainly two mechanisms of vesicle formation.

Fig.1: the budding theory

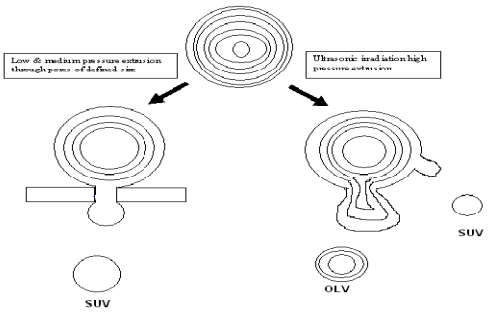
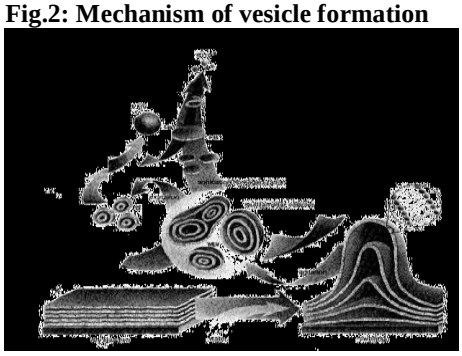


Fig.2: Mechanism of vesicle formation



a) The budding theory

Stress induced hydration of phospholipids
Organization into lamellar arreys
Results in to budding of lipid bilayer leading to downsizing. (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).

Classification :

1. Based on size and number of lamellae:

Type of vesicles	Unit of size (nm)
Multilameller vesicles	500
Oligolameller vesicles	100 to 1000
Unilameller vehicles	20 to 1000
Multi vesicular system	>1000
Doble liposomes	>1000

2.Based on method of preparation:
Reverse phase evaporation method
Stable plurilamelar vesicles
Frosen and thawed multilameller vesicles
Vesicles prepared by extrusion technique
Dehydration-Rehydration technique

3. Based on composition:
Conventional liposomes

Fusogenic liposomes
pH sensitive liposomes
Immunoliposomes

Methods of liposome preparation[13]
General methods of preparation
All the methods of preparing the liposomes involve four basic stages:
1. Drying down lipids from organic solvent.
2. Dispersing the lipid in aqueous media.
3. Purifying the resultant liposome.
4. Analyzing the final product.

Method of liposome preparation and drug loading
The following methods are used for the preparation of liposome:
Liposome may be prepared by two techniques
a) Passive loading technique.
b) Active loading technique.

1 Passive loading technique
A) Mechanical dispersion method
- Lipid hydration by hand shaking or freeze drying
- Micro emulsification
- Sonication
- French pressure cell
- Membrane extrusions
- Dried reconstituted vesicle
- Freeze thawed liposome

B) Solvent dispersion method
- Ethanol injesction
- Ether injection
- Double emulsion vesicle
- Reverse phase evaporation vesicle
- Stable plurilamellar vesicle

C) Detergent removal method
- Detergent (cholate, alkylglycoside, Tritonx-100) removed from mixed micelles
- Dialysis
- Column chromatography
- Dilution

2) Active loading technique
phase, the organic solvent is immiscible with the aqueous phase, the latter being in excess and
the case where the organic solvent is in excess, and immiscible with the aqueous phase.

1) Passive loading technique:
Passive loading techniques include three different group of method working on different principles namely mechanical dispersion, solvent dispersion and detergent solubilization.

A) Mechanical dispersion method of passive loading
All method covered under this category begin with a lipid solution in organic solvent and end up with lipid dispersion in water. The various components are typically combined by codissolving the lipid in organic solvent and organic solvent is then removed by film diposition under vacuum.

When all solvent is removed, the solvent dispersion mixture is hydrated using aqueous buffer. The film spontaneously swell and hydrate to form liposomes. At this point method incorporate some diverge processing parameters in various way to modify their ultimate properties. The post hydration treatments include vortexing, sonication, freeze thawing and high- pressure extrusion.

B) Solvent dispersion method of passive Loading:

Ether injection (solvent vaporization) :

A solution of lipids dissolved in diethyl ether or ether-methanol mixture is gradually injected to an aqueous solution of the material to be encapsulated at 55°C to 65°C or under reduced pressure. The consequent removal of ether under vacuum leads to the creation of liposomes. The main disadvantages of the technique are that the population is heterogeneous (70 to 200 nm) and the exposure of compounds to be encapsulated to organic solvents at high temperature.

Ethanol injection :

A lipid solution of ethanol is rapidly injected to a huge excess of buffer. The MLVs are at once formed. The disadvantages of the method are that the population is heterogeneous (30 to 110 nm), liposomes are very dilute, the removal all ethanol is difficult because it forms into azeotrope with water, and the probability of the various biologically active macromolecules to inactivate in the presence of even low amounts of ethanol is high.

Reverse phase evaporation method:

This method provided a progress in liposome technology, since it allowed for the first time the preparation of liposomes with a high aqueous space-to-lipid ratio and a capability to entrap a large percentage of the aqueous material presented. Reverse-phase evaporation is based on the creation of inverted micelles. These inverted micelles are shaped upon sonication of a mixture of a buffered aqueous phase, which contains the water-soluble molecules to be encapsulated into the liposomes and an organic phase in which the amphiphilic molecules are solubilized. The slow elimination of the organic solvent leads to the conversion of these inverted micelles into viscous state and gel form. At a critical point in this process, the gel state collapses, and some of the inverted micelles were disturbed. The excess of phospholipids in the environment donates to the formation of a complete bilayer around the residual micelles, which results in the creation of liposomes. Liposomes made by reverse phase evaporation method can be made from numerous lipid formulations and have aqueous volume-to-lipid ratios that are four times higher than hand-shaken liposomes or multilamellar liposomes

Briefly, first, the water-in-oil emulsion is shaped by brief sonication of a two-phase system, containing phospholipids in organic solvent such as isopropyl ether or diethyl ether or a mixture of isopropyl ether and chloroform with aqueous buffer. The organic solvents are detached under reduced pressure, resulting in the creation of a viscous gel. The liposomes are shaped when residual solvent is detached during continued rotary evaporation under reduced pressure. With this method, high encapsulation efficiency up to 65% can be obtained in a medium of low ionic strength for example 0.01 M NaCl. The method has been used to encapsulate small, large, and macromolecules. The main drawback of the technique is the contact of the materials to be encapsulated to organic solvents and to brief periods of sonication. These conditions may possibly result in the breakage of DNA strands or the denaturation of some proteins . Modified reverse phase evaporation method was presented by Handa et al., and the main benefit of the method is that the liposomes had high encapsulation efficiency (about 80%)

C) Detergent removal method of passive loading

In this method the phospholipids are brought into intimate contact with the aqueous phase via detergent, which associate with phospholipids molecule and serve to screen the hydrophobic portion of the molecule from water. The structure formed as result of this association is known as micelles, and can be composed of several hundreds of component molecule. Their size and shape depend on the chemical nature of detergent, the concentration and other lipid involved. The concentration of detergent of in water at which micelles just start to form is known as ‘critical micelle concentration’. Below the critical micelle concentration, micelle the detergent molecule exists entirely in free solution.

As detergent is dissolved in water in concentration higher than the CMC, micelle form in more and more numbers, while the concentration of detergent in the free from remain essentially the same as it is at the CMC. Micelle containing other participating component in addition to detergent (or composed of two or more detergent in their formulation known as "mixed micelle".

Dialysis:

The detergents at their critical micelle concentrations (CMC) have been used to solubilize lipids. As the detergent is detached, the micelles become increasingly better-off in phospholipid and lastly combine to form LUVs. The detergents were removed by dialysis [34-36]. A commercial device called LipoPrep (Diachema AG, Switzerland), which is a version of dialysis system, is obtainable for the elimination of detergents. The dialysis can be performed in dialysis bags engrossed in large detergent free buffers (equilibrium dialysis)

Gel-permeation chromatography:

In this method, the detergent is depleted by size special chromatography. Sephadex G-50, Sephadex G-l 00 (Sigma-Aldrich, MO, USA), Sepharose 2B-6B, and Sephacryl S200-S1000 (General Electric Company, Tehran, Iran) can be used for gel filtration. The liposomes do not penetrate into the pores of the beads packed in a column. They percolate through the inter-bead spaces. At slow flow rates, the separation of liposomes from detergent monomers is very good. The swollen polysaccharide beads adsorb substantial amounts of amphiphilic lipids; therefore, pretreatment is necessary. The pre-treatment is done by pre-saturation of the gel filtration column by lipids using empty liposome suspensions.

Dilution:

Upon dilution of aqueous mixed micellar solution of detergent and phospholipids with buffer, the micellar size and the polydispersity increase fundamentally, and as the system is diluted beyond the mixed micellar phase boundary, a spontaneous transition from poly-dispersed micelles to vesicles occurs.

2) Active loading technique

The utilization of liposomes as drug delivery system is stimulated with the advancement of efficient encapsulation procedures. The membrane from the lipid bilayer is in general impermeable to ions and larger hydrophilic molecules. Ions transport can be regulated by the ionophores while permeation of neutral and weakly hydrophobic molecule can be controlled by concentration gradients. Some weak acid or bases however, can be transported through the membrane due to various transmembrane gradient, such as electric, ionic (pH) or specific salt (chemical potential) gradient. Several method exist for improved loading of drugs, including remote (active) loading method which load drug molecules into preformed liposome using pH gradient and potential difference across liposomal membrane. A concentration difference in proton concentration across the membrane of liposomes can drive the loading of amphipathic molecule. Active loading method have the following advantages over passive encapsulation technique:

- A high encapsulation efficiency and capacity.
- A reduced leakage of the encapsulated compounds.
- "bed side" loading of drugs thus limiting loss of retention of drugs by diffusion, or chemical degradation during storage.
- Flexibility of constitutive lipid, as drug is loaded after the formation of carrier unit.
- Avoidance of biological active compounds during preparation step in the dispersion thus reducing safety hazards.
- The transmembrane pH gradient can be developed using various method depending upon the nature of drug to be encapsulated.

Mechanism of formation of liposomes

In order to understand why liposomes are fomed when phospholipid are hydrated, it requires a basic understanding of physiochemical featured of phospholipid. Phospholipids are amphipatic (having affinity for both aqueous and polar moieties) molecules as they have a hydrophobic tail and a hydropdilic or polar head. The hydrophilic tail composed of two fatty acid chains containing 10-24 carbon atoms and 0-6 double bound in each chain.

The polar end of molecule is mainly the phosphoric acid bound to a water soluble molecule. The hydrophilic and hydrophobic domain/segment within the molecular geometry of amphiphilic lipid orient and self organize in ordered supramolecular structure when confronted with solvent. In aqueous medium the molecule in self assembled structure is oriented in such a way that the polar portion of molecule remain in contact with the polar environment and at the same time shield the non-polar part. Among the amphiphiles used in drug delivery, such as soap, detergent, polar lipid, the latter (polar lipid) are often employed to form concentric bilayer structure. However, in aqueous medium these molecule are able to form various phases, some of them are stable and others remain in the metastable state. At high concentration of these polar lipids, liquid-crystalline phases are formed that upon dilution with an excess of water can be dispersed into relatively stable colloidal particles. The macroscopic structure most often formed include lamellar, hexagonal or cubic phases dispersed as colloidal nanoconstruct (artificial membrane) referred to as liposomes, hexasomes or cubosomes respectively. The most common natural polar phospholipids are phosphatidylcholine. These are amphipathic molecule in which a glycerol bridge links to a pair of hydrophobic acyl chains with a hydrocarbon chains with a hydrophilic polar head group, phosphocholine. Thus the amphipathic (amphiphilic) nature of the phospholipid and their analogues render them the ability to form closed concentric bilayers in the presence of water.

Stealth liposomes and conventional liposomes[9]:

Although liposomes are like biomembranes, they are still foreign objects of the body. Therefore, liposomes are known by the mononuclear phagocytic system (MPS) after contact with plasma proteins. Accordingly, liposomes are cleared from the blood stream. These stability difficulties are solved through the use of synthetic phospholipids, particle coated with amphipathic polyethylene glycol, coating liposomes with chitin derivatives, freeze drying, polymerization, micro-encapsulation of gangliosides. Coating liposomes with PEG reduces the percentage of uptake by macrophages and leads to a prolonged presence of liposomes in the circulation and, therefore, make available abundant time for these liposomes to leak from the circulation through leaky endothelium. A stealth liposome is a sphere-shaped vesicle with a membrane composed of phospholipid bilayer used to deliver drugs or genetic material into a cell. A liposome can be composed of naturally derived phospholipids with mixed lipid chains coated or steadied by polymers of PEG and colloidal in nature. Stealth liposomes are attained and grown in new drug delivery and in controlled release. This stealth principle has been used to develop the successful doxorubicin-loaded liposome product that is presently marketed as Doxil (Janssen Biotech, Inc., Horsham, USA) or Caelyx (Schering- Plough Corporation, Kenilworth, USA) for the treatment of solid tumors. Recently impressive therapeutic improvements were described with the useof corticosteroidloaded liposome in experimental arthritic models. The concerning on the application of stealth liposomes has been on their potential to escape from the blood circulation. However, long circulating liposome may also act as a reservoir for prolonged release of a therapeutic agent. Pharmacological action of vasopressin is formulated in long circulating liposome .

Name	Trade name	Company	Indication
Liposomal amphotericin B	Abelcet	Enzon	Fungal infections
Liposomal amphotericin B	Ambisome	Gilead Sciences	Fungal and protozoal infections
Liposomal cytarabine	Depocyt	Pacira (formerlySkyePharma)	Malignant lymphomatous meningitis
Liposomal daunorubicin	DaunoXome	Gilead Sciences	HIV-related Kaposi's sarcoma
Liposomal doxorubicin	Myocet	Zeneus	Combinationtherapy with cyclophosphamide in metastatic breast cancer
Liposomal Vaccine	Epaxal	Berna Biotech	Hepatitis A
Liposomal Vaccine	Inflexal V	Berna Biotech	Influenza
Liposomal morphine	DepoDur	SkyePharma, Endo	Postsurgical analgesia

Liposomal verteporfion	Visudyne	QLT, Novartis	Age-related macular degeneration, pathologic myopia, ocular histoplasmosis
Liposome-PEG doxorubicin	Doxil/Caelyx	Ortho Biotech, Schering-Plough	HIV-relatedKaposi's sarcoma, metastatic breastcancer, metastatic ovarian cancer
Micellular estradiol	Estrasorb	Novavax	Menopausal therapy

Therapeutic application of liposomes[3]:

Liposomes in anticancer therapy Numerous different liposome formulations of numerous anticancer agents were shown to be less toxic than the free drug . Anthracyclines are drugs which stop the growth of dividing cells by intercalating into the DNA and, thus, kill mainly rapidly dividing cells. These cells are not only in tumors but are also in hair, gastrointestinalmucosa, and blood cells; therefore, this class of drug is very toxic. The most used and studied is Adriamycin (commercial name for doxorubicin HCl; Ben Venue Laboratories, Bedford, Ohio). In addition to the above-mentioned acute toxicities, its dosage is limited by its increasing cardio toxicity. Numerous diverse formulations were tried. In most cases, the toxicity was reduced to about 50%. These include both acute and chronic toxicities because liposome encapsulation reduces the delivery of the drug molecules towards those tissues. For the same reason, the efficiency was in many cases compromised due to the reduced bioavailability of the drug, especially if the tumor was not phagocytic or located in the organs of mononuclear phagocytic system.

Benefits of drug load in liposome Examples

1. Improved solubility of lipophilic and amphiphilic drugs Amphotericin B, porphyrins, minoxidil, some peptides, and anthracyclines, respectively; hydrophilic drugs, such as anticancer agent doxorubicin or acyclovir
2. Passive targeting to the cells of the immune system, especially cells of the mononuclear phagocytic system Antimonials, amphotericin B, porphyrins, vaccines, immunomodulators
3. Sustained release system of systemically or locally administered liposomes
Doxorubicin, cytosine arabinoside, cortisones, biological proteins or peptides such as vasopressin
4. Site-avoidance mechanism Doxorubicin andamphotericin B
5. Site-specific targeting Anti-inflammatory drugs, anti-cancer, anti-infection
6. Improved transfer of hydrophilic, charged molecules Antibiotics, chelators, plasmids, and genes
7. Improved penetration into tissues Corticosteroids, anesthetics, and insulin

Applications of liposomes in medicine and pharmacology:

Advances in liposome design are leading to new applications for the delivery of new biotechnology products, for example antisense oligonucleotides, cloned genes, and recombinant proteins. A vast literature define the viability of formulating wide range of conservative drugs in liposomes, frequently resultant in improved therapeutic activity and/or reduced toxicity compared with the free drug. As a whole, changed pharmacokinetics for liposomal drugs can lead to improved drug bioavailability to particular target cells that live in the circulation, or more prominently, to extravascular disease sites, for example, tumors. Recent improvements include liposomal formulations of all-trans-retinoic acid and daunorubicin, which has received Food and Drug Administration consent as a first-line treatment of AIDS-related advanced Kaposi's sarcoma. Distinguished examples are vincristine, doxorubicin, and amphotericin B.

Liposomes In Cosmetics:

Now cosmetic products have reached the stage where liposomes can encapsulate active ingredients thought to be necessary for the skin so they may be directly applied to the skin cells. The liposome wall is very similar, physiologically, to the material of cell membranes. Liposomes are typically manufactured from various fatty substances that are used to encapsulate, or to create a sphere around, cosmetic materials. They act as a delivery system. Liposomes are believed to be deformed and transformed into fragments as a rule. Therefore size, shape, and lamallarity are not so relevant for the application, but for the chemical composition of the total formulation. A patent involves a skin whitening lotion in which liposomes, consisting of vitamin E and complex lipids are dispersed in alcohol and water. The patent claims that vitamin E remains stable long enough to exhibit reducing action.

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.

Liposome as drug/protein delivery vehicle:

They used for controlled and sustained drug release in the formulation also helps in Enhaned drug solubilization. Liposomes as drug delivery which helps to altered pharmacokinetic and biodistribution Enzyme replacement therapy and lysosomal disorders.

Liposomes for targeted drug delivery:

Liposomal entrapment of these drugs showed reduced cardiotoxicity, dermal toxicity and better survival of ex-perimental animals compared to the controls receiving free drugs. DXR entrapped in liposomes shows reduced non-specific toxicity and maintains or enhances anticancer effect. hydrophilic stealth coating, which allows the DoxilTM liposomes to circulate in the blood stream for prolonged periods. The lipid matrix and an internal buffer system combine to keep virtually all the DXR encapsulated during liposome residence in the circulation. This means that the drug is not free to exert its toxic effects . Sustained release and passive tumor targeting can explain the enhanced efficacy.

Characterization of liposomes[2]:

1. Physocochemical methods:

a. Particle size determination:

Determination of particle size of liposomes done by laser light scattering

b. Transmission electron microscopy:

Osmium tetroxide is used in TEM for maintaining temperature of -196˚c

c. X-ray scattering:

It determines size and shape of liposomes.

d. Differential scanning colourimetry:

Crystalline to liquid crystalline and liquid crystalline to amorphous transitions can be studied.

e. Rheology:

Flow characteristics exhibited by rheology are plastic and pseudoplastic.Mechanical oscillation measurement is the method of choice for determining liquid crystalline get elasticity.

2. Percent entrapment:

Minicolumn centrifugation method is mostly used.

3. Determination of percent capture:

It is clearly essential to measure the quantity of material entrapped inside liposomes before studying the behavior of this entrapped material in physical or biological systems .

Leakage through phase separation:

Lipophilic compounds may phase separate from liposomal bilayers .A simple light microscope is a very suitable tool to identify these crystals or amorphous precipitate in liposomal dispersions.the larger ones

form Maltose crosses when viewed under a light microscope with two cross-positioned polarizing light filters.When phase separation is observed may be estimated by measuring the drug contain phospholipid in the supernatant after 10 to 30 sec.centrifugation nthen filter it.

Lekage via permeation membrane:
Leakage of water soluble compounds out of the liposomes might be estimated by ultracentrifugation followed by quantification of the free drug contain in the supernatant.

4. Determination of percentage release:
Choice of marker: For release of water soluble markers from liposomes.One would like to use a molecule:
a. That does not pass through interact membranes.
b.That is highly water soluble.
c. With a very low solubility in organic media.
d. That does not associate with membranes in any way so as to destabilize or aggreagate them.
e. That can be easily sepearated from liposomes by conventional methods.

Detection method	Marker	Molecular weight
Optical density	Sodium chromate	162
	Cytochrome C	13000
Enzymatic	Glucose	181
	Isocitrate	258
Radiolabel	DNA	millions
	Inulin	5000
Fluroscence	Calcein	620
	Flurescein	319

5. Determination of entrapped volume:
Entrapped volume(lit/mol) = 500/3.A.N.R
A= Area of the membrane occupied by one lipid
N=Avegadros no.
R=Radious

6. Electron microscopy:
Electron microscopy is alternative to estimate the lamillarity of liposomes.A sample is quickly frozen to about -200˚c and subsequently fractured with a sharp knife in vaccum.The fracture plane falls often in the middle of a membrane ,which is one of the weakest region.Finally, an ultrathin metal layer.

7. Phospholipid quantification:
The Bartlett assay:
The principle of the Bartlett assay is based on the colorimetric determination of inorganiv phosphate.The phospholipid content of liposopmes can be determined after destruction of the phospholipid with perchloric acid to inorganic phosphate is converted to phosphomolybdic acid. This compound can be determined colorimetrically at 830nm.

The Stewart assay:
In the Stewart assay for phospholipid, the ability of phospholipids to form a complex with ammonium ferrothiocynate in organic solution is utilized.The advantage of this method is that the presence of inorganic phosphate does not interfare with the assay.

Limitation in liposome technology[1]:
1. Stability
2. Sterilization
3. Encapsulation efficiency
4. Active targeting
5. Gene therapy
6. Lysosomal degradation

CONCLUSION

Liposomes have been used in a broad range of pharmaceutical applications. Liposomes are acceptable and superior carriers and have ability to encapsulate hydrophilic and lipophilic drugs and protect them from degradation. Liposomes are used in sustain release, diagnostic purpose, intracellular delivery systems for proteins/peptides, antisense molecules, ribozymes and DNA.

Development will continue to explore the validity of liposomes for the delivery of peptide and proteins, although progress in thid particular field has been meager. These developments will hopefully safeguard against the overoptimistic and unrealistic ideas and promises of the past and lead into another highly productive and innovative phase of liposome research.

REFERENCES

1. Abolfazl Akbarzadeh *et al* , Nano Review Liposome: classification, preparation, and applications, Nanoscale Research Letters 2013, **8**:102.
2. Chauhan Tikshdeep *et al*, Liposome Drug Delivery: A Review, Department of Pharmacy, Manav Bharti Univesity, Solan, Himachal Pradesh, India. *International journal of pharmaceutical and chemical sciences*.
3. Mukul Kumar Basu,Liposomes in drug targeting,Biomembrane Division,Indian institute of chemical biology,Culcatta,India.
4. Agarwal, K. Bali, A. and Gupta,C.M., Influence of phospholipid structure on the stability of liposomes in serum.Biochimica et. Biophysica Acta 856, 36-40 (1986a)
5. Gabizon, A. Goren, D. Cohen, R. Barenholz Y: Development of liposomal anthracyclines: from basics to clinical applications. *J Control Release* **53**: 275–279 (1998)
6. Pick U: Liposomes with a large trapping capacity prepared by freezing and thawing of sonicated phospholipid mixtures. Arch Biochem Biophys, **212**: 186–194 (1981)
7. Chrai, S.S. Murari, R. Imran A: Liposomes: a review. Bio Pharm, **14(11)**: 10–14 (2001)
8. Wilson, T. Papahadjopoulos, D. Taber, R., The introduction of poliovirus RNA into cells via lipid vesicles-liposomes, Cell, **17**: 77-84 (1979)
9. Kataria Sahil *et al*, Stealth Liposomes-Review, *Intrnational Journal of Ayurveda and Pharmacy*, **2(5)**: 1534-1538 (2011)
10. Shrinath, P. Diwan, P.V. Stealth Liposomes: An overview" *Indian Journal of pharmacology*, **26**:179-184 (1994)
11. Banerjee, R. Tyagi, P. Li, S. Huang, L., Anisamide-targeted stealth liposomes: a potent carrier for targeting doxorubicin to human prostate cancer cells. *Int J Cancer* **112**:693–700 (2004)
12. Dexi, Liu and Young, K. Song: 'Cationic liposome-mediated transfection *in vivo* (review)' Gene Ther Mol Biol. **2**: 59-68 (1998)
13. Himanshu Anwekar *et al*, Liposome- as drug carriers, *International Journal of Pharmacy and Life Sciences*, **2(7)**: 0976-7126 (2011)
14. Riaz M: Liposome preparation method. *Pak J Pharm Sci.* **9(1)**: 65–77 (1996)
15. Allen, T.M. & Hansen, C. Pharmacokinetics of stealth versus conventional liposomes: effect of dose. *Biochim. Biophys. Acta* **1068**: 133–141 (1991)
16. Nicolas, K. Van der Neut, R. Fok, J. J., Dekruyff B., Biochim. Biophys Acta, **819**: 55-65 (1985)