Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2014, 6 (3):110-128
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Progress with liposomal drug delivery systems: Formulation to therapy

Anjan K. Mahapatra*[1], P. N. Murthy[2], S. Chandana[1], Ranjit P. Swain[1] and Narahari Polei[3]

[1]Dept. of Pharmaceutical Technology, Maharajah's College of Pharmacy, Vizianagaram, India
[2]Dept. of Pharmaceutics, Royal College of Pharmacy and Health Sciences, Berhampur, India
[3]Dept. of Pharmaceutics, Avanti Institute of Pharmaceutical Sciences, Visakhapatnam, India

---

ABSTRACT

The closed bilayer phospholipid systems likely called liposomes, were first described in 1965 by Alec Bangham and soon were accepted as drug delivery systems. Work on liposomes by number of researchers led the technical advances. These advances have led to numerous clinical trials and studies in such diverse areas as the delivery of anti-cancer, anti-fungal and anti-biotic drugs, the delivery of gene medicines and drug delivery to site of action, long circulating PEGylated liposomes, triggered release liposomes and liposomes containing combinations of drugs. This review is a focus on recent advances and some of the relevant challenges faced in developing clinically relevant liposomal drug carriers. The main objective of pharmaceutical science is to design and develop dosage forms with fulfilling the therapeutic need of the patients effectively. The writing highlights all aspects of liposomes starting from compositions to therapeutic applications and strategies through preparation and characterization. It is discussed in-depth on the role of lipids in bioavailability, design of lipid based drug delivery systems, and understanding of morphological characteristic of liposomes etc. Lipids as carrier have the potential of providing endless opportunities due to their ability to enhance intestinal solubilization and absorption via selective lymphatic uptake of poorly bioavailable drugs. Their use provides improved pharmacokinetic properties, controlled or sustained release of drugs with less systemic toxicity. Liposomes, which emerged as the most relevant model for biological membranes and for understanding lipid biophysics, later became the most successful drug delivery system with more number of FDA approved products.

Key words: Liposomes, Drug delivery, Targeting, Therapy, PEGylation, Stability and Characterization.

INTRODUCTION

An advance with drug delivery technology is a prospect to medicine and healthcare system. New inventions in materials chemistry have initially excited the advance of drug delivery systems (DDS), creating carriers that are biocompatible, biodegradable, targeting, and stimulus-responsive [1]. The studies on the variety of enclosed phospholipid bilayer structures consisting of single bilayers (bangosomes) by Alec Bangham and colleagues lead to the discovery of "Liposomes". Many novel developments have been occuring recently in this region, from clinically acceptable products to new tentative applications, with *gene* delivery and cancer therapy still being the foremost areas of interest [2- 4].

Liposomes are closed lipid bilayer structures of microscopic carriers characterized by unilamellar or multilamellar vesicles surrounding with one or more distinct internal aqueous compartments [5]. This amphiphilic nature enables

loading of hydrophilic and hydrophobic therapeutic agents in the core and the bilayer, respectively. The tiny size enables quick assimilation into the bloodstream and delivering at specific site, thus making them significant for modifying toxicity, solubility, stability and converting drugs into ideal candidates of improved pharmacokinetic and pharmacodynamic (PK-PD) profiles. The issue with stability, high cost and limited shelf life due to the rancidification of lipids poses major limitations. [6-9].

STRUCTURAL COMPONENTS

PHOSPHOLIPIDS

Glycerol containing phospholipids are mostly used in liposomal formulations which contain glycerol moiety as backbone in their structure and are derived from phosphatidic acid. The main head group organic molecules are glycerol, choline, ethanolamine, serine and inositol. The long chain fatty acids afford lipid nature to the phospholipid. Differences at fatty acid part can changes the phospholipid molecules characteristics. Saturated fatty acids are mostly used than unsaturated fattyacids for better liposomal stability. Most liposomes are prepared by using lecithin of egg or vegetable (soya bean) origin. Also a number of synthetic phospholipids are utilized in the preparation of liposomes [10-11]. A structural illustration and chemical components of liposomes is shown in **Figure 1.**

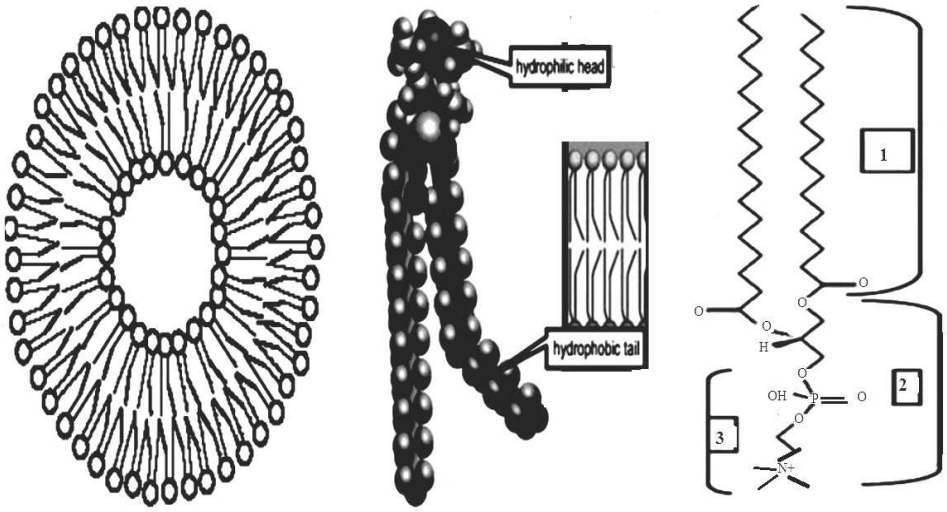

Fig.1. Schematic illustration of liposome and chemical structure of liposome component phospholipid.1)Fattyacid part 2) Glycerol back bone 3)phosphorylated alcohol (Head group)

SPHINGOLIPIDS

Sphingolipids are the membrane components containing sphingoid base [12]. Natural gangliosides(GM_1)class of sphingolipids are included in liposome formulations to provide a layer of surface charged groups, to prolong the lifetime of liposomes in the blood and to prevent their uptake by the reticuloendothelial system (RES). Sphingomyelins (SMs) are important phospholipids useful in regulation of cholesterol distribution within membranes [13].

STEROIDS

Cholesterol (Chol) is one of the major components in liposomal formulations whose incorporation increases the rigidity of the lipid bilayer, improves fluidity of the membrane, improve stability, increases the time of circulation in the blood stream [14,15].

CATIONIC LIPIDS

These are amphiphiles, analogous to natural phospholipids except for the presence of a cationic charge. It consists of long hydrocarbon chains (largely comprised of alkyl chains or cholesterol); hydrophilicity is by charged group (quaternary nitrogen) and linker bond (ester, carbamate *etc*). Due to their amphiphilicity nature upon hydration, self-assemble into lamellar vesicular structures with interior aqueous phase [16, 17]. Different types of lipids are given in **Table 1.**

Table1. Different types of lipids: [18-19]

Types of lipids	Name	Abbreviation
Natural phospholipids	Phosphatidylcholine(lecithin)	PC
	Phosphatidylethanolamine (Cephalin)	PE
	Phosphatidylglycerol	PG
	Phosphatidylserine	PS
	Phosphatidylinositol	PI
Synthetic phospholipids	1,2-distearoylphosphatidylcholine	DSPC
	EGG Yolk Phosphatidylcholine	EYPC
	1-palmitoyl-2-oleoyl-phosphatidylcholine	POPC
	Distearoylphosphatidylcholine	DSPC
	Dipalmitoylphosphatidylglycerol	DPPG
	Diphosphatidylglycerol (Cardiolipin)	DPG
Sphingolipids	Sphingomyelin	SM
	Glycosphingolipids	GSLs
	Gangliosides	
Cationic lipids	2,3-bis(oleoyl)oxipropyltrimethylammonium chloride	DOTMA
	1, 2-dioleoyl-sn-glycero-3-phosphoethanolamine	DOPE
	Dioctadecyldimethylammoniumbromide	DODAB
	N-[1-(2,3-Dioleoyloxy)propyl]N,N,Ntrimethylammoniummethylsulfate	DOTAP
	Dioctadecylamidoglycylspermine	DOGS
	2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,Ndimethyl-l-propanaminium trifluoroacetate (Lipofectamine)	DOSPA
	3β[N-(N',N'-dimethylaminoethane)carbamoyl]cholesterol	DC-Chol
	Dimyristoyltrimethylammoniumpropane	DMTAP
	1,3-di-oleoyloxy-2-(6-carboxyspermyl)-propylamide)	DOSPER
pH-titratable lipids	Palmitoylhomocyteine.	PHC
	Oleic acid.	OA
	Aspartic acid-derived artificial lipids	ADL
	Cholesterylhemisuccinate.	CHEMS
	poly(glycidol)s	PGs
	N-isopropylacrylamide	NIPAM

CLASSIFICATION OF LIPOSOMES

Liposomes are classified into three categories [20]; based on-

1. Method of their preparation
2. Based on their lamellarity and size (Figure 2).
3. Based on their composition and application (Figure 3).

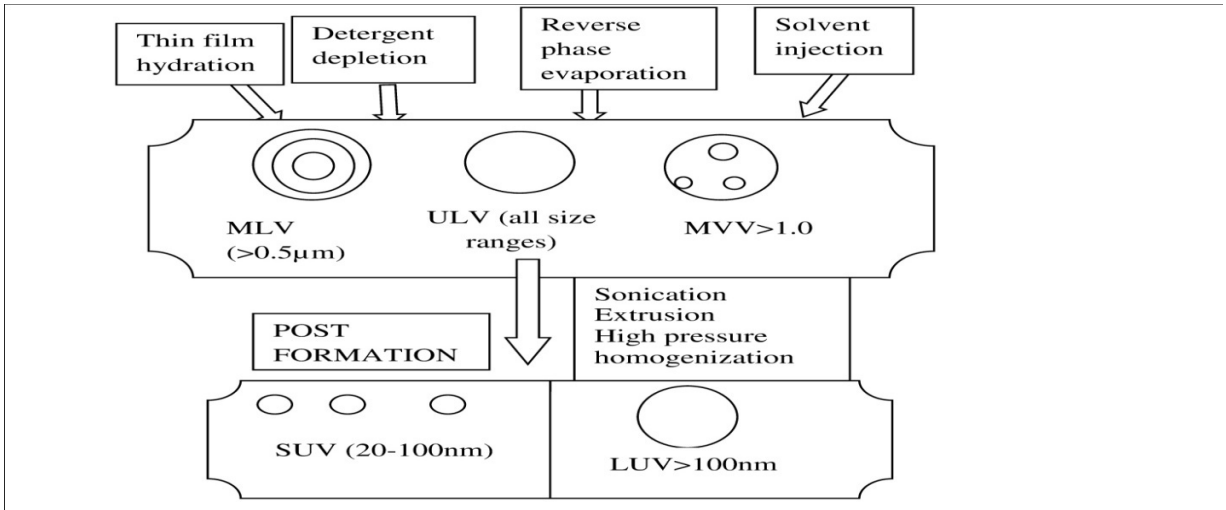

Fig.2. Representation of liposome Classification of based on lamellarity, size and method of preparations.MLV-Multi Lamellar vesicles; ULV-Unilamellar vesicles; MVV-Multi vesicular vesicles; SUV-Small unilamellar vesicles; LUV –Large unilamellar vesicles.

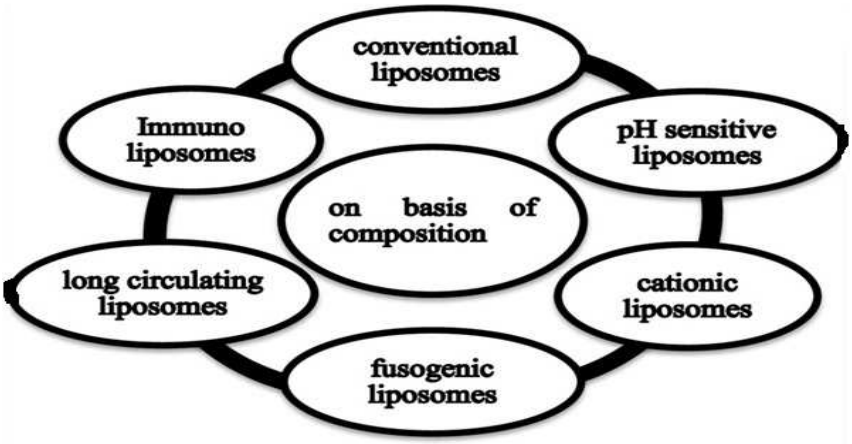

Fig.3. Types of liposomes based on composition and application

DESIGNING OF LIPOSOMES

CONVENTIONAL LIPOSOMES

Conventional liposomes known as classical liposomes comprise of neutral or anionic phospholipids without any surface modification (excluding polyethylene glycol) are short-circulating in nature. When given intravenously, gets quickly coated with plasma proteins and hastily cleared by the Mononuclear Phagocyte System (MPS).Liposomes accumulate mostly in liver and spleen due to their rich blood supply and the abundance of tissue-resident phagocyte cells.

Liposomes act as reservoirs encapsulating the drug and protecting it from the degradation and reducing the unintended side effects. Liposomes provide suitable environment, which enhances the solubility of the hydrophobic molecule of Paclitaxel (PTX) by liposomes to MCF-7 breast cancer cells resulted in a considerable increase of the intracellular PTX level and was more efficient in arresting cells in mitosis in comparison with PTX delivered by Taxol® [21- 23].

LONG CIRCULATING "STEALTH" LIPOSOMES:

Liposome gets rapidly cleared by the reticulo-endothelial system (RES) due to van der Waals and short range hydrophobic interactions with macromolecules (Fibronectin, C-reactive protein) in the blood [24]. Sterically stabilized Liposomes with hydrophilic polymer such as PEG display longer circulation times in blood as compared to conventional liposomes due to reduced identification rate by cells and macrophages onto the liposome surface owing to water shell surrounding the liposome. This attachment of PEG is known as PEGylation [25-27]. Also while Inclusion of specific natural glycolipids such as monosialoganglioside (GM_0) or hydrogenated soyaphosphatidylinositol (HPI) improved their prolonged circulation. Recently liposomes containing PE derivatives (PEG-PE) exhibited long circulation through mechanisms like repulsion, enhanced binding of disosponins and molecular cloud formation [28-30]. Polymer brushes sterically stabilize the liposomes. The PEG liposomal doxorubicin showed increased efficacy in cancer treatment [31, 32]. The experimental work on liposomal formulations containing 4 mol% of Paclitaxel (PTX) was done by considering conventional ones made up of PC/PG/cholesterol (molar ratio, 9:1:2) and PEGylated ones composed of PC/PG/cholesterol/1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol**)** (DSPE-PEG) (molar ratio, 9:1:2:0.7). It was found that both are physically stable only for less than 1 day and retained only 50% of the initial PTX content. PEGylated PTX liposomes were long-circulating with increased half-life time (48.6 hr) due to reduced clearance compared to conventional liposomes showing 9.3hr [33].

A schematic presentation on the process of PEGylation is shown in **Figure 4.**

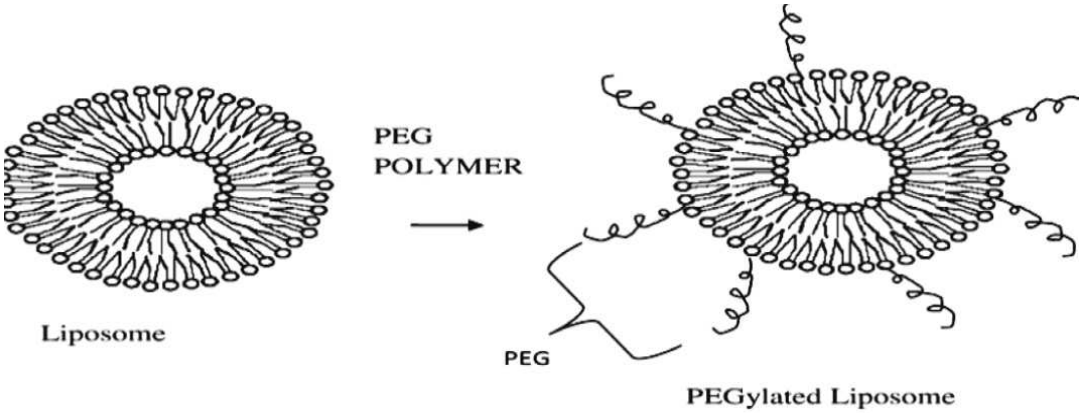

Fig.4. Representation of PEGylation

IMMUNOLIPOSOMES

Immunoliposomes gained importance by concept of "magic bullet" coined by Paul Ehrlich, who regarded that one part has moiety capable of recognizing and binding the target and other furnishing a therapeutic action at the target. These Nanosized DDS obtained by hydrophobic interaction of antibodies exclusively modified with hydrophobic residues on the surface of liposome showed high specificity for ligand [34]. Successful targeting of the immunoliposomes (with enclosed drug) to the appropriate target cells was the first step to induce a therapeutic effect. Promising results expected in the treatment of diseases with target sites located inside the blood circulation as in the case of lymphomas, leukemias, sickle cell disease and malaria, in reducing toxic levels of circulating drugs. In improving the evaluation of gamma scintigraphic scans RES uptake as well as the barrier function of the endothelium are major factors counteracting the extra vascular disposition of immunoliposomes after intravenous administration [35]. For this reason long circulation liposomes have been designed, which were sterically stabilized by coating the liposome surface with amphipathic PEG derivatives *i.e.*, use of PEG to sterically interfere with the antibody's ability in recognizing the antigen and enhanced the circulation time. However, the steric barrier of PEG decreases the protein coupling efficiency at the liposome surface as well as the target recognition, especially where higher concentrations of PEG (with high molecular weight) are used. In order to overcome these problems, antibodies were coupled to the terminal ends of PEG to increase antibody accessibility [36]. PEG-immunoliposomes was developed with monoclonal antibodies or their fragments attached at the distal ends of the PEG chains. These liposomes showed improved binding to their specific target when compared to both Type 1 and Type 2 due to the combined benefits of steric stabilization by PEG and improved antibody accessibility [37]**.**Illustration of conjugation of antibodies to liposomes is shown in **Figure5.**

Majority of immunoliposomes are targeted for delivery of anticancer drugs. Thus, the recent clinical success of doxorubicin-loaded long-circulating PEGylated liposomes (Doxil®/Caelyx®) in the treatment of metastatic breast cancer, progressive ovarian cancer, multiple myeloma and AIDS-related Kaposi's sarcoma motivated numerous experimental attempts for the improvement of their targeting properties by surface immobilization of different antibodies or their fragments against specific tumor antigens. CD19 (targeting antigen), an internalizing receptor overexpressed in most types of B-lymphoid malignancies. Introduction of anti-CD19 monoclonal antibodies (mAbs) or its Fab′ fragments to PEG-liposomes loaded with doxorubicin enhanced targeting and therapeutic efficacy in mice bearing a human CD19+ B-lymphoma .The cytotoxic efficiency of immunoliposomes is also dependent on the surface density of the membrane antigen against which liposomes were targeted. It was calculated that about 4×104 antigen sites per single cell are required to apply the immunoliposomal targeting effect. The extent of heterogeneous expression of antigens in the targeting area suggested that a co-mobilization of antibodies against different antigens on a single immunoliposome will provide better and more uniform targeting of all cells within the tumor. Alternatively, the antigen-negative cells may also be killed by so called "bystander" effect, *i.e.* an action of the drug released from the immunoliposomes attached to a cancer cell expressing a particular antigen on neighboring cancer cells devoid of a similar receptor. PEG liposomes modified with different antibodies have been successfully applied for targeted delivery of siRNA and DNA. Another exciting application of immunoliposomes includes targeted delivery of "bioenergic" substrates, such as ATP, to the ischemic myocardium [36].

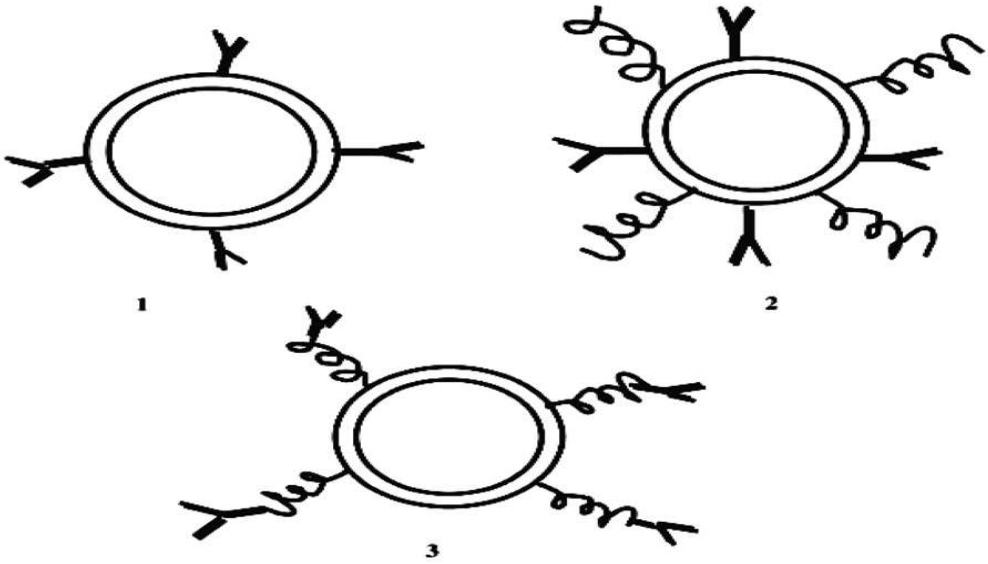

Fig.5. Illustration of conjugation of antibodies to liposomes .1) Type 1: "PEG-free' immunoliposomes with antibody directly linked to the lipid. 2) Type 2: PEG-immunoliposomes with antibody directly linked to the lipid. 3) Type 3: PEGimmunoliposomes with antibody conjugated to the distal end of the PEG chain.

CATIONIC LIPOSOMES

These are new non-viral carriers, useful as delivery systems for genetic materials. The electrostatic interactions between these positively charged cationic lipid complexes and negatively charged DNA, RNA, short single-stranded antisense sequences as well as some proteins forms lipoplexes which significantly improved their uptake by cells, leading to improved nucleic acid delivery. It does not require any encapsulation process that limited the development of conventional liposomes as carriers [38, 39].

A schematic presentation on formation of lipoplex is given in Figure 6**.**

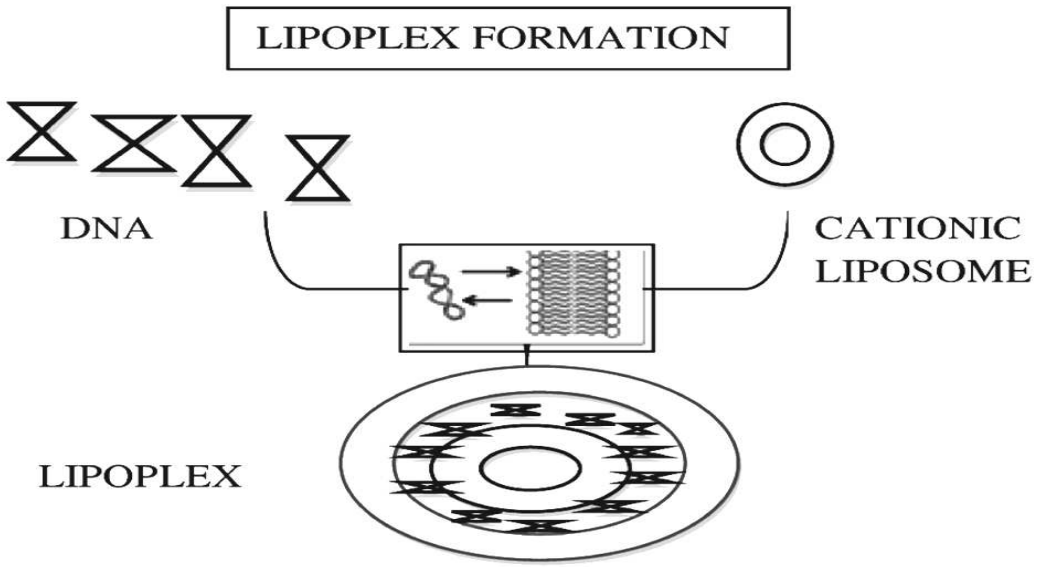

Fig.6.Representation of Lipoplexes formation

Since the first two important lipids used and synthesized were DODAB and the DOTAP family respectively. Lipofectin (DOGS) was mostly used and several cationic lipids have been designed to promote DNA transfer. Since their first use by Felgner et al. in 1987, a large number of cationic liposomes have been synthesized and used for delivery of nucleic acids into cells in culture, in animals and even in patients enrolled in phases I and II clinical trials

Efficacy has been demonstrated in melanoma patients injected with lipoplexes, delivering the Hela (HLA-B7) gene. Therefore, they are effective vehicles for human gene therapy. Most cationic liposomal formulations require the inclusion of a neutral lipid, or helper lipid, in order to increase transfection efficiency. The most commonly used neutral lipids are DOPE and Chol. The aim of gene therapy is to deliver DNA, RNA or antisense sequences to appropriate cells in order to alleviate symptoms or prevent the occurrence of a particular disease, i.e. repair the defect and also its cause. The major approaches to gene therapy include gene replacement, addition of genes for production of natural toxins, stimulation of the immune system or overexpression of highly immunogenic genes for immune self-attack and sensitization of cells to other treatments [40-42].

A list of commercially available cationic liposomes for gene transfection is given in **Table 2.**

Table 2.Commercially available cationic liposomes for *gene* transfection: [40]

Name	Composition(w/w)
Lipofectin	DOTMA:DOPE(1:1)
Lipofectamine	DOSPA:DOPE(3:1)
Lipofectace	DOBAB:DOPE(1:2.5)
DOTAP	DOTAP
Transfectam	DOGS
Oligofectamine	DOSPER

In mouse myeloid dendritic cells, several cationic liposomes (DMTAP, DOTAP, Lipofectamine (DOSPA/DOPE) *etc*) induced expression of co-stimulatory molecules CD80 and CD86 (considered as activation markers) while pro-inflammatory cytokine secretion is not affected. Another lipid-based transfection reagent, called HiPerFect (Qiagen, Hilden, Germany), was especially synthesized for transfection of siRNA.

Cationic lipids have also been used in combination with immunostimulatory adjuvants, including TLR (toll like receptors) agonists (lipid A, CpG (cytosine being 5 prime to the guanine base) DNA, etc.), saponins, and cytokines, to enhance the immunogenicity of the vaccines by improving protection of some adjuvant components against cytoplasmic enzymes and their delivery into cells. However, most lipids have no specific chromophore and fail to be monitored by routine spectrophotometric detection but fluorescence based Techniques are used to characterize cationic liposomes .The major disadvantage with *in vivo* use of cationic lipids was low transfection efficiency by heterogeneity and instability in serum containing environments [43, 44].

FUSOGENIC LIPOSOMES

Fusogenic activity helps to stabilize liposome membranes by inclusion of viral fusion proteins, peptides and synthetic polymers. To achieve efficient transfection of cells, gene vectors must possess an ability to promote gene transferral from the endosome to the cytosol before degradation in the lysosome. Various methods have been used to enhance the endosomal escape of the entrapped gene, A PEG derivative with carboxyl groups, succinylated poly (glycidol) (SucPG), conjugated with EYPC to modified SucPG liposome complexes to generate fusogenic activity at mildly acidic pH.PEG chains grafted to the liposome surface have shown to stabilize the liposome and reduce its interaction with cells. These transferrin bearing SucPG modified liposomes complexes with lipoplexes to achieve cell transfection through efficient internalization into cells through transferrin receptors and release their contents into the cytoplasm by fusing with lysosome or endosome. Transfection activity of DC-chol lipoplexes was also enhanced by complexation with SucPG-modified liposomes. These polymer-modified liposomes as a cytoplasmic delivery vehicle, now attempted to apply for production of potent vaccines, which delivered antigenic proteins (ovalbumin) into cytosol of dendritic cells and activated cellular immune response through their administration via nasal mucosa. Recently another polymer 3-methylglutarylated poly (glycidol) (MGluPG) which has hydrophobic side chains, exhibited higher fusion ability than SucPG [45, 46]. As now conjugation of liposomes with mixture of EYPC and DOPE were used to increase the fusion ability of liposomes. Liposomes containing negatively charged phospholipids become fusogenic in presence of calcium. New PEG based hydrophobically modified PEG polymers (HMPEGs) in combination with fusogenic liposomes shield them from complement binding [47-49]. In 1985,

Okada et al. found Sendai virus and developed a hybrid delivery system called fusogenic Liposomes composed of conventional liposomes and Sendai virus along with Hemagglutinating and neuraminidase (HANA) envelope proteins but later modified with F protein envelope instead of HANA protein. Nakanishi et al. further developed it by using ultraviolet Sendai virus. These are developed for the induction of antigen-specific cytotoxicT-lymphocyte (CTL) responses of antigens into cytoplasm [50]. A schematic presentation of fusogenic liposome preparation along with targeting strategy is shown in **Figure7.**

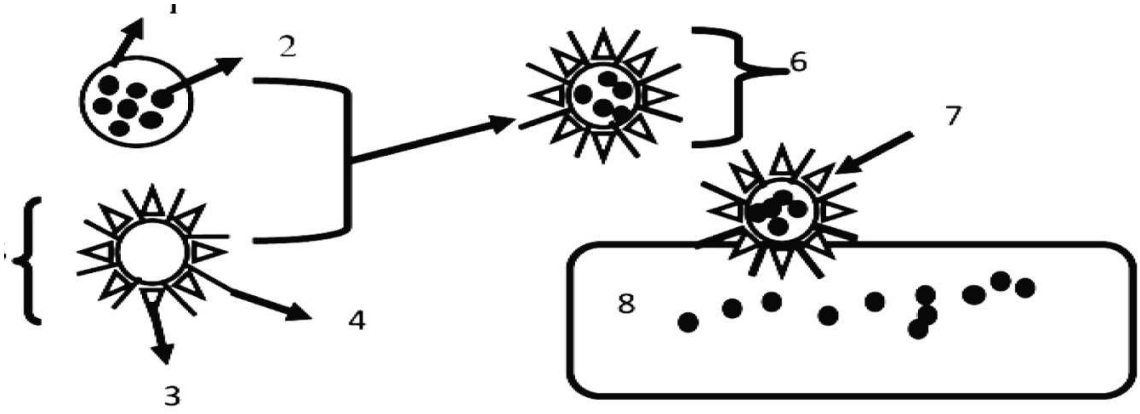

Fig.7. Representation of fusogenic liposome preparation along with targeting strategy 1) Conventional liposome 2) contents like DNA, RNA or proteins 3) F-protein 4) HANA protein 5) Ultraviolet-inactivated Sendai virus 6) Fusogenic liposome 7) Attachment to cell membrane by Fusion and introduced contents into cytoplasm 9) cell

pH SENSITIVE LIPOSOMES

The initial rationale of pH sensitive liposomes was to precise the acidic environment to trigger destabilization of liposomal membranes which undergoes pH induced fusion with endosomal membrane, destabilization and releases drug contents into cytosol. They act as vehicles for cytoplasmic delivery of drugs of weak bases, macromolecules and nucleotides. These pH sensitive liposomes usually contain Phospholipids such as PE, DOPE along with few titrable amphiphiles (stabilizers). The liposomes based on these bilayered components are destabilized in the acidic environment of the endosomes and rapidly released their contents. Recent studies mainly focus on the construct of new lipid compositions that attribute pH-sensitivity to liposomes or modification of liposomes with various pH-sensitive polymers and imparting hydrophilicity to the liposomal surface for longevity and ligand-mediated targeting. The modification of liposomes with stimuli-sensitive polymers is an effective method and evident work of Tirrell et al. by using pH-sensitive polymer, poly (alkyl acrylic acid), which destabilize membranes at low pH values because protonation of the carboxylate ions increases the hydrophobicity of the polymers, allowing the hydrophobic segments to penetrate the lipid bilayer and to induce defects in the membrane. Fusion, on other side, would result the insertion of the hydrophobic segments of the polymer into the membrane of neighboring liposomes and/or endosomes. This would lead to close vesicle–vesicle contacts, facilitating local dehydration at the contact site, causing defects in the packing of the membrane lipids, and eventually promoting fusion (to promote drug efflux to the cytosol) [51-55]. The current classes of pH-sensitive liposomes are pH-titratable polymers which destabilize membranes followed by change in polymer conformation at low pH mentioned in Table 1.

TARGETING STRATEGIES

PASSIVE TARGETING

It mostly involves physiological body features such as bulk recognition by RES, MPS and Enhanced permeability and retention effect (EPR-effect) trapping liposomes in extracellular space due to the ineffective lymphatic drainage within tumor tissue and is referred as non-targeted or passive targeting i.e. targeting without targeting ligand [56]. A

pictorial representation on passive targeting of liposomes by EPR effect utilizing the anatomical and pathophysiological abnormalities of tumor vasculature is given under Figure 8.

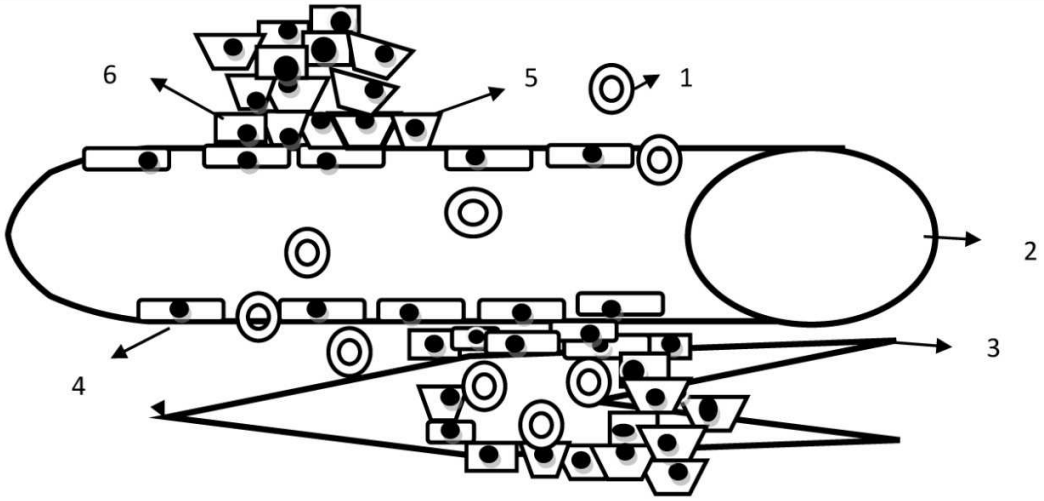

Fig.8. Passive targeting of liposomes by EPR effect utilizing the anatomical and pathophysiological abnormalities of tumour vasculature.1.Drug loaded liposomes 2.Blood vessel 3.Lymphatic vessel 4.Endothelial cell 5.Tumor cell 6.Normal cell

ACTIVE TARGETING: This targeting occurs through direct and specific interaction between a specific recognition site on the liposomal surface and a receptor on the cytoplasm membrane of the target cell based on molecular recognition mechanism. The targeting ligands include antibodies, antibody fragments, peptides, aptamers and small molecules such as folic acid or carbohydrates that target the cancer cells or tissue [57].

TEMPORAL CONTROL OVER PARTICLE PROPERTIES:
TRIGGERED RELEASE
These lipidic drug carriers are now being endowed with specific targeting and transmembrane exchange mechanism. Liposomes showed trigger release of liposomal contents upon reaching the targeted site to increase bioavailability and reduce the toxic effects of drugs. Three main types of remote triggers are heat, ultrasound and light, and local trigger which are native to the disease site or cellular organelles such as enzymes and pH changes.

First trigger drug release concept by Yatvin and Weinstein showed temperature-triggered local drug delivery using temperature-sensitive liposomes (TSLs) composed of 1,2-dipalmitoyl-sn-glycerophosphocholine (DPPC,T_m=41.5 $^{\circ}$C) and 1,2-distearoyl-sn-glycerophosphocholine (DSPC,T_m=54.9°C) which releases encapsulated drugs during melting phase transition temperature (T_m). At T_m structural diversities in the lipid membrane occurs as it transfers from a gel to the liquid-crystalline phase. Liposomal membranes in the gel (solid-like) phase are less permeable to water and drugs compared to the liquid-crystalline phase. At T_m, the membrane permeability of the lipid bilayer increases by several folds, facilitating the release of the liposomal content. From temperature induced delivery of drugs (neomycin and methotrexate) in TSLs composed of DPPC showed slow drug release kinetics and no quantitative drug release at the melting phase transition temperature. Incorporation of lysolipids (e.g. 1-Palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine (P-Lyso-PC)) in the membrane led to TSL with an ultra fast drug release. Preclinical experiments with doxorubicin (DOX)-loaded temperature-sensitive systems along with externally applied temperature showed an improved efficacy and reduced toxicity [58-60]. An illustration of temperature triggered drug release is shown in **Figure 9.**

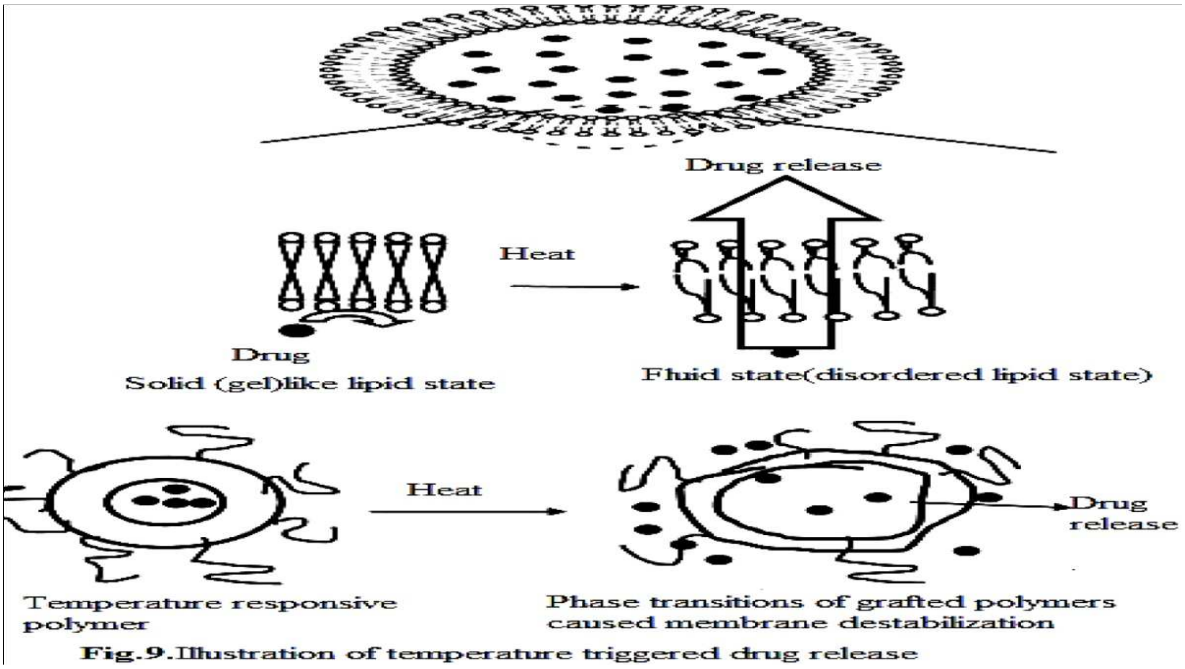

Fig.9.Illustration of temperature triggered drug release

Drug content release in the confined areas can be exaggerated by focusing ultrasound. The ultrasound-triggered release has been established for liposome entrapped gas bubbles and drugs. Low frequency ultrasound (LFUS) increases the permeability of liposomes because phospholipid bilayer simulates biological membranes. Exposing of Stealth cisplatin to LFUS at an intensity of 3.3 W/cm^2 for different periods of time (30 to 180 s), resulted in a time-dependent release, reaching 62% after 180s of LFUS irradiation. The chemical integrity and biological potency of drug was not affected by LFUS [61-63].

Table 3: Preparation methods for liposomes

Classification methods	Sub-classification of methods	Liposomes obtained	References
Mechanical dispersion	Lipid hydration method	MLV	[66,67]
	Proliposomes		
	Freeze drying method		
Physical hydration or post formation processing	Micro-emulsification	MLV	[68,69]
	Sonication (bath or probe type sonicator)	SUV	
	French pressure cell extrusion	SUV	
	Membrane extrusion	LUV	
	Dried reconstitute	LUV or MLV	
	Freeze-thawing sonication	SUV or LUV	
	Dehydration-rehydration cycle(DRV)	SUV	
	Calcium induced vesiculation	LUV	
	High pressure extrusion technique	SUV or LUV	
	Microfluidizer technique	SUV or LUV	
Solvent dispersion methods	Ether injection	SUV or LUV	[70,10]
	Double emulsification	LUV	
	Ethanol injection	SUV or LUV	
	Reverse-phase evaporation (REV	LUV	
	Inkjet injection	SUV or LUV	
Detergent removal method	Detergent removal/dialysis	SUV or LUV	[71]
	Reconstituted sendai virus enveloped vesicle	SUV or LUV	
Novel methods	Microfluidic channel	MLV or ULV	[72]
	Supercritical fluid injection and decompression	MLV or ULV	[73]
	Supercritical liposome method	SUV or MLV	[74]
	Improved/ supercritical reverse phase evaporation	LUV or MLV	[75]
	Membrane contactor	SUV or LUV	[76]
	Rapid solvent exchange	SUV or LUV	[77]

Photodynamic therapy (PDT) exploits the light for controlled delivery of the PDT compounds with the sources of reactive oxygen species. Some recent advances include new class of liposomes containing 1, 2 bis-(tricosa-10, 12-dinoyl)-Sn-glycero-3-phosphocholine($DC_{8,9}PC$) that have photo-cross linkable triple bonds are used to deliver DOX. DOX, when irradiated with 514 nm light for 0–7 min, showed 22% higher release compared to the non-irradiated samples and was the first drug to release photochemically from liposome [64-65].

PREPARATION METHODOLOGY: A tabular presentation on classification of preparation methods for different type of liposomes is given in Table 3.

DRUG LOADING TECHINIQUES

Liposomal formulations are designed to achieve high and stable drug loading [during storage and circulation]. This is not an easy task, to achieve drug to lipid mole ratio and to reach the intra-liposome drug concentration range of hundreds of mM. This can be done by passive or active loading. The methods by which drugs can be loaded into liposomes depend on the properties of the drugs and the lipids. Loading of liposomes is typically based on non-covalent interactions of the cargo with either the hydrophilic aqueous interior or the hydrophobic membrane.

PASSIVE LOADING

Passive entrapment of drugs in liposomes involves preferential partitioning of the drug either in the aqueous compartment or by association with the lipids. It involves different methods working on different principles namely mechanical dispersion, solvent dispersion and detergent solubilization to getting active drug into target cells. This cannot be achieved due to the poor drug solubility, so that therapeutic levels of drug cannot be reached. This loading inefficiency leads to great loss of the active agent and a need to remove unloaded drug. Therefore, the use of liposomes as a vehicle becomes inefficient as well as uneconomical.

ACTIVE LOADING

The analysis of the available loading approaches revealed clearly that the active loading approach is of another choice to achieve a viable formulation, and in many cases the only way to achieve the desired intraliposome drug concentration, usually defined as drug to lipid mole ratio.

Several methods exist for improved loading of drugs, including remote [active] loading method which loads drug molecules into preformed liposome using pH gradient and potential difference across liposomal membrane. Deamer and co-workers were the first to demonstrate remote loading of amphipathic weak bases [such as catecholamines] by a pH gradient. [Which was extensively used by Cullis and co-workers for doxorubicin remote loaded into liposomes by pH gradient method]. Many anticancer and antibiotic drugs are weak bases and can be accumulated in liposomes in response to a transmembrane pH gradient.

Trans membrane ion gradients described as nano-chemical loading engines are pre-fabricated into the liposomes, to exhibit the desired pH and/or ion gradient. These nanoengines are achieved by using salts composed of either weak bases [e.g., ammonium sulfate] or weak acids [e.g., acetic acid]. The approach for efficient and stable remote loading of amphipathic weak bases into preformed liposomes based on a transmembrane gradient of ammonium sulfate [$[NH4]_2SO_4$ liposome$\gg$ $[NH_4]_2SO_4$] medium which that acts as the driving force for drug loading. It is based on the strategy of fabricating liposomes by exhibiting a transmembrane intra-liposome high/extra-liposome medium low ion gradient, which acts as the driving force for the remote loading of amphipathic weak base drugs. Amphipathic weak acids can also be remote loaded by similar approach but driving force is transmembrane gradient of calcium acetate. The counter ion should also be selected for gradient-forming ion [e.g., sulfate in the case of ammonium or calcium in the case of acetate gradient] so that it will control the state of aggregation and precipitation/crystallization of the drug-counter ion salt in the intra-liposome aqueous phase, thus by control the efficiency and stability of remote loading, as well as drug release rate at various temperatures [78, 79].

STABILITY

Stability is a critical factor that must be considered during formulation design and development. Physical and chemical instability of liposomes often limit their widespread use in medical applications. Chemical instability is caused by hydrolysis or oxidation of the phospholipid molecules and is indicated by leakage of the encapsulated drug and alterations in vesicle size due to fusion and aggregation. These effects can be minimized by adding antioxidants such as tocopherol or BHT, by storing the liposome preparation under an atmosphere of nitrogen or argon; ensuring that peroxide forming solvents are completely removed from the preparation prior to storage.

Buffers at neutral pH decrease hydrolysis. Another physical property that affects liposomal targetability has been size and preparation method. Extrusion technique is preferred than sonication to prevent lipid degradation by oxygen during liposome preparation.

Physical instability may be caused by drug leakage from the vesicles or aggregation or fusion of vesicles to form larger particles. Charge inducing lipids such as PG, Chol and sphingomyelins are integrated into the liposome bilayer to decrease fusion, permeability and leakage of encapsulated drug. Lyophilisation mainly focus on the protection of the lipid bilayers from damage by ice crystals during freezing, inhibition of vesicle fusion/aggregation following dehydration and the avoidance of a phase transition during rehydration. Cryo- and lyoprotectants such as carbohydrates, glycerol dimethylsulfoxide, and glycerol, quaternary amines and sugars such as sucrose or trehalose interact with the head groups of the phospholipids and counteracting fusion or membrane disruption Stabilization of photosensitive materials i.e. protective effect may gained by using multilamellar vesicles comprising of hydrophilic cyclodextrin which capable of forming an inclusion complex with the photosensitive material in the aqueous phase and a combination of light absorbing (chemical absorbers and physical blockers of UV radiation) and antioxidant agents in the lipid bilayer (beta carotene quench both singlet oxygen driven photochemical reactions and free radical reactions) [80-85].

CHARACTERIZATION OF LIPOSOMES

MEAN VESICLE SIZE AND SIZE DISTRIBUTION

Liposome size is dependent on the preparation technique i.e. sonication times, extrusion pressures, lipid composition and measuring liposome–complement interactions [86]. A number of methods are used to determine size and size distribution, among which light-scattering analysis is commonly used. The recently used methods are atomic force microscopy, ultracentrifugation, Coulter counter, gel exclusion chromatography, laser diffraction, and light microscopy [87]. The rate of liposome uptake by RES was found to increase with the size of the vesicles (< 0.1/µm). The spleenic sinusoids and kupffer cells filtering rate of size is normally between 150-250 nm more than this size particle may get entrapped in MPS system. Liu *et al*. studies on biodistribution of phosphatidylcholine liposomes ranging in size from ~40 to 450 nm in mice. Showed results after 4hrs as significant accumulation in the liver for all sizes of liposomes, and liposomes larger than 100 nm showed increasing accumulation in the spleen. Fang *et al*. studies showed effects of particle size on serum protein binding. PEG-functionalized particles less than 100 nm in size bound less than 6% of the total serum proteins, while ~250 nm particles bound more than 34%.Hence it shows that smaller particles offered higher surface PEG chain density and stealth properties to diminish opsonization than larger particles [88].

BILAYER ORGANIZATION

Lipids have a characteristic phase transition temperature (T_c). The stability of liquid-crystalline bilayers can be increased through incorporation of Chol at high concentrations that eliminate phase transition and decrease the membrane fluidity at a temperature > T_c, and makes the liposomes more stable and less leaky after systemic administration. The substitution of egg sphingomyelin for phospholipids such as DSPC in liposomes, increasing the drug-to-lipid ratio and altering the fatty acyl chain length and saturation of the lipids results in improved drug retention properties and longer circulation lifetime properties [89]. Lamellarity determined by electron, Cryo-electron and freeze fracture microscopy.

SURFACE CHARGE

The nature and density of charge on the liposome surface are important parameters which influence the mechanism and extent of liposome-cell interaction. Negatively charged liposomes are removed more rapidly from the circulation than neutral or positively charged liposomes. PEG functionalisation is a well-recognized technique to mask the particle surface and limit non-specific protein binding [90].

ENCAPSULATION EFFICIENCY (EE)

The EE is defined as the percentual amount of drug entrapped in the vesicles in relation to the total amount of drug present during the vesicle formation and entrapment procedure. Methods for determining the extent of drug entrapped or encapsulated within liposomes usually rely on destruction of the lipid bilayer using the methods column chromatography technique or other assay methods, gel filtration, exhaustive dialysis and centrifugation and subsequent quantification of the released material. It was calculated as follows (Ishii and Nagasaka) [91].

Encapsulation Efficiency (%) = $\frac{C_{total}-C_{out}}{C_{total}} X100$

C_{total}=Total amount of drug entrapped, which can be quantitated by disruption of liposomes completely and release of components; C_{out}= is the amount of drug quantitated by the liposome suspension diluted with water and ultrafiltered through a millipore filter.

THERAPEUTIC APPLICATIONS OF LIPOSOME

LIPOSOMES IN GENE THERAPY

Cationic liposomes can retain drug agents at the tumor vascular site and facilitate interaction of liposomes with subcellular targets prior to releasing their payload. They can also be used to target non-intracellular targets as well as cell-membrane bound molecules other than proteoglycans. This is promising given that many anti-angiogenic agents have been confirmed to exert their action by each mechanism. For example, SU5416, an inhibitor of tyrosine kinase activity of vascular endothelial growth factor (VEGF), requires direct access to a specific endothelial cell membrane-associated receptor (Flk-1/KDR) in order to suppress neovascular growth of tumors. Although SU5416 is suggested to exert long-lasting effects on VEGF phosphorylation and function, cationic liposome-assisted drug delivery could enhance interactions with specific endothelial cell targets. Effective anti-angiogenic therapy requires the continuous presence of drugs in circulation (92, 94). The inclusion of PEG in cationic liposome preparations can extend circulation half-life of SU5416 compared to SU5416 alone. The duration of drug (SU5416) exposure with tumor target can be enhanced using PEG in cationic liposome.

LIPOSOMES FOR CANCER THERAPY

RGD-Modified Liposomes for Cancer Therapy

RGD-modified immunoliposomes was developed for targeting the antivascular drug combretastatin to irradiated mouse melanomas [95].Combretastatin was incorporated into liposomes with surfaces modified by the addition of cyclo (Arg-Gly-Asp-D-Phe-Cys) (RGD) to create an immunoliposomes. Pattillo et al. 2005 found immunoliposomes of antivascular drugs preferential targeting to irradiated tumors results in significant tumor growth delay. Cyclic RGD peptide, cyclo(Arg-Gly-Asp-Phy-Lys) anchored sterically stabilized liposomes (RGD-SL) were investigated for selective and preferential presentation of carrier contents at angiogenic endothelial cells over-expressing $\alpha_v\beta_3$ integrins on and around tumor tissue and for assessing their targetabilty [96].RGD-modified sterically stabilized liposomes have also been evaluated to improve the antitumor efficacy of doxorubicin [97,98]. Holig et al. have isolated from phage display RGD motif libraries with novel high affinity cyclic RGD peptides on the basis of their selectivity towards endothelial or melanoma cells [99]. Administration of large amounts of synthetic peptides based on the Arg-Gly-Asp (RGD) sequence has been shown to suppress tumor metastasis. To overcome the rapid degradation of peptides in the circulation, an RGD mimetic, L-arginyl-6-aminohexanoicacid (NOK), was synthesized and conjugated with phosphtidylethanolamine (PE) (NOK-PE) for liposomalization [100].

RGD-MODIFIED LIPOSOMES IN CANCER GENE THERAPY

Gene therapy is aimed in order to modify the genetic program of a cell toward a therapeutic or prophylactic goal. It is the modification of the host immune response toward the tumor; the disruption of the tumor neovascularization; the lysis of tumor cells with replication-competent viruses, and suicide gene therapy where an inactive prodrug is converted into a cytotoxic drug by gene-expressed enzymes. RGD peptides have been used to target the lipid-protamine-DNA (LPD) lipopolyplexes to tumor cells (MDA-MB-231), expressing appropriate integrin receptors [101]. The incorporation of PEGylated lipid into Lipid-Protamine-DNA (LPD-PEG) lipopolyplexes causes a decrease of their in vitro transfection activity. Fahr et al. 2002 developed a novel liposomal vector (Artificial Virus Particles; AVPs) for cancer gene therapy [102]. Artificial virus-like particles (AVPs) represent a novel type of liposomal vector, resembling retroviral envelopes. AVPs are serum-resistant and non-toxic and can be endowed with a peptide ligand as a targeting device. AVPs carrying cyclic peptides with an RGD integrin-binding motif (RGD-AVPs) were suitable for the specific and efficient transduction of human melanoma cells.

Folate Receptor (FR) Targeted Liposomes for Cancer Therapy

FR, also known as folate-binding proteins (FBP), is an N-glycosylated protein with high binding affinity to folate. The selective amplification of FR expression in both human solid tumors and leukemia suggests its utility as a potentially valuable target for drug and gene delivery. targeted liposomes are folate-conjugated liposomes targeting to acute myelogenous leukemia, CD19-targeted immunoliposomes for non-Hodgkin's lymphoma (NHL) therapy [103], and anti-HER2 immunoliposomal doxorubicin targeting to HER2-overexpressing breast cancer cells [104].

Cellular uptake of FR-targeted liposomes has been characterized using KB cells, a FR-a (C) human oral carcinoma cell line [105]. Drug delivery properties of FR-targeted liposomes have been studied in vitro using liposomes loaded with chemotherapeutic agents such as doxorubicin, daunorubicin, and cisplatin. Lee et al. first reported the in vitro effect of doxorubicin and showed these targeted liposomes showed w86-fold greater cytotoxicity in KB cells compared to non-targeted control liposomes. The enhancement in cytotoxicity was correlated with the increase in doxorubicin uptake and could be blocked by excess free folate [106-108].

Liposomal Vaccination System for Immunity-Modulating Antitumor Therapy:
Liposomes with encapsulated protein or peptide antigen are phagocytosed by macrophages and eventually accumulate in lysosomes. Once in the lysosomes, degraded peptides are presented to the major histocompatibility complex class II (MHCII) complex on the macrophage surface. This results in the stimulation of specific T-helper cells, and, ultimately, stimulation of specific B cells, which results in the subsequent secretion of antibodies [109]. A fraction of the liposomal antigen can escape from endosomes into the cytoplasm (for example, when pH-sensitive liposomes are used) and in this case the liberated antigen is processed and presented in association with the MHCI complex, which induces a cytotoxic T-lymphocyte (CTL) response; this provides liposomes with certain benefits over traditional adjuvants (such as Freund's adjuvant) that do not induce any significant CTL response.

Magnetic liposomes for cancer treatment
An interesting approach for targeted drug delivery under the action of magnetic field is the use of liposomes loaded with a drug and a ferromagnetic material. Magnetic liposomes containing doxorubicin were intravenously administered to osteosarcoma-bearing hamsters.When the tumor-implanted limb was placed between two poles of a 0.4 Tesla magnet, the application of the field for 60 minutes resulted in a fourfold increase in drug concentration in the tumour [110]. In the same osteosarcoma model in which the magnet was implanted into the tumour, magnetic liposomes loaded with adriamycin demonstrated better accumulation in tumour vasculature and resulted in enhanced tumor-growth inhibition [111].

Liposome-Based DNA/Protein Vaccines
More recently, co-entrapment of the plasmid DNA vaccine together with the protein vaccine it encodes in the same liposome by the use of the same technology leads, after only one injection, to even stronger immune responses than those seen with liposomes containing the DNA or the protein vaccine alone [112]. This approach to genetic immunization mimics the way by which immunity is achieved in viral infections where both the viral DNA and the envelope proteins it encodes contribute to the immune responses against the virus. The coating liposomes containing the DNA and protein vaccines with mannose residues (via the incorporation into the bilayers of a mannosylated lipid) further potentiates immune responses to the vaccine, presumably by the targeting of such liposomes to the mannose receptors on the surface of APCs [113]. Here, we describe the methodology for the incorporation of plasmid DNA and/or protein into liposomes of varying lipid composition, vesicle size, and surface charge, as well as immunization studies with cationic liposomes (with or without incorporated mannosylated lipid) co-entrapping DNA and the protein it encodes.

Mitochondriotropic Liposomes
The mitochondrion is an essential organelle for all eukaryotic cells. Mitochondria are unique in comparison to all other organelles as they contain their own genome (mtDNA) and the necessary transcription and translation systems. The accumulation of somatic mutations in the mitochondrial genome has been suggested to be involved in aging, in age-related neurodegenerative diseases, as well as in cancer. The term ''stoichiometric carriers''are composed of biologically active molecules and the mitochondriotropic triphenylphosphonium (TPP) cation. In a series of extensive in vitro studies performed by Murphy and coworkers, bioactive molecules linked to TPP were shown to accumulate up to several hundredfold inside mitochondria in comparison to the corresponding native, i.e., free bioactive molecules. More recently they also tested the potential of TPP as a mitochondria-specific drug carrier for in vivo administrations by investigating the mode of delivery, tissue distribution, and clearance of three different TPP conjugates within mice [114]. They could show that relatively high doses of TPP conjugates can be fed safely to the animals over long periods of time resulting in steady-state distributions within heart, brain, liver, and muscle. A potential drawback of the use of stoichiometric carriers is the need for covalent linkage between carrier and bioactive molecule, which may influence its biological activity.

Virosomes for Drug Delivery

Special attention has been paid to the delivery of influenza vaccine using virosomes containing the spike proteins of influenza virus [115], used because it elicits high titres of influenza-specific antibodies. Trials of virosome influenza vaccine in children showed that it is highly immunogenic and well tolerated [116] Virosomes can be used for cell- and organ- or tissue-specific delivery of pharmaceutically active substances in the body. The unique properties of virosomes partially relate to the presence of HA in their membrane. This viral protein not only confers structural stability and homogeneity to virosomal formulations, but also significantly contributes to the fusion activity of virosomes, which induces the endolysosomal pathway. On the virosomal surface, ligands can be attached. This function is crucial for the targeted delivery of drugs. Therefore, virosomes selectively bind with their ligands to the target cell. Likewise, the virosomal HA promotes binding to the target cell and receptor-mediated endocytosis. In the endosome, the virosomal HA— triggered by an acidic environment—mediates membrane fusion, and therapeutically active substances escape from the endosome into the cytoplasm of the target cell. This concept has been validated in vivo. The cytotoxic drug doxorubicin was encapsulated into the virosomes. On their surface, monoclonal antibodies were cross-linked, mediating specific targeting of the carrier to cancer cells. The virosome-formulated cytostatics were delivered to the target cells and dramatically reduced the tumor volume [117]. The specificity of the targeting as well as the efficiency of cellular uptake can be highly modulated and adapted to the preferred conditions In general, virosomes can provide an excellent opportunity for the efficient delivery of both various antigens and many drugs (including nucleic acids, cytotoxic drugs and toxoids) [118,119] although they might present certain problems associated with their stability/leakiness and immunogenicity.

Cytoskeleton-specific immunoliposomes

Specific anticardiac myosin monoclonal antibodies have an excellent capacity to recognize and bind hypoxic cells with damaged plasma membranes when intracellular myosin is exposed into extracellular space [120]. This property of the antimyosin antibody has been successfully used for the delivery of antibody-bearing liposomes in the field of experimental myocardial infarction. In addition, immunoliposomes specifically targeting ischaemically damaged cardiomyocytes (cytoskeleton-specific immunoliposomes) seal membrane damage and decrease the level of cell death both in vitro and in the isolated rat heart model (121,122).

Liposomes for Pulmonary Delivery

From a toxicological viewpoint, liposomes are an appropriate drug delivery system for administration to the lungs. They prepared with phospholipids endogenous to the lung as surfactants. Both animals and humans Studies have shown that liposomes can modulate the fate of pulmonary deposited materials, increasing their residence time within the airways and potentially decreasing systemic adverse effects. A wide range of liposome associated materials have been administered to the airways of both animals and humans.

Radiolabeling of Liposomes for Scintigraphic Imaging

Scintigraphic imaging is a noninvasive imaging technique commonly applied in nuclear medicine. Radiolabeled compounds such as ^{67}Ga, ^{99m}Tc, ^{111}In, ^{123}I, ^{125}I (called radiopharmaceuticals or radiotracers) are administered intravenously to patients for diagnostic or, in certain cases, therapeutic purposes. GAMMA-SCINTIGRAPHY and MRI both require a sufficient quantity of radionuclide or paramagnetic metal to be associated with the liposome. There are two possible routes to improve the efficacy of liposomes as contrast mediums for gamma-Scintigraphic and MRI: increasing the quantity of carrier-associated reporter metal (such as ^{111}In), and/or enhancing the signal intensity. To increase the load of liposomes with reporter metals, amphiphilic chelating polymers, such as N, α-(DTPA-polylysyl) glutaryl phosphatidyl ethanolamine, were introduced [123]. These polymers easily incorporate into the liposomal membrane and markedly increase the number of chelated in atoms attached to a single lipid anchor. In the case of MRI, metal atoms chelated into these groups are directly exposed to the water environment, which enhances the signal intensity of the paramagnetic ions and leads to corresponding enhancement of the vesicle contrast properties.

CONCLUSION

It require one-to-two more years to complete golden fifty years of effort by scientists and formulators on liposomes from the concept of clinical utility to its acceptance as a novel drug delivery system. Incorporating drugs inside engineered colloidal carriers is a promising approach that can lead to improved drug delivery. Colloidal carriers such as liposomes can be used to improve the therapeutic index of both established and new drugs by modifying their distribution. Thus increasing their efficacy and/or reducing their toxicity. The liposomes utilized in the therapeutic

applications of drug targeting, imaging tumors, gene medicine and vaccine delivery, cancer treatment (chemotherapy), topical applications and lung diseases. They have the clinical benefit of their passive accumulation at the site of increased vasculature permeability if have diameter of less than 200 nm and ability to reduce the toxic effect of entrapped drug relative to free drug. The liposomal preparations provide increase in therapeutic index, a measure of efficacy over toxicity and significant reduction in side effects. Their clinically essential properties include- biocompatibility, improved bioavailability of hydrophobic drugs with poor aqueous solubility, low toxicity and reduced side effects, lower clearance rate, targetability, controlled release at requirements and more importantly better PK-PD profiles.

If these delivery systems are carefully designed with respect to the target and route of administration, they may provide one solution to some delivery problems posed by new classes of active molecules such as peptides, proteins, genes and oligonucleotides. Liposomal drug delivery is now an established technology and its scope for the clinical products has no limit. Nevertheless, challenges still remain. More human clinical studies are needed to establish *in vitro- in vivo* correlation, which may help in understanding the solubilization mechanism of lipids in the formulation. Good product quality and product performance can be achieved with rational design of a lipid based dosage form. The issue with long term stability and high production cost, which leads to a limitation before have been solved with improvements in technology.

Acknowledgements
The authors are very grateful to Dr. Anil K. Mahapatra, M.D (Medicine), D.M (Cardiology), Interventional cardiologist, Indus Hospital, Visakhapatnam and Dr. Rashmi Rath, M.S. (Ophthalmology), F.I.C.S, Ophthalmologist, Shankar Foundation Eye Hospital, Visakhapatnam for their revision of this manuscript. We thank Dr. Sudarsan Biswal (Drugs control Department, Govt. of Odisha) and Dr. Phani Bhushan, Dept. of Biotechnology, Maharajah's College of Pharmacy for their discussions on some aspects of the manuscript.

REFERENCES

[1] K.W. Leong, Y. Zhang, H. F. Chan, *Adv Drug Delivery Rev*, **2013**, 65, 104–120.
[2] T. M. Allen, P. R. Cullis. *Adv Drug Delivery Rev*, **2013**, 65, 36–48.
[3] J. Klein, R. Goldberg, *Chem Phys Lipids*, **2012**, 165**,** 374– 381.
[4] V. P. Torchilin, *Nature Rev Drug Discovery*, **2005**, 4, 145-160.
[5] D. Paolino, D. Cosco, M. Roberto, C. Christian, F. Massimo, *Drug Discovery Today*, **2011**, 16, 7-8.
[6] J. A. Zasadzinski, B. Wong, N. Forbes, B. Gary, G. Wu, *Curr Opin Colloid Interface Sci*, **2011**, 16, 203–214.
[7] S. Mitragotri, J.W. Yoo, N. Doshi, *Advanced Drug Delivery Reviews*, **2011**, 63, 1247–1256.
[8] S. Singh, S. Chakraborty, D. Shukla, B. Mishra, *Eur J Pharm Sci*, **2009**, 73, 1–15.
[9] K. P. Gannu, P. R. Rao, *Acta Pharm Sinica B*, **2011**, 1(4), 208–219.
[10] S. R. Vemuri, C. T. Rhodes, *Pharm Acta Helv*, **1995**, 70**,** 95-111.
[11] S. N. Perttu, M. T. Hyvonen, I. Vattulainen, *Biochim Biophys Acta*, **2009**, 1788, 122–135.
[12] F. M. Goni, A. Alonso, *Biochim Biophys Acta*, **2006**, 1758, 1902–1921.
[13] T. M. Allen, *Adv Drug Delivery Rev*, **1994**, 13, 285-309.
[14] J. D. Plessis, C. Ramachandran, N. Weiner, D. G. Moiler, *Int J Pharm*, **1996**, 127, 273-278.
[15] A.V. Popova, K. H. Dirk, *Biophys J*, **2007**, 93, 1204–1214.
[16] N. M. Rao, *Chem Phys Lipids*, **2010**, 163, 245–252.
[17] C. Lonez, M. Vandenbranden, J. M. Ruysschaert, *Adv Drug Delivery Rev*, **2012**, 64, 1749–1758.
[18] D. Zhi, S. Zhang, F. Qureshi, Y. Zhao, S. Cui, B. Wang, H. Chen, Y. Wang, D. Zhao, *Bioorg Med Chem Lett***, 2012**, 22, 3837–3841.
[19] B. K. Kim, K. O. Doh, J. H. Nama, H. Kang, J. G. Park, I. J. Moon, Y. B. Seu, *Bioorg Med Chem Lett*, **2009**, 19, 2986–2989.
[20] M. N. Jones, *Adv Colloid Interface Sci*, **1995**, 54, 93-128.
[21] M. D. Joshi, W. J. Unger, G. Storm, Y. V. Kooyk, M. Enrico, *J Contr Release*, **2012**, 161, 25-37.
[22] D. Gutman, G. Golomb, *J Contr Release*, **2012**, 161, 619–627.
[23] S. Koudelka, J. Turanek, *J Control Release*, **2012**, 163, 322–334.
[24] L. D. Danilo, B. Ceh, M. Winterhalterb, P. M. Frederik, J. J. Vallnerd, *Adv. Drug Delivery Rev*, **1997**, 24, 165-177.
[25] K. Sen, M. Mandal, *Int. J .Pharm*, **2013**, 448, 28– 43.

[26] T. Shehata, K. I. Ogawara, K. Higaki, T. Kimura, *Inter J of Pharma*, **2008**, 359, 272–279.
[27] M. C. Woodle, L. R. Collins, E. Sponsler, N. Kossovsky, D. Papahadjopoulos, F. J. Martin, *Biophys J*, **1992**, 61, 902-910.
[28] Y. K. Malam, M. Loizidou, A. M. Seifalian, *Trends Pharmacol Sci*, **2009**, 30(11), 592-598.
[29] M. C. Woodle, *Chem. Phys. Lipids*, **1993**, 64, 249-262.
[30] R. R. Sawanta, V. P. Torchilin, *The AAPS Journal*, **2012**, 14, 303-314.
[31] M. I. Papisov, *Adv. Drug Delivery Rev*, **1998**, 32, 119–138.
[32] J. A. O. Shaughnessy, *Clin Breast Cancer*, **2003**, 4(5), 318-328.
[33] M. L. Immordino, P. Brusa, S. Arpicco, B. Stella, F. Dosio, L. Cattel, *J. Control. Release*, **2003**, 91, 417–429.
[34] A. Koshkaryev, R. Sawant, M. Deshpande, V. Torchilin, *Adv. Drug. Delivery. Rev*, **2013**, 65, 24–35.
[35] P. A. M. Pierre, S. Gert, D. J. A. B. Crommelln, *Adv. Drug Delivery Rev*, **1987**, 1, 249-266.
[36] G. Bendas, A. Krause, U. Bakowsky, J. Vogel, U. Rothe, *Int. J. Pharm*, **1999**, 181, 79–93.
[37] D. Kozlowska, P. Foran, P. M. Mahon, J. Martin, S. S. Eustace, R. O. Kennedy, *Adv. Drug Delivery Reviews*, **2009**, 61, 1402–1411.
[38] C. Lonez, M. Vandenbranden, J. M. Ruysschaert, *Prog Lipid Res*, **2008**, 47, 340–347.
[39] A. Percot, D. Briane, R. Coudert, P. Reynier, N. Bouchemal, N. Lievre, E. Hantz, J. L. Salzmann, A. Cao, *Inter. J. Pharm*, **2004**, 278, 143–163.
[40] R. I. Zhdanov, O. V. Podobed, V.V. Vlassov, *Bioelectrochemistry*, **2002**, 58, 53– 64.
[41] Y. Kyoung, T. G. Park, *Adv Drug Delivery Rev*, **2009**, 61, 850–862.
[42] D. D. Lasic, N. S. Templeton. *Adv Drug Deliv Rev*, **1996**, 20, 221-266.
[43] F. S. Mehrabadi, W. Fischer, R. Haag, *Curr Opin Solid St M*, **2012**, 16, 310–322.
[44] C. M. Luís, M. S. Loura, R. Maria, A. Barros, M. Prieto, *Biochim Biophys Acta*, **2011**, 1808, 2694–2705.
[45] K. Kono, M. Iwamoto, R. Nishikawa, H. Yanagie, T. Toru, *J Contr Release*, **2000**, 68, 225–235.
[46] S. Naoki, C. Kojima, A. Harada, K. Koiwai, K. Shimizu, N. Emic, K. Kono, *Int J Pharm*, **2006**, 325, 186–190.
[47] E. Yub, C. Kojim, A. H. Tana, S. Watarai, K. Kono, *Biomaterials*, **2010**, 31, 943–951.
[48] M. E. Baraka, E. I. Pecheur, D. F. H. Wallach, J. R. Philippot, *Biochim Biophys Acta*, **1996**, 1280: 107-114.
[49] D. T. Auguste, R. K. Prudhomme, P. L. Ahl, P. Meers, J. Kohn, *Biochim Biophys Acta*, **2003**, 1616, 184– 195.
[50] J. Kunisawa, S. Nakagawa, T. Mayumi, *Adv Drug Deliv Rev*, **2001**, 52, 177–186.
[51] D. C. Drummond, M. Zignani, J. C. Leroux, *Prog Lipid Res*, **2000**, 39, 409-460.
[52] Y. Ogawa, M. Kodaka, H. Okuno, *Chem Phys Lipids*, **2002**, 119, 51-68.
[53] T. Ishida, M. J. Kirchmeier, E. H. Moase, S. Zalipsky, T. M. Allen, *Biochim Biophys Acta*, **2001**, 1515, 144-158.
[54] A. E. Felber, M. H. Dufresne, J. C. Leroux, *Adv Drug Delivery Rev*, **2012**, 64, 979–992.
[55] E. Roux, M. Francis, M. F. Winnik, J. C. Leroux, *Inter J Pharm*, **2002**, 242, 25–36.
[56] T. Sato, J. Sunamoto, *Prog Lipid Res*, **1992**, 31(4), 345-372.
[57] S. Muro, *J Controlled Release*, **2012**, 164, 125–137.
[58] H. Grüll, S. Langereis, *J Contr Release*, **2012,** 161, 317–327.
[59] D. Needhama, M. W. Dewhirst, *Adv Drug Delivery Rev*, **2001**, 53, 285–305.
[60] M. D. Smeta, S. L. Sandra, V. D. Bosch, H. Grüll, *J. Controlled Release*, **2010**, 143, 120–127.
[61] M. N. Antipina, G.B. Sukhorukov, *Adv Drug Delivery Rev*, **2011**, 63, 716–729.
[62] A. Schroeder, R. Honen, K. Turjeman, A. Gabizon, J. Kost, Y. Barenholz, J. *Contr Release*, 2009, 137, 63–68.
[63] A. Schroedera, J. Kostb, Y. Barenholza, *Chem Phys Lipids*, **2009**, 162, 1–16.
[64] N. Fomina, J. Sankaranarayanan, A. Almutairi, *Adv Drug Delivery Rev*, **2012**, 64, 1005–1020.
[65] P. Rai, S. Mallidi, X. Zheng, R. Rahmanzadeh, Y. M. S. E. A. Khurshid, T. Hasan, *Adv Drug Delivery Rev*, **2010**, 62, 1094–1124.
[66] D. A. Tyrrell, T. D. Heath, C. M. Colley, E. R. Brenda, *Biochim Biophys Acta*, **1976**, 457, 259-302.
[67] D. D. Lasic, .*Biochem J*, **1988**, 256, 1-11.
[68] S. Batzri, E. D. Korn, *Biochim Biophys Acta*, **1973**, 298, 1015-1019.
[69] R. L. Hamilton, J. J. Goerke, L. S. S. Guo, M. C. Williams, R. J. Havel, *J Lipid Res*, **1980**, 2(1), 981-992.
[70] M. Pons, M. Foradada, J. Estelrich, *Int J Pharm*, **1993**, 95, 51-56.
[71] S. N. Chatterjee, S. Agarwal, *Free Radic Biol Med*, **1988**, 4, 51-72.
[72] K. Thompson, H. Singh, *J.Dairy Sci*, **2006**, 89, 410–419.
[73] M. Bahrami, S. Ranjbarian, *J Supercritical Fluids*, **2007**, 40, 263–283.
[74] C. C. Beh, R. Mammucari, N. R. Foster, *Chem Eng J*, **2012**, 188, 1– 14.
[75] L. Lesoin, O. Boutin, C. Crampon, E. Badens, *Colloids and Surfaces A Physicochem Eng Aspects*, **2011**, 377, 1–14.

[76] L. A. Meure, N. R. Foster, F. Dehghani, *AAPS PharmSciTech*, **2008**, 9(3), 798-809.
[77] J. T. Buboltz, G. Feigenson, *Biochim Biophys Acta*, **1999**, 1417, 232-245.
[78] Y. C. Barenholz, *J of Contr Release*, **2012**, 160, 117-134.
[79] A. V. Yadav, M. S. Murthy, A. S. Shete, S. F. Sakhare, *Ind J Pharm Edu Res*, **2011**, 45(4), 402-413
[80] M. Gangwar, R. Singh, R. K. Goel, G. Nath, *Asian Pac J Trop Biomed*, **2012**, 1176-1188.
[81] F. Hallouard, N. Anton, P. Choquet, A. C. T. Vandamme, *Biomaterials*, **2010**, 31, 6249-6268.
[82] C. Chen, D. Han, C. Cai, X. Tang, *J Control Release*, **2010**, 142, 299–311.
[83] B. Heurtault, P. Saulnier, B. Pech, J. E. Proust, J. P. Benoit, *Biomaterials*, **2003**, 24, 4283–4300.
[84] B. Stark, G. Pabst, R. Prass, *Eur J Pharm Sci*, **2010**, 41, 546–555.
[85] L. Y. Loukas, V. Vraka, G. Gregoriadis, *Int J Pharm*, **1998**, 162, 137–142.
[86] K. A. Edwards, A. J. Baeumner, *Talanta*, **2006**, 68, 1432–1441.
[87] S. Bibi, R. Kaura, M. H. Lacey, S. E McNeil, J. Wilkhu, E. Lattmann, D. Christensen, A. R. Mohammed, Y. Perrie, *Inter J Ph.arma*, **2011**, 417, 138– 150.
[88] A. P. R. Johnston, G. K. Such, S. Leen, F. Caruso, *Curr. Opin. Colloid Interface Sci*, **2011**, 16, 171–181.
[89] A. Sharma, U. S. Sharma, *Inter J Pharma*, **1997**, 154, 123-140.
[90] A. Chonn, P. R. CuBis, D. V. Devine, *J Immunol.*, **1991**, 146, 4234-4241.
[91] N. K. Uchiyamab, H. Kiwadab, N. Tomoko, *Inter J Pharma*, **2005**, 298, 198–205.
[92] L. M. Strawn, L. K. Shawver, *Expert Opin Investig Drugs*, **1998**, 7, 553–573.
[93] D. B. Mendel, R. E. Schreck, D. C. West, G. Li, L. M. Strawn, S. S. Tanciongco, S. Vasile, L. K. Shawver, J. M. Cherrington, *Clin Cancer Res*, **2000**, 6, 4848–4858.
[94] V. Brower, *Nat Biotechnol*, **1999**, 17, 963–968.
[95] C. B. Pattillo, F. S. Sarraf, N. Ramakrishna, B. M. Moore, C. George, *Pharm. Res*, **2005**, 22, 1117.
[96] P. K. Dubey, V. Mishra, S. Jain, S. Mahor, S. P. Vyas, *J Drug Target*, **2004**, 12, 257.
[97] R. M. Schiffelers, G. A. Koning, T. L. T. Hagen, M. H. Fens, A. J. Schraa, A. P. Janssen, R. J. Kok, G. Molema, G. Storm, *J Contr Release*, **2003**, 91, 115.
[98] X. B. Xiong, Y. Huang, W. L. Lu, H. Zhang, X. Zhang, Q. Zhang, *Pharm Res*, **2005**, 22, 933.
[99] P. Holig, M. Bach, T. Volkel, T. Nahde, S. Hoffmann, R. Muller, E. Kontermann, *Protein Eng Des Sel*, **2004**, 17, 433.
[100] K. Kurohane, Y. Namba, N. Oku, *Life Sci.*, **2000**, 68, 273.
[101] P. Harvie, B. Dutzar, T. Galbraith, S. Cudmore, O. M. Anklesaria, P. R. Paul, *Liposome Res*, **2003**, 13, 231.
[102] A. Fahr, K. Muller, T. N. Muller, S. Brusselbach, *J Liposome Res*, **2002**, 12, 37.
[103] T. M. Allen, D. R. Mumbengegwi, G. J. R. Charrois, *Clinical Cancer Research*, **2005**, 11(9), 3567–3573.
[104] J. W. Park, K. Hong, D. B. Kirpotin, G. Colbern, R. Shalaby, J. Baselga, Y. Shao, U. B. Nielsen, J. D. Marks, D. Moore, D. Papahadjopoulos, C. C. Benz, *Clin Cancer Res*, **2002**, 8(4), 1172–1181.
[105] R. J. Lee, P. S. Low, *J Biolog Chem*, **1994**, 269(5), 3198–3204.
[106] R. J. Lee, P. S. Low, *Biochim Biophys Acta*, **1995**, 1233(2), 134–144.
[107] X. Q. Pan, R. J. Lee, *Anticancer Research*, **2005**, 25(1A), 343–346.
[108] A. Gabizon, *Bioconjug Chem*, **1999**, 10(2), 289–298.
[109] M. Friede, *Boca Raton: CRC Press*, **1995**, 189.
[110] H. Nobuto, T. Sugita, T. Kubo, S. Shimose, Y. Yasunaga, T. Murakami, M. Ochi, *Int J Cancer*, **2004**, 109, 627–635.
[111] T. Kubo, T. Sugita, S. Shimose, Y. Nitta, Y. Ikuta, T. Murakami, *Int J Oncol*, **2001**, 18, 121–125.
[112] A. Bacon, D. H. Hreczuk-Hirst, B. McCormack, *In: Proceedings of 30th Annual Meeting of the Controlled Release Society, Abstract*, **2003**, 441:884.
[113] N. Garcon, G. Gregoriadis, M. Taylor, J. Summerfield, *Immunology*, **1988**, 64,743–745.
[114] R. A. Smith, C. M. Porteous, A. M. Gane, M. P. Murphy, *Proc Natl Acad Sci USA*, **2003**, 100(9), 5407–5412.
[115] A. Huckriede, L. Bungener, T. Daemen, J. Wilschut, *Meth. Enzymol*, **2003**, 373, 74–91.
[116] C. Herzog, I. C. Metcalfe, U. B. Schaad, *Vaccine*, **2002**, 20 (5), 24–28.
[117] E. Waelti, N. Wegmann, R. Schwaninger, A. Wetterwald, C. Wingenfeld, B. Rothen-Rutishauser, C. D. Gimmi, *Cancer Res*, **2002**, 62(2), 437–444.
[118] R. Gluck, C. Moser, I. C. Metcalfe, *Expert Opin Biol Ther*, **2004**, 4, 1139–1145.
[119] C. Moser, I. C. Metcalfe, J. F. Viret, *Expert Rev Vaccines*, **2003**, 2, 189–196.
[120] B. A. Khaw, J. A. Mattis, G. Melincoff, H. W. Strauss, H. K. Gold, E. Haber, *Hybridoma*, **1984**, 3, 11–23.
[121] B. A. Khaw, V. P. Torchilin, I. Vural, J. Narula, *Nature Med*, **1995**, 1, 1195–1198.
[122] T. Khudairi, B. A. Khaw, *J Am Coll Cardiol*, **2004**, 43, 1683–1689.
[123] V. P. Torchilin, *Current Pharm Biotech*, **2000**, 1, 183–215.

Table 4: List of Abbreviations

Abbreviations used in text	Full name
PEG	Poly ethylene glycol
RES	Reticulo endothelial system
MPS	Mononuclear phagocyte system
PTX	Paclitaxel
HPI	Hydrogenated soya Phosphatidyl inositol
DSPE	Distearoyl phosphatidyl ethanolamine
mAbs	Monoclonal antibodies
DOGS	Di octadecylamidoglycylspermine
DODAB	Di octadecyl dimethyl ammonium bromide
DOTAP	Di oleoyloxypropyl trimethyl ammoniummethyl propane
GM_0	Monosialoganglioside
CpG	Cytosine being 5 prime to the guanine base
TLR	Toll like receptors
Suc PG	Succinylated poly glycidol
EYPC	EGG Yolk Phosphatidylcholine
DC-Chol	Dimethyl amino ethane carbamoyl cholesterol
MGGluPG	Mehtyl glutarylated poly glycidol
HANA	Hemagglutinating and neuraminidase
CTL	Cytotoxic T- lymphocyte
PE	Phosphatidyl ethanolamine
DOPE	Di oleoyl phosphor ethanolamine
EPR	Enhanced permeability and retention
TSL	Temperature sensitive liposome
DPPC	Di palmitoyl phosphatidyl choline
DSPC	Distearoyl phosphatidyl choline
P-Lyso-PC	Palmitoylhydroxyl-lyso- phosphotidyl choline
DOX	Doxorubicin
LFUS	Low frequency ultra sound
PDT	Photo dynamic therapy
BHT	Butylated hydroxyl toluene
PG	Phosphatidylglycerol