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A COMPREHENSIVE REVIEW ON NOVEL DRUG DELIVERY VIA UNIQUE PROPERTY OF LIPOSOME

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ABSTRACT
Liposomes (blister) are polymeric nanoparticles used for drug delivery due to their unique properties. Liposomes can encapsulate both hydrophobic (water hating) and hydrophilic drug (water loving). Liposomes deliver the drugs into cells by fusion or endocytosis mechanisms. In the last few decades, liposomes have been considered as ideal models for mimic biological membranes and also they are suitable carriers for drugs, diagnostics, vaccines, and other bioactive agents.Among several talented new / Novel delivery systems, liposomes characterize an novel technology to deliver active molecules to the site of action, and at present, several formulations are in clinical use. Research on liposome technology has progressed from conventional vesicles to'second-generation liposomes', in which long-circulating liposomes are obtained by modulating the lipidcomposition, size, and charge of the vesicle. Various aspects related to mechanism of liposome formation, characterization and stability of the liposomal drug product were alsodiscussed in the article. Liposomes can be used as a therapeutic tool in the fields like tumor targeting, genetic transfer, immune modulation, skinand topical therapy.

KEYWORDS: Liposomes, Drug Delivery, Nanoparticles.

INTRODUCTION
The word liposome comes from two Greek words, lipos (fat) and soma (body or structure).[1, 2] Liposomes are small artificial vesicles of spherical shape that can be created from cholesterol and natural nontoxic phospholipids. Due to their size and hydrophobic and hydrophilic character (besides biocompatibility), liposomes are promising systems for drug delivery. Liposome properties differ considerably with lipid composition, surface charge, size, and the method of preparation. Furthermore, the choice of bilayer components determines the 'rigidity' or 'fluidity' and the charge of the bilayer. For instance, unsaturated phosphatidylcholine species from natural sources (egg or soybean phosphatidylcholine) give much more permeable and less stable bilayers, whereas the saturated phospholipids with long acyl chains (for example, dipalmitoylphosphatidylcholine) form a rigid, rather impermeable bilayer structure.[3-5]

They were firstly introduced by British hematologist, Bangham and his students in the mid-1960s. Bangham was found phospholipids combined with water immediately formed a sphere because one end of each molecule is water soluble, while the opposite end is water insoluble.[6] Liposomes were introduced as drug-delivery vehicles in the 1970s.[7]

It has been displayed that phospholipids impulsivelyform closed structures when they are hydrated in aqueoussolutions. Such vesicles which have one or morephospholipid bilayer membranes can transport aqueousor lipid drugs, depending on the nature of those drugs. Because lipids are amphipathic (both hydrophobic andhydrophilic) in aqueous media, their thermodynamic phase properties and self assembling characteristics influence entropically focused confiscation of their hydrophobicsections into spherical bilayers. Those layers are referred to as lamellae.[8] Generally, liposomes are definiteas spherical vesicles with particle sizes ranging from30 nm to several micrometers. They consist of one ormore lipid bilayers surrounding aqueous units, wherethe polar head groups are oriented in the pathway of theinterior and exterior aqueous phases. On the other hand, self-aggregation of polar lipids is not limited to conventionalbilayer structures which rely on molecular shape,temperature, and environmental and preparation conditionsbut may self-assemble into various types of colloidal particles.[9]

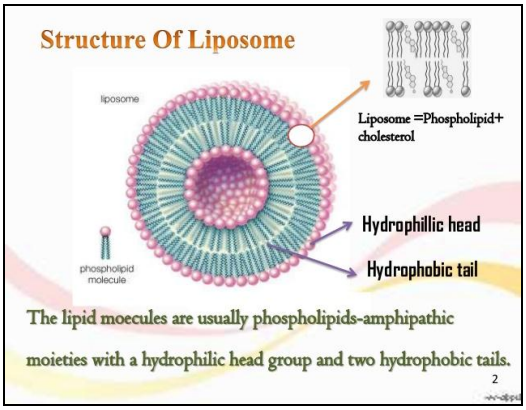

Fig 1: Structure of Phospho-cholestro liposomal vesicle.

HISTORY OF LIPOSOMES

The history of liposomes can be divided into three periods: genesis, middle age and modern era.

Genesis (1968-75): The physiochemical characterizations of liposomes have been investigated. In this period liposomes were used to study the nature of biological membrane and thin lipid film hydration method was developed to prepare liposomes.

Middle age (1975 – 85): In this period, advantages, stability and interaction characteristic, liposomes, physico-chemical properties of liposomes, their interaction with the cells and their behavior within the body were studied. Also, various methods for the preparation of liposomes discovered.

Modern era (1985 onwards): Today, liposomes are used in different scientific fields such as biophysics (properties of cell membranes and channels), mathematics, biochemistry (function of membrane proteins), theoretical physics (topology of two-dimensional surfaces floating in a three dimensional continuum) and biology (excretion, cell function, signaling, gene delivery and function).[10]

ADVANTAGES AND DISADVANTAGES OF LIPOSOME[11]

Advantage of liposome

- Non ionic
- Can carry both water and lipid solubledrugs
- Biodegradable drugs can be stabilizedfrom oxidation
- Improve protein stabilization
- Controlled hydration
- Provide sustained release
- Targeted drug delivery or site specificdrug delivery
- Stabilization of entrapped drug fromhostile environment
- Alter pharmacokinetics and pharmacodynamics of drugs
- Can be administered through variousroutes
- Can incorporate micro and macromolecules
- Act as reservoir of drugs
- Therapeutic index of drugs is increased
- Site avoidance therapy
- Can modulate the distribution of drug
- Direct interaction of the drug with cell
- Biodegradable and flexible

Disadvantages

- Less stability
- Low solubility
- Short half life
- Phospholipids undergoes oxidation,hydrolysis
- Leakage and fusion
- High production cost
- Quick uptake by cells of R.E.S
- Allergic reactions may occur to liposomalconstituents
- Problem to targeting to various tissuesdue to their large size

MECHANISM OF LIPOSOME FORMATION:

The basic part of liposome is formed byphospholipids, which are amphiphilicmolecules(having a hydrophilic head and hydrophobictail). The hydrophilic part is mainly phosphoricacid bound to a water soluble molecule,whereas, the hydrophobic part consists of twofatty acid chains with 10 – 24 carbon atoms and0 – 6 double bonds in each chain.[12]

When these phospholipids are dispersed inaqueous medium, they form lamellar sheets byorganizing in such a way that, the polar headwhich permits unrestrictedgroup faces outwards to the aqueous regionwhile the fatty acid groups face each other andfinally form spherical/ vesicle like structures calledas liposomes. The polar portion remains incontact with aqueous region along with shieldingof the non-polar part (which is oriented at anangle to the membrane surface).[13]

When phospholipids are hydrated in water, alongwith the input of energy like sonication, shaking,heating, homogenization, etc. it is thehydrophilic/ hydrophobic interactions betweenlipid – lipid, lipid – water molecules that lead to the formation of bilayered vesicles in order toachieve a thermodynamic equilibrium in theaqueous phase.[14] The reasons for bilayered formation include.

- The unfavourable interactions createdbetween hydrophilic and hydrophobic phase can be minimized by folding intoclosed concentric vesicles.
- Large bilayered vesicle formation promotesthe reduction of large free energydifference present between the hydrophilicand hydrophobic environment.
- Maximum stability to supramolecular selfassembled structure can be attained byforming into vesicles.[15]

STRUCTURAL COMPONENTS[16,17]

a) Phospholipids

Glycerol containing phospholipids are mostcommon used component of liposomeformulation and represent greater than 50% ofweight of lipid in biological membranes. These are derived from phosphatidic acid.

The backbone of the molecule is glycerol moiety. At C_3position OH group is esterified to phosphoricacid. OH at C_1 & C_2 are esterified with long chain. Fatty acid giving rise to the lipidic nature. One ofthe remaining OH group of phosphoric acid maybe further esterified to a wide range of organicalcohols including glycerol, choline, ethanolamine, serine and inositol. Thus theparent compound of the series is the phosphoricester of glycerol.

Examples of phospholipids are

- Phosphatidyl choline (Lecithin) – PC
- Phosphatidyl ethanolamine (cephalin) –PE
- Phosphatidyl serine (PS)
- Phosphatidyl inositol (PI)
- Phosphatidyl Glycerol (PG)

For stable liposomes, saturated fatlyacids are used. Unsaturated fatty acidsare not used generally.

b) Sphingolipids

Backbone is sphingosine or a related base. These are important constituents of plant andanimal cells. A head group that can vary fromsimple alcohols such as choline to very complex carbohydrates.

Most common Sphingolipids -Sphingomyelin. Glycosphingo lipids.
Gangliosides – found on grey matter, used as aminor component for liposome production.
This molecule contain complex saccharides withone or more Sialicacid residues in their polarhead group & thus have one or more negative charge at neutral pH. These are included inliposomes to provide a layer of surface chargedgroup.

c) Sterols

- Cholesterol & its derivatives are often included in liposomes for
- decreasing the fluidity or micro viscocity of the bilayer
- reducing the permeability of the membrane to water soluble molecules
- Stabilizing the membrane in the presence of biological fluids such as plasma.(This effect used in formulation of i.v. liposomes)

d) Synthetic phospholipids

E.g.: for saturated phospholipids are

- Dipalmitoylphosphatidyl choline (DPPC)
- Distearoylphosphatidyl choline (DSPC)
- Dipalmitoylphosphatidyl ethanolamine (DPPE)
- Dipalmitoylphosphatidyl serine (DPPS)
- Dipalmitoylphosphatidic acid (DPPA)
- Dipalmitoylphosphatidyl glycerol (DPPG)

E.g.: for unsaturated phospholipids

- Dioleoylphosphatidyl choline (DOPC)
- Dioleoylphosphatidyl glycerol (DOPG)

e) Polymeric materials

Synthetic phospholipids with diactylenic group in the hydrocarbon chainpolymerizes when exposed to U.V,leading to formation of polymerizedliposomes having significantly higherpermeability barriers to entrappedaqueous drugs. e.g. for other Polymerisable lipids are – lipidscontaining conjugated diene, methacrylate etc..

f) Other Substances

- Variety of other lipids of surfactants are used to form liposomes.
- Many single chain surfactants can form liposomes on mixing with cholesterol.
- Non ionic lipids.
- A variety of poly glycerol and poly ethoxylated mono and dialkyl amphiphiles used mainly in cosmetic preparations.
- Single and double chain lipids having fluoro carbon chains can form very stable liposomes.
- Sterylamine and dicetyl phosphate.
- Incorporated into liposomes so as to impart either a negative or positive surface charge to these structures.
- A number of compounds having a single long chain hydrocarbon and an ionic head group found to be capable of forming vesicles. These include quaternary ammonium salts of dialkyl phosphates.

CLASSIFICATION OF LIPOSOMES

The liposome size can vary from very small (0.025 µm)to large (2.5 µm) vesicles. Moreover, liposomes may haveone or bilayer membranes. The vesicle size is an acuteparameter in determining the circulation half-life ofliposomes, and both size and number of bilayers affectthe amount of drug encapsulation in the liposomes. Onthe basis of their size and number of bilayers, liposomescan also be classified into one of two categories: (1)multilamellar vesicles (MLV) and (2) unilamellar vesicles.Unilamellar vesicles can also be classified into twocategories: (1) large unilamellar vesicles (LUV) and (2)small unilamellar vesicles (SUV).[18]

In unilamellarliposomes, the vesicle has a single phospholipid bilayersphere enclosing the aqueous solution. In multilamellarliposomes, vesicles have an onion structure. Classically,several unilamellar vesicles will form on the inside of theother with smaller size, making a multilamellar structureof concentric phospholipid spheres separated by layersof water.[19]

METHODS OF LIPOSOME PREPARATION

General methods of preparation

All the methods of preparing the liposomes involve fourbasic 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 oflipsome.
A. Passive loading techniques
B. Active loading technique.

Passive loading techniques include three differentmethods.
1. Mechanical dispersion method.
2. Solvent dispersion method.
3. Detergent removal method (removal of non-encapsulated material).

Mechanical dispersion method
The following are types of mechanical dispersionmethods.
1.1. Sonication.
1.2. French pressure cell: extrusion.
1.3. Freeze-thawed liposomes.
1.4. Lipid film hydration by hand shaking, non-hand.shaking or freeze drying.
1.5. Micro-emulsification.
1.6. Membrane extrusion.
1.7. Dried reconstituted vesicles.[20,21]

Sonication: Sonication is perhaps the most extensivelyused method for the preparation of SUV. Here, MLVsare sonicated either with a bath type sonicator or aprobesonicator under a passive atmosphere. The maindisadvantages of this method are very low internal volume/encapsulation efficacy, possible degradation ofphospholipids and compounds to be encapsulated, eliminationof large molecules, metal pollution from probetip, and presence of MLV along with SUV.[20]

There are two sonication techniques
a) Probe sonication: The tip of a sonicator is directlyengrossed into the liposome dispersion. The energyinput into lipid dispersion is very high in thismethod. The coupling of energy at the tip results inlocal hotness; therefore, the vessel must be engrossedinto a water/ice bath. Throughout the sonication upto 1 h, more than 5% of the lipids can be deesterified.Also, with the probe sonicator, titaniumwill slough off and pollute the solution.
b) Bath sonication: The liposome dispersion in acylinder is placed into a bath sonicator. Controllingthe temperature of the lipid dispersion is usuallyeasier in this method, in contrast to sonication bydispersal directly using the tip. The material beingsonicated can be protected in a sterile vessel, dissimilar the probe units, or under an inertatmosphere.[22]

French pressure cell: extrusion French pressure cellinvolves the extrusion of MLV through a small orifice.[20] An important feature of the French press vesiclemethod is that the proteins do not seem to be significantlypretentious during the procedure as they are insonication.[23] An interesting comment is that Frenchpress vesicle appears to recall entrapped solutes significantlylonger than SUVs do, produced by sonication ordetergent removal.[24-26]

The method involves gentle handling of unstablematerials. The method has several advantages over sonicationmethod.[27] The resulting liposomes are rather larger than sonicated SUVs. The drawbacks of themethod are that the high temperature is difficult to attain, and the working volumes are comparatively small(about 50 mL as the maximum).[20,21]

Freeze-thawed liposomes SUVs are rapidly frozen andthawed slowly. The short-lived sonication dispersesaggregated materials to LUV. The creation of unilamellar vesicles is as a result of the fusion of SUVthroughout the processes of freezing and thawing.[28-30] This type of synthesis is strongly inhibited byincreasing the phospholipid concentration and by increasingthe ionic strength of the medium. The encapsulationefficacies from 20% to 30% were obtained.[28]

Solvent dispersion method
Ether injection (solvent vaporization)A solution oflipids dissolved in diethyl ether or ether-methanol mixtureis gradually injected to an aqueous solution of thematerial to be encapsulated at 55°C to 65°C or underreduced pressure. The consequent removal of etherunder vacuum leads to the creation of liposomes. Themain disadvantages of the technique are that the populationis heterogeneous (70 to 200 nm) and the exposureof compounds to be encapsulated to organic solvents at high temperature.[31,32]

Ethanol injection A lipid solution of ethanol is rapidlyinjected to a huge excess of buffer. The MLVs are atonce formed. The disadvantages of the method are thatthe population is heterogeneous (30 to 110 nm), liposomes are very dilute, the removal all ethanol isdifficult because it forms into azeotrope with water, and the probability of the various biologically activemacromolecules to inactivate in the presence of evenlow amounts of ethanol is high.[33]

Reverse phase evaporation method: This methodprovided a progress in liposome technology, since itallowed for the first time the preparation of liposomeswith a high aqueous space-to-lipid ratio and a capabilityto entrap a large percentage of the aqueous materialpresented. Reverse-phase evaporation is based on thecreation of inverted micelles. These inverted micelles areshaped upon sonication of a mixture of a buffered aqueousphase, which contains the water-soluble moleculesto be encapsulated into the liposomes and an organicphase in which the amphiphilic molecules are solubilized. The slow elimination of the organic solventleads to the conversion of these inverted micelles intoviscous state and gel form. At a critical point in thisprocess, the gel state collapses, and some of the

invertedmicelles were disturbed. The excess of phospholipids inthe environment donates to the formation of a completebilayer around the residual micelles, which results in thecreation of liposomes. Liposomes made by reverse phaseevaporation method can be made from numerous lipidformulations and have aqueous volume-to-lipid ratiosthat are four times higher than hand-shaken liposomesor multilamellar liposomes[21,22]

Briefly, first, the water-in-oil emulsion is shaped bybrief sonication of a two-phase system, containingphospholipids in organic solvent such as isopropyl etheror diethyl ether or a mixture of isopropyl ether andchloroform with aqueous buffer. The organic solventsare detached under reduced pressure, resulting in thecreation of a viscous gel. The liposomes are shapedwhen residual solvent is detached during continued rotaryevaporation under reduced pressure. With thismethod, high encapsulation efficiency up to 65% can beobtained in a medium of low ionic strength for example0.01 M NaCl. The method has been used to encapsulatesmall, large, and macromolecules. The main drawback ofthe technique is the contact of the materials to beencapsulated to organic solvents and to brief periods ofsonication. These conditions may possibly result in thebreakage of DNA strands or the denaturation of someproteins.[34]

Modified reverse phase evaporationmethod was presented by Handa et al., and the main benefit of the method is that the liposomes had high encapsulationefficiency (about 80%).[35]

Detergent removal method (removal of non-encapsulatedmaterial)

Dialysis The detergents at their critical micelleconcentrations (CMC) have been used to solubilise lipids. As the detergent is detached, the micelles becomeincreasingly better-off in phospholipid and lastly combineto form LUVs. The detergents were removed bydialysis.[36-38] A commercial device called LipoPrep (Diachema AG, Switzerland), which is a version ofdialysis system, is obtainable for the elimination ofdetergents. The dialysis can be performed in dialysisbags engrossed in large detergent free buffers (equilibriumdialysis).[19]

Detergent (cholate, alkyl glycoside, Triton X-100) removalof mixed micelles (absorption) Detergentabsorption is attained by shaking mixed micelle solutionwith beaded organic polystyrene adsorbers suchas XAD-2 beads (SERVA Electrophoresis GmbH,Heidelberg, Germany) and Bio-beads SM2 (Bio-RadLaboratories, Inc., Hercules, USA). The great benefit of using detergent adsorbers is that they can eliminatedetergents with a very low CMC, which are not entirelydepleted.

Gel-permeation chromatography In this method, thedetergent 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 usedfor gel filtration. The liposomes do not penetrate intothe pores of the beads packed in a column. They percolatethrough the inter-bead spaces. At slow flow rates,the separation of liposomes from detergent monomers isvery good. The swollen polysaccharide beads adsorbsubstantial amounts of amphiphilic lipids; therefore, pretreatmentis necessary. The pre-treatment is done bypre-saturation of the gel filtration column by lipids usingempty liposome suspensions.

Dilution

Upon dilution of aqueous mixed micellar solution of detergentand phospholipids with buffer, the micellar sizeand the polydispersity increase fundamentally, and asthe system is diluted beyond the mixed micellarphaseboundary, a spontaneous transition from poly-dispersedmicelles to vesicles occurs.

Stealth liposomes and conventional liposomes

Although liposomes are like biomembranes, they are stillforeign objects of the body. Therefore, liposomes areknown by the mononuclear phagocytic system (MPS)after contact with plasma proteins. Accordingly, liposomesare cleared from the blood stream.These stability difficulties are solved through the useof synthetic phospholipids, particle coated with amphipathicpolyethylene glycol, coating liposomes with chitinderivatives, freeze drying, polymerization, micro-encapsulationofgangliosides.[19]

Coating liposomes with PEG reduces the percentage ofuptake by macrophages and leads to a prolonged presenceof liposomes in the circulation and, therefore, makeavailable abundant time for these liposomes to leak fromthe circulation through leaky endothelium.A stealth liposome is a sphere-shaped vesicle with amembrane composed of phospholipid bilayer used to deliverdrugs or genetic material into a cell. A liposomecan be composed of naturally derived phospholipids withmixed lipid chains coated or steadied by polymers ofPEG and colloidal in nature. Stealth liposomes areattained and grown in new drug delivery and in controlledrelease. This stealth principle has been used todevelop the successful doxorubicin-loaded liposomeproduct that is presently marketed as Doxil (JanssenBiotech, Inc., Horsham, USA) or Caelyx (Schering-Plough Corporation, Kenilworth, USA) for the treatmentof solid tumors. Recently impressive therapeutic improvementswere described with the useofcorticosteroidloadedliposome in experimental arthritic models. Theconcerning on the application of stealth liposomes hasbeen on their potential to escape from the blood circulation.However, long circulating liposome may also act asa reservoir for prolonged release of a therapeutic agent.Pharmacological action of

vasopressin is formulated inlong circulating liposome.[39,40]

Drug loading in liposomes

Drug loading can be attained either passively (i.e., thedrug is encapsulated during liposome formation) oractively (i.e., after liposome formation). Hydrophobicdrugs, for example amphotericin B taxol or annamycin,can be directly combined into liposomes during vesicleformation, and the amount of uptake and retention isgoverned by drug-lipid interactions. Trapping effectivenessof 100% is often achievable, but this is dependenton the solubility of the drug in the liposome membrane.Passive encapsulation of water-soluble drugs depends onthe ability of liposomes to trap aqueous buffercontaining a dissolved drug during vesicle formation.Trapping effectiveness (generally <30%) is limited by thetrapped volume delimited in the liposomes and drugsolubility. On the other hand, water-soluble drugs that have protonizable amine functions can be actively entrappedby employing pH gradients[41], which can resultin trapping effectiveness approaching 100%.[42]

Freeze-protectant for liposomes (lyophilization)

Natural excerpts are usually degraded because of oxidationand other chemical reactions before they aredelivered to the target site. Freeze-drying has been astandard practice employed to the production of manypharmaceutical products. The overwhelming majority ofthese products are lyophilized from simple aqueoussolutions. Classically, water is the only solvent that must bedetached from the solution using the freeze-drying process,but there are still many examples where pharmaceuticalproducts are manufactured via a process that requiresfreeze-drying from organic co-solvent systems.[43]

Freeze-drying (lyophilization) involves the removal ofwater from products in the frozen state at tremendouslylow pressures. The process is normally used to dryproducts that are thermo-labile and would be demolishedby heat-drying. The technique has too muchpotential as a method to solve long-term stability difficultieswith admiration to liposomal stability. Studiesshowed that leakage of entrapped materials may takeplace during the process of freeze-drying and on reconstitution.Newly, it was shown that liposomes whenfreeze-dried in the presence of adequate amounts of trehalose(a carbohydrate commonly found at highconcentrations in organism) retained as much as 100%of their original substances. It shows that trehalose is anexcellent cryoprotectant (freeze-protectant) for liposomes.Freeze-driers range in size from small laboratorymodels to large industrial units available from pharmaceutical equipment suppliers.[44]

CHARATERIZATION OF LIPOSOMES

Liposomes produced by different methods havevarying physicochemical characteristics, whichleads to differences in their in vitro (sterilizationand shelf life) and in vivo (disposition)performances.[45-47] Rapid, precise andreproducible quality control tests are required forcharacterizing the liposomes after theirformulation and upon storage for a predictablein vitro and in vivo behavior of the liposomal drugproduct.[48,49] A liposomal drug product can becharacterized for some of the parameters thatare discussed below.

Size and size distribution

When liposomes are intended for inhalation orparenteral administration, the size distribution is ofprimary consideration, since it influences the invivo fate of liposomes along with theencapsulated drug molecules.[50-54] Various techniques of determing the size of the vesiclesinclude microscopy (optical microscopy[55], negative stain transmission electron microscopy[46], cryo-transmission electron microscopy[56], freeze fracture electron microscopy andscanning electron microscopy[49]), diffractionand scattering techniques (laser light scatteringand photon correlation spectroscopy)[49] andhydrodynamic techniques (field flowfractionation[57], gel permeation[58] andultracentrifugation).

Percent drug encapsulation

The amount of drug encapsulated/ entrapped inliposome vesicle is given by percent drugencapsulation. Column chromatography can beused to estimate the percent drug encapsulationof liposomes.[59] The formulation consists of both free (un-encapsulated) and encapsulated drug. So as to know the exact amount of drugencapsulated, the free drug is separated fromthe encapsulated one.Then the fraction ofliposomes containing the encapsulated drug istreated with a detergent, so as to attain lysis, which leads to the discharge of the drug from thevesicles into the surrounding medium. Thisexposed drug is assayed by a suitable techniquewhich gives the percent drug encapsulated fromwhich encapsulation efficiency can becalculated.[60-63]

Trapped volume per lipid weight can also givethe percent drug encapsulated in a liposomevesicle. It is generally expressed as aqueousvolume entrapped per unit quantity of lipid,µl/µmol or µg/mg of total lipid.[45,47] Inorder todetermine the trapped volume, various materialslike radioactive markers, fluorescent markers and spectroscopically inert fluid[64] can be used.Radioactive method is mostly used fordetermining trapped volume.[45] It is determinedby dispersing lipid in an aqueous mediumcontaining a non-permeable radioactive solutelike[22Na] or [14C] inulin.[65] Alternatively, watersoluble markers like 6-carboxyfluorescein, 14C or3H-glucose or sucrose can be used to determinethe trapped volume.[49] A novel method of determining intravesicular volume by salt entrapment was also reported in literature.[66]

Surface charge

Since the charge on the liposome surface plays akey role in the in vivo disposition, it is essential toknow the

surface charge on the vesicle surface.Two methods namely, free-flow electrophoresisand zeta potential measurement can be used toestimate the surface charge of the vesicle. Thesurface charge can be calculated by estimatingthe mobility of the liposomal dispersion in asuitable buffer (determined using Helmholtz–Smolochowski equation).[67]

Vesicle shape and lamellarity

Various electron microscopic techniques can beused to assess the shape of the vesicles. Thenumber of bilayers present in the liposome, i.e., lamellarity can be determined using freezefractureelectron microscopy[45] and 31P-Nuclearmagnetic resonance analysis. Apart fromknowing the shape and lamellarity, the surfacemorphology of liposomes can be assessed usingfreeze-fracture and freeze-etch electronmicroscopy.[68]

Phospholipid identification and assay

The chemical components of liposomes must be analyzed prior to and after the preparation.[49] Barlett assay[69], Stewart assay[70] and thin layerchromatography[71] can be used to estimate thephospholipid concentration in the liposomalformulation. A spectrophotometric method toquantify total phosphorous in a sample was givenin literature, which measure the intensity of blue color developed at 825 nm against water.[72] Cholesterol oxidase assay or ferric perchloratemethod[73] and Gas liquid chromatographytechniques can be used to determine thecholesterol concentration.[74]

APPLICATION OF LIPOSOME[75]

1) Liposome as drug/protein delivery vehicle.
- Controlled and sustained drug release in situ
- Enchaned drug solubilization
- Altered pharmacokinetic and biodistribution
- Enzyme replacement therapy and lysosomal disorders

2) Liposome in antimicrobial, antifungal andantiviral therapy.
- Liposomal drugs
- Liposomal biological response modifier

3) Liposomes in tumour therapy
- Crrrier of small cytotoxic molecule
- Vehicle for macromolecule as cytokines or genes

4) Liposome in gene therapy
- Gene and antisence therapy
- Genetic (DNA) vaccination

5) Liposome in immunology
- Immunoadjuvant
- Immunomodulator
- Immunodiagnosis

6) Liposome as artificial blood surrogates
7) Lipososmes as radiopharmaceutical and radiodiagnostic carrier
8) Liposomes in cosmetics and dermatology
9) Liposomes in enzyme immobilization and bioreactor technology.

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