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LIPID-BASED NANOPARTICULATE DRUG DELIVERY SYSTEMS: AN OVERVIEW

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ABSTRACT

Liposomes are microparticulate lipid vesicles that are under extensive investigation as drug carriers for improving the delivery of therapeutic agents. They are by far the most advanced nanoparticle systems for the systemic delivery of biologically active agents, with over seven approved products and many others in advanced clinical trials. There are four major areas of activity. The first, and best developed, area concerns the delivery of established, small molecule “conventional” drugs with the intent of reducing toxicity and/or increasing efficacy. The second concerns the utility of liposomal nanoparticles for delivery of plasmid DNA for gene therapy applications, whereas the third area involves the application of liposomal formulations of oligonucleotides for immunostimulatory applications. Finally, there are considerable opportunities for the use of liposomes for delivery of oligonucleotides for “gene silencing” applications. This review discusses advantages of liposomes, preparation and characterization, applications, formulation strategies and limitations of liposomes.

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INTRODUCTION

Phospholipids, upon hydration, spontaneously form bilayer membrane vesicles (liposomes) or may act as surfactants in forming micro- or nanoemulsions or solid-lipid nanoparticles. Phospholipids, triglycerides, and cholesterol are the main ingredients of liposomes and lipid nanoparticles. They are natural components of biological membranes and lipoproteins and are, therefore, presumed to be highly biocompatible (1). Drugs and cell-targeting ligands can be incorporated into these structures by encapsulation (for hydrophilic molecules), lipid-phase solubilization (for lipophilic molecules), conjugation to a lipid anchor (as a lipid-derivatized prodrug), or electrostatic complexation (for poly-anionic molecules such as nucleic acids), depending on their specific physicochemical properties. Liposomes and lipid nanoparticles smaller than 300 nm are potentially suitable for systemic administration.

LIPOSOMES:

Liposomes were discovered in the early 1960's by Bangham and colleagues and subsequently became the most extensively explored drug delivery system. Structurally liposomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic phospholipids. Liposomes are formed when phospholipids are hydrated. The most common natural phospholipids are phosphatidylcholine (PC). These are amphiphilic molecules in which a glycerol bridge links to a pair of hydrophobic acyl hydrocarbon chains with a hydrophilic polar head group phosphocholine. Amphiphilic nature of phospholipids and their analogues render them the ability to form closed concentric bilayers in the presence of water. Liposomes are formed when thin films of amphiphilic nature 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 (MLVs). Sonification is done to get small unilamellar vesicles (SUVs). Extrusion is also done to get large unilamellar vesicles (LUVs). Several methods exist for improved loading of drugs using pH gradients and potential difference across liposomal membranes. The pH gradient is created by preparing liposomes with a

low pH inside the vesicles followed by the addition of the base to the extra liposomal medium. Accumulation occurs at the low pH side. So the unprotonated form of basic drug can diffuse through the bilayer. At the low pH side, the molecules are predominately protonated which lowers the concentration of drug in the unprotonated form and thus promotes the diffusion of more molecules at the low pH side of the bilayer. Stealth liposomal technology is designed for the intravenous drug delivery.

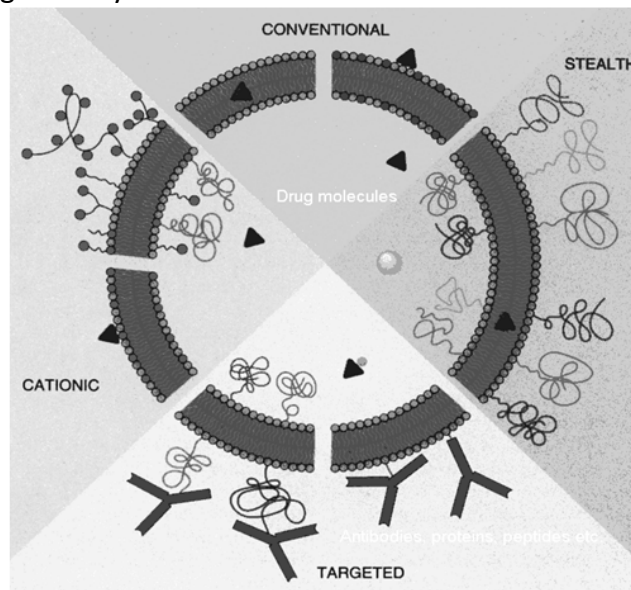


Figure 1: Schematic presentation of four types of liposomes (Small unilamellar vesicles).

ADVANTAGES OF LIPOSOMES:

- Provide controlled drug delivery
- Biodegradable, biocompatible, flexible
- Non ionic
- Can carry both water and lipid soluble drugs
- Drugs can be stabilized from oxidation
- Improve protein stabilization
- Controlled hydration
- Provide sustained release
- Targeted drug delivery or site specific drug delivery
- Stabilization of entrapped drug from hostile environment

- Alter pharmacokinetics and pharmacodynamics of drugs
- Can be administered through various routes
- Can incorporate micro and macro molecules
- 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

PREPARATION AND CHARACTERIZATION OF LIPOSOMES:

Liposomes form spontaneously when phospholipids are hydrated. Additional steps are often necessary to modify the size distribution and lamellarity of liposomes. Several methods have been established for liposome preparation based on the scale of the preparation and other considerations, such as drug encapsulation efficiency. The first step in liposome preparation is to dissolve lipid ingredients in a suitable solvent; this is then followed by lipid hydration and particle size reduction. For example, lipids are first dissolved in chloroform/methanol and dried into a thin film on a rotary evaporator and are then hydrated in an aqueous buffer above the phase-transition temperature (2). This process will result in the formation of heterogeneous multilamellar vesicles (MLVs.) The lamellarity of the MLVs can be reduced by repeated cycles of freezing and thawing. If phospholipids are dissolved in a water-miscible solvent such as ethanol and rapidly diluted into an aqueous buffer, liposomes with relative small particle sizes can be generated directly. This is then followed by removal of the solvent from the liposome preparation and/or further size- modifying steps (3).

Particle size can then be reduced by a number of methods, including sonication, extrusion, and homogenization. Sonication, typically using an ultrasonic probe sonicator, can reduce liposome particle size by inducing cavitation (4). Extrusion is performed using a high-pressure filter unit, such as the Lipex™ extruder by Northern Lipids, Inc., containing a track-etched polycarbonate membrane at a temperature above the phase-transition temperature of the liposomal bilayer

(5). The polycarbonate membrane has straight through cylindrical pores of precise diameters and can withstand pressure of 3000 psi with proper support. Liposomes extruded through the polycarbonate membranes typically have narrow particle size distribution. Large-scale production of liposomes is possible using the extrusion method employing high-surface-area extrusion filter units. Another potentially scalable method for liposome particle size reduction is high-pressure homogenization usually at pressures above 5000 psi (2). This method can be used for continuous production of liposomes or nanoparticles at large scale. In the lab-scale, liposomes can be synthesized by detergent dialysis in which lipids are first solubilized in a dialyzable detergent, such as octylglucoside and then dialyzed against a buffer (6). Alternatively, reversed-phase evaporation method may be used to form a water-in-oil emulsion in a volatile organic solvent followed by phase inversion upon solvent removal. This method was designed to maximize entrapment efficiency of water-soluble agents (7).

Methods suitable for drug loading into liposomes depend on the properties of the drug. Lipophilic drugs can be codissolved with the lipids during liposome preparation. Hydrophilic drugs can be passively entrapped into liposomes during liposome formation. Alternatively, drugs can be incorporated into liposomes by a pH gradient-driven remote loading procedure. For example, an inward-directed pH gradient could be established by entrapment of a low pH buffer (e.g., pH 4 sodium citrate) or by entrapping ammonium sulfate followed by external buffer exchange resulting in reduction of intraliposomal pH. Addition of a weakly basic drug, such as doxorubicin and vincristine, results in near-quantitative loading of the drug into the liposomes due to intraliposomal protonation of the drug molecules and complexation with entrapped counterion (8,9). For polyelectrolytes such as DNA, loading into liposomes can be achieved by electrostatic complexation with incorporation of cationic lipids into the liposome composition (10,11). Structure of DNA complexes of cationic liposomes may not be similar to the structure of typical liposomes and is highly dependent on lipid head

group structure, cationic-to-anionic charge ratio, and kinetics of complex formation (12).

Liposomes can be purified by a number of methods. At the lab scale, liposomes can be purified based on their size by high-speed centrifugation, size exclusion chromatography, or dialysis (3). At a larger scale, liposomes can be purified by tangential flow diafiltration (13). Liposomes can also be lyophilized in the presence of a cryoprotectant, typically a disaccharide such as sucrose, lactose, or trehalose, which can prevent vesicle fusion and particle size increase, although significant leakage of aqueous content may occur upon rehydration of the liposomes (14).

A liposomal formulation can be characterized by a number of established methods. First, particle size distribution can be measured by dynamic light scattering, by cryo- or negative-staining electron microscopy, or by atomic force microscopy. Surface electrical property of liposomes can be measured by zeta potential measurement. Other useful analyses include colloidal stability and rate of drug release in storage and in plasma by dialysis, kinetics of uptake, and internalization of fluorescence labeled liposomes in cultured cells by fluorescence microscopy and flow cytometry. Cytotoxicity of drug-carrying and empty liposomes can be studied in tissue culture. Furthermore, plasma clearance kinetics, tissue biodistribution, toxicity, and therapeutic efficacy of drug-carrying liposomes can be assessed in appropriate animal models.

Table 1: preparation methods of liposomes

Method	
Vesicles	
Mechanical methods	
Vortex or hand shaking of phospholipid dispersions	MLV
Extrusion through polycarbonate filters at low or medium pressure	OLV, LUV
Extrusion through a French press cell "Microfluidizer" technique	Mainly SUV
High-pressure homogenization	Mainly SUV

Ultrasonic irritation
SUV of minimal size
Bubbling of gas
BSV

Methods based on replacement of organic solvent(s) by aqueous media

Removal of organic solvent(s)	MLV, OLV, SUV
Use of water-immiscible solvents:	
ether and petroleum	MLV, OLV, LUV
Ethanol injection method	LUV
Ether infusion (solvent vaporization)	LUV, OLV, MLV
Reverse-phase evaporation	

Methods based on detergent removal

Gel exclusion chromatography	SUV
"Slow" dialysis	LUV, OLV, MLV
Fast dilution	LUV, OLV
Other related techniques	MLV, OLV, LUV, SUV

LIPOSOMES AS DRUG CARRIERS:

The application of liposomes as a drug-delivery system has become more popular over the last decades, because of their biocompatibility and versatility in carrying systemically administered drugs such as chemotherapeutics and antibiotics with narrow therapeutic windows. A variety of therapeutic agents have been incorporated into liposomes. Several have reached clinical use. These include liposomal doxorubicin (15) (Doxil™), daunorubicin (16) (Daunoxome™), amphotericin B (17) (Amphotec™, Ambisome™, and Abelcet™), cytarabine (18) (Depocyte™), and verteporfin (19) (Visudyne™). Numerous liposomal formulations are in clinical trial, including those for vincristine, all-trans retinoic acid, topotecan, and cationic liposome-based therapeutic gene transfer vectors. Many more are in preclinical evaluation including liposomal formulations of chemotherapeutics, neutron capture agents, oligonucleotides, plasmid DNA, photosensitizers, antibiotics, and vaccines (20). Besides potential use in systemic gene delivery, cationic liposomes are routinely used as transfection reagents for plasmid DNA and oligonucleotides in the laboratory.

Liposomal delivery of anticancer drugs has been shown to greatly extend their systemic circulation time, reduce toxicity by lowering plasma free drug concentration, and facilitate preferential localization of drugs in solid tumors based on increased endothelial permeability and reduced lymphatic drainage, or enhanced permeability and retention (EPR) effect (21–23). For example, liposomal entrapment of doxorubicin greatly reduces its dose-limiting cardiotoxicity. Clearance of drugs in a liposomal formulation is mediated by phagocytic cells of the reticuloendothelial system (RES), primarily located in liver and spleen. Liposomal entrapment can protect drugs such as nucleic acids from rapid metabolism by plasma enzymes (24). In addition, liposomal delivery of drugs appears to mediate bypassing of P-glycoprotein (Pgp)-related multidrug resistance in tumor cells. Liposomes also present a platform for delivery of drug combinations. For example, Wang et al. (25) coencapsulated doxorubicin and verapamil (a Pgp inhibitor) into liposomes and studied their *in vitro* cytotoxicity. The result demonstrated effective reversal of multidrug resistance in doxorubicin-resistant cell lines.

FORMULATION STRATEGIES FOR LIPOSOMES:

Sterically Stabilized Liposomes:

RES clearance of liposomes is mediated by adsorption of plasma proteins to the bilayer surface. Incorporation of 3 to 10 mol% of polyethylene glycol (PEG)-conjugated lipid, such as monomethoxy-PEG (molecular weight 2000)-distearoyl phosphatidylethanolamine (mPEG2000-DSPE) has been shown to greatly extend the circulation time of liposomes by providing steric hindrance on the bilayer surface (21,22). PEGylated liposomes exhibit circulation half-life of up to two days compared to several hours for non-PEGylated liposomes. The prolongation in mean residence time of PEGylated liposomes is due to slower clearance of these liposomes by RES organs (26). This can, in addition, increase EPR effect-mediated tumor localization and antitumor therapeutic efficacy.

Lipid Composition for Increased Stability In Vivo:

As drug permeability over the lipid bilayer is reduced in the “gel” state compared to “fluid” state, stability of liposomal entrapment can be maximized by selecting high-phase-transition phospholipids, for example, phosphatidylcholines (PCs) with long and saturated fatty acyl chains, such as distearoyl PC and hydrogenated soy PC, which remain in a gel state at physiological temperature. Addition of 30 to 50 mol% of cholesterol can further improve stability of the lipid bilayers by filling in gaps between PC molecules. Having a tight bilayer also reduces insertion of plasma proteins and reduces RES clearance of the liposomes.

pH-Sensitive Liposomes:

Although high stability of liposomes prior to reaching the cellular target is generally desirable, efficient release of liposomal drug in the target tissue and/or cell is essential for its therapeutic activity. Environmentally sensitive liposomes are designed to take advantage of the differences in the microenvironment of a solid tumor or inside the cell and to undergo destabilization on reaching their target. pH-sensitive liposomes are designed to destabilize at mildly acidic pH found in solid tumors and in endosomal compartments. These are typically composed of dioleoyl phosphatidylethanolamine (DOPE), which has a cone-shaped geometry that favors transition from bilayer to HII phase, and a weakly acidic amphiphile, such as oleic acid or cholesteryl hemisuccinate, which stabilizes the bilayer structure at neutral pH but not at mildly acidic pH (27–29). pH-sensitive liposomes have been shown to be much more effective in facilitating endosomal release of membrane-impermeable drugs from internalized liposomes in cells. Other nonbilayer-favoring lipids, such as oleyl alcohol and diolein, are also effective in forming pH-sensitive liposomes (30,31). Alternatively, a pH-sensitive oligopeptide that undergoes coil to α -helix conformational transition, such as glutamic acid-alanine-leucine-alanine (GALA), influenza fusion peptides, and pH-responsive polymer poly-2-ethyl-acrylic acid linked to a lipid anchor, can also mediate intracellular disruption of the endosomal membrane (32–34).

Fusogenic and Endosomolytic Liposomes:

These liposomes can be constructed by reconstitution of envelope proteins of viruses into liposomes or encapsulation of hemolysins from bacteria with varying degrees of pH dependence (35). In addition to pH, the reducing and enzymatic environment inside the endosomal compartment can be utilized to trigger the cleavage of disulfide or enzyme-sensitive linker that may be incorporated into a bilayer-stabilizing lipid (36,37). For example, gelonin, a type I plant toxin, was coencapsulated inside pH-sensitive liposomes with listeriolysin O (LLO), the pore-forming protein that mediates escape of the intracellular pathogen *Listeria monocytogenes* from the endosome into the cytosol (38). Such a strategy resulted in a dramatic improvement on the cytotoxicity of encapsulated gelonin against the murine B16 melanoma cell line, over free gelonin or gelonin encapsulated in non-LLO-containing pH-sensitive liposomes. In another study, Mastrobattista et al. (35) have demonstrated that coencapsulation of a pH-dependent fusogenic peptide (diINF-7) and diphtheria toxin A chain (DTA) in non-pH-sensitive immunoliposomes promotes efficient cytosolic delivery of DTA.

Temperature-Sensitive Liposomes:

Local release of liposomal drug can also be triggered by hyperthermia by adopting a bilayer composition with phase-transition temperature slightly above 37°C, such as dipalmitoyl phosphatidylcholine or conjugation to a thermosensitive polymer (39,40). Temperature-sensitive liposomes that show phase transition at 40°C can be synthesized by incorporating lipid-conjugated copolymers of *N*-isopropylacrylamide and *N*-acryloylpyrrolidine (41). A thermosensitive liposome formulation entrapping doxorubicin (ThermoDox™) is currently

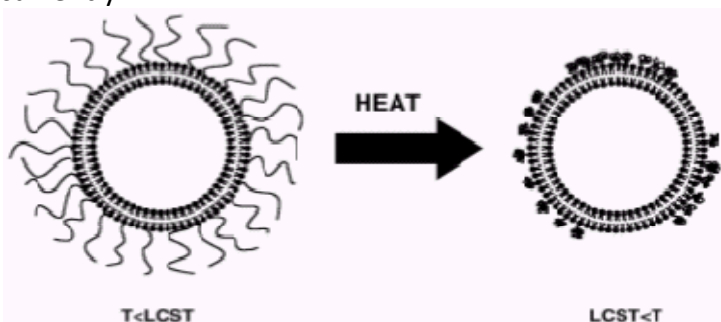


Figure2: Schematic illustration of design of temperature sensitive liposomes using a thermosensitive polymer.

Cationic Liposomes:

These liposomes can form electrostatic complexes with plasmid DNA and facilitate gene transfer (42). A wide variety of cationic lipids have been synthesized including those with a monovalent headgroup, such as 1,2-dioleoyl-3-trimethylammoniumpropane, *N*-[2,3-(dioleoyloxy)propyl]-*N,N,N*-trimethylammonium chloride, and 3-β-[*N*-(*N,N*'-dimethylaminoethyl)carbonyl]-cholesterol, and those with a multivalent headgroup, such as 2,3-dioleoyloxy-*N*-[2(spermine-carboxamido)ethyl]-*N,N*-dimethyl-1-propanaminium trifluoroacetate. DOPE is often used as a helper lipid in these liposomes to enhance their fusogenicity (43). Cationic liposomes with multivalent cationic lipids form particles with condensed structure with plasmid DNA, whereas those with monovalent cationic lipids have been shown to form extended spaghetti-like structures. Although cationic liposomes exhibit efficient gene transfer activity in tissue culture and are currently commonly used reagents for in vitro transfection, only low-level transfection in select tissues, typically the lung, can be obtained upon systemic administration of cationic liposome/DNA complexes in murine models (44,45). This might be due to the trapping of the cationic complexes in the capillary blood vessels in the lung, which is the first-pass organ encountered by intravenously administered liposome complexes. In addition to plasmid DNA, cationic liposomes have been used in the delivery of antisense oligodeoxyribonucleotides (ODNs) and siRNA into cells.

Liposomes with a weakly basic cationic lipid, such as 1,2-dioleoyl-3-dimethylammoniumpropane, can efficiently incorporate plasmid DNA or ODNs at acidic pH, where the lipids are largely cationic and return to a mostly neutral zeta potential at pH 7.4, where the lipids are mostly deprotonated (46). These liposomes have longer circulation time than cationic liposomes carrying permanently charged cationic lipids and may be useful for systemic delivery of DNA to solid tumors. Cationic liposome complexes with plasmid DNA,

ODNs, or siRNA can also activate the innate immune system and may play a role in immunotherapy (47). In addition to nucleic acid delivery, cationic liposomes carrying chemotherapy agent paclitaxel have been shown to preferentially target tumor endothelium, suggesting a possible role for these liposomes in tumor-targeted drug delivery. Schmitt-Sody et al. (48) showed that cationic liposomal paclitaxel exhibits high selectivity for tumor endothelium and is highly efficacious in tumor growth inhibition.

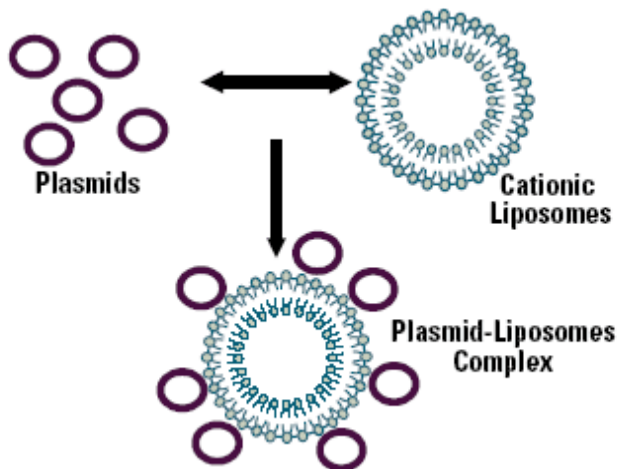


Figure 3: formation of Plasmid- Liposomes Complex

Targeted Liposomes:

Liposomes can be targeted to specific cell populations via incorporation of a targeting moiety. The targeting ligand can consist of a lipid-anchored antibody or antibody fragment, transferrin, folate, or carbohydrate. Immunoliposomes are synthesized by conjugation of the liposome to an antibody [e.g., anti-HER2 (49), antitransferrin receptor (50,51), anti-CD20 (52), and anti-CD19 (53)] or an antibody fragment such as Fab (54) and scFv (55). HER2 is a receptor tyrosine kinase, a product of the HER2 (c-erbB2) proto-oncogene, which has been shown to play an important role in the development and progression of breast and other cancers. Park et al. (56, 57) reported that anti-HER2 immunoliposomes, with encapsulated doxorubicin, displayed significantly enhanced therapeutic efficacy in four different breast cancer xenograft models when compared to nontargeted liposomes, free drug, or free antibody. For targeting PEGylated liposomes, it is helpful to incorporate a long PEG-based linker between the

targeting ligand and the lipid anchor. Incorporation of the targeting moiety can be accomplished during liposome formation by detergent dialysis, or postliposome formation by conjugation to reactive lipids or postinsertion of ligands from micelles of lipid-derivatized antibodies (58, 59). The last method seems highly promising for future clinical development. In addition to antibodies, other targeting moieties such as transferrin (51,60) and folic acid (61–63) have been frequently evaluated in targeting liposomes to tumor cells that overexpress the transferrin or the folate receptor.

Targeted liposomes are specifically taken up by target cells and have been shown to be highly efficient in drug delivery and to bypass multidrug resistance in cell lines (64). Tumor localization of targeted liposomes often does not greatly exceed that of nontargeted liposomes because biodistribution of liposomes is dictated by vascular permeability and the associated EPR effect. Furthermore, there is concern that liposomes, due to their size, cannot penetrate into solid tumors, which typically have high interstitial pressure. Nevertheless, targeted liposomes such as anti-HER2 immunoliposomes and folate receptor-targeted liposomes have shown improved antitumor efficacy in murine models over nontargeted control liposomes (51, 63). Leukemias, which have greater accessibility from circulation, are also potentially good targets for targeted liposomes, as suggested by recent reports. The advantage of using immunoliposome for MAb-based targeted therapy in leukemia exists in: (i) liposomes containing high payload of cytotoxic agents have unrestricted access to malignant cells and (ii) applications of a chemotherapeutic agent that has already shown clinical efficacy can potentially bring synergistic effect with therapeutic MAb, based on a different killing mechanism. Pan et al. (65) studied the therapeutic efficacy of folate receptor-targeted liposomes in combination with upregulation of FR- β expression in an ascitic xenograft model of acute myelogenous leukemia using all-trans retinoic acid. In vivo antitumor activity of folate receptortargeted liposomal daunorubicin in the L1210-JF ascitic murine leukemia model has also been reported by Pan and Lee

(66). The result showed that folate receptor targeted liposomes could effectively target receptor-positive leukemia cells in vivo.

Lipid Nanoparticles:

Lipid nanoparticles are nanoscale spherical particles composed of lipids with a lipidic core. These are suitable for delivery of lipophilic therapeutic agents. The molecule of interest can be formulated to lipid nanoparticle matrix through lipid phase dissolution. They have considerable utility as controlled delivery system for drugs and vaccines. Lipid nanoparticles can be synthesized by combining an oil phase (e.g., triolein) with phospholipids as emulsifiers. The oily core can be used to incorporate lipid-soluble drugs such as paclitaxel (67, 68), hematoporphyrin (69), and lipid-conjugated prodrugs (70). They can be synthesized by similar methods as those used in liposomes, such as high-pressure homogenization. Like liposomes, these particles are cleared by RES, localized in tumors via EPR effect, and can be made long circulating by incorporation of PEGylated lipid.

LIMITATIONS OF LIPOSOME TECHNOLOGY:

As described above, liposomes have a great potential in the area of drug delivery. However, liposome-based drug formulations have not entered the market in great numbers so far. Some of the problems limiting the manufacture and development of liposomes have been stability issues, batch to batch reproducibility, sterilization method, low drug entrapment, particle size control, and production of large batch sizes and short circulation half-life of vesicles. Some of the problems are discussed in detail below.

1. Stability:

One of the major problems limiting the widespread use of liposomes is stability—both physical and chemical. Depending on their composition, the final liposome formulations may have short shelf-lives partly due to chemical and physical instability. Chemical instability may be caused by hydrolysis of ester bond and/or oxidation of unsaturated acyl chains of lipids. Physical instability may be caused by drug leakage from the vesicles and/or aggregation or fusion of vesicles to form larger particles (71). Both of these processes (drug

leakage and change in liposome size) influence the in vivo performance of the drug formulation, and therefore may affect the therapeutic index of the drug. For instance, large liposomes may be rapidly cleared by RES leading to sub therapeutic plasma concentrations of the drug and reduced AUCs (area under the plasma concentration-time curve).

Physical instability may also occur due to partitioning out of a hydrophobic drug from the bilayer into the solvent on standing (or long term storage). Some of the stability problems may be overcome by lyophilization in which the final liposome product is freeze-dried with a cryoprotectant (mostly a sugar like Trehalose) and is reconstituted with vehicle immediately prior to administration. Lyophilization increases the shelf-life of the finished product by preserving it in a relatively more stable dry state. Some liposome products on market or in clinical trials are provided as a lyophilized powder. For example, AmBisome™, the first liposome product to be marketed in several countries is supplied as a lyophilized powder to be reconstituted with sterile water for injection (72). Recently, lyophilized paclitaxel-liposome formulations have been developed which show good stability (73).

2. Sterilization:

Identification of a suitable method for sterilization of liposome formulations is a major challenge because phospholipids are thermo labile and sensitive to sterilization procedures involving the use of heat, radiation and/or chemical sterilizing agents. The method available for sterilization of liposome formulations after manufacture is filtration through sterile 0.22, µm membranes (74). However, filtration is not suitable for large vesicles (>0.2 µm) and also is not able to remove viruses. Sterilization by other approaches such as γ-irradiation and exposure to chemical sterilizing agents are not recommended because they can cause degradation of liposome components and may leave toxic contaminants (75). Suitable methods for sterilization of liposome formulations are being explored by several groups. For instance, it has been shown that under certain conditions, liposomes with thermo stable, lipophilic drugs could be sterilized by autoclaving

without substantial loss of contents and/or degradation of phospholipids (76).

3. Encapsulation efficiency:

Liposome formulation of a drug could only be developed if the encapsulation efficiency is such that therapeutic doses could be delivered in a reasonable amount of lipid, since lipids in high doses may be toxic and also cause non-linear (saturable) pharmacokinetics of liposomal drug formulation. Some new approaches that provide high encapsulation efficiencies for hydrophilic drugs have been developed. For instance, active loading of the amphipathic weak acidic or basic drugs in empty liposomes can be used to increase the encapsulation efficiency (77). However, active loading is not suitable for hydrophobic drugs such as paclitaxel for which encapsulation efficiency is < 3 mole% mainly due to the low affinity of drug for the lipid bilayers (78).

4. Active targeting:

One of the major limitations of active targeting using ligand-directed immunoliposomes has been their rapid clearance due to non-specific uptake by the cells of RES. The development of LCL conjugated with ligands has revived interest in this field since LCL are not as rapidly cleared by RES. However, many problems still remain to be overcome. For instance, foreign immunoglobulin-ligands conjugated to immunoliposomes may induce immunogenicity and increase clearance on subsequent exposure. The ligand (antibodies) conjugated with liposomes may increase the liposome size and reduce extravasation and thus could limit targeting to intravascular targets (78). It has been shown that size of LCI may be increased in the blood circulation by interaction of the antibodies with serum components, which in turn can increase their size-dependent uptake by spleen. Moreover, immunoliposome enter the cells by endocytosis and if liposome contents are not released from the endosome, they would ultimately be degraded in the lysosomes. This would only be true for drugs sensitive to lysosomal enzymes.

5. Gene therapy:

A number of technical problems have to be overcome before cationic liposome-mediated transfection can be fully exploited. For instance, liposomes are significantly less efficient than viral vectors in their transfection

ability. Furthermore, the DNA-lipid complexes are not stable in terms of particle size (79, 80) for long periods of time. In addition, there is lack of in vivo targeting after systemic administration, and the toxicity of cationic lipids limits the administered dose of the DNA lipid complex. Plasmid-liposomes complexes may be more suited to delivery of genetic material by local administration.

6. Lysosomal degradation:

Once the liposome has reached the target cell, the efficacy is determined not only by the amount of drug associated with the cell, but also by the amount of drug reaching the 'target molecule' inside the cells. Immunoliposomes may deliver the drug to the cells selectively but the pharmacological activity depends on the ability of intact drug to diffuse into cytoplasm from the endosomes in sufficient amounts.

CONCLUSIONS:

Lipid-based nanoparticulates are versatile drug carriers with significant potential for clinical applications. Technological advances such as introduction of remote loading methods, PEGylated liposomes, and targeted liposomes provided additional advantages. In addition to modulating toxicity, pharmacokinetics, and biodistribution, liposomal delivery has shown promise as a mechanism to overcome multidrug resistance. Furthermore, liposomes are promising delivery vehicles for novel therapeutic agents such as siRNA and drugs that lack aqueous solubility. Particularly promising for future development are targeted liposomes, which have yet to be thoroughly evaluated in clinical studies. Given current trends, lipid-based nanoparticulates are likely to have an expanding role in drug delivery in the clinical setting.

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