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STEALTH LIPOSOMES: AN ADVANCEMENT OF LIPOSOME TECHNOLOGIES

Vibha Bhut*[1],
Anil Prajapati[1], Dipti Rathod[1], Kunal Patel[1], Poras Patel[1]

[1]Shree Swaminarayan Sanskar Pharmacy College, Zundal, Dist. Gandhinagar, Gujarat, India-382421.

ABSTRACT

A liposome is a microscopic vesicle consisting of an aqueous core enclosed in one or more phospholipid layers with a diameter ranging from 25 nm to 10000 nm, used to convey vaccines, drugs, enzymes, or other substances to target cells or organs. The resulting closed sphere may encapsulate aqueous soluble drugs within the central aqueous compartment or lipid soluble drugs within the bilayer membrane. The most promising results of liposome modification were achieved by covalent attachment of hydrophilic polymer groups to the liposome surface. The presence of PEG on the surface of the liposomal carrier has been shown to extend blood-circulation time while reducing mononuclear phagocyte system uptake (stealth liposomes). This technology has resulted in a large number of liposome formulations encapsulating active molecules. Further by synthetic modification of the terminal PEG molecule, stealth liposomes can be actively targeted with monoclonal antibodies or ligands. This review focuses on stealth technology and it also discusses emerging trends of this promising technology.

Correspondence to Author

Vibha Bhut

Shree Swaminarayan Sanskar Pharmacy College, Zundal, Dist. Gandhinagar, Gujarat, India-382421.

Email: bhut_vibha@yahoo.Com

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INTRODUCTION

Liposomes include controlled retention of entrapped drug in the presence of biological fluids, prolonged vesicle residence in circulation and enhanced vesicle uptake by target cells. Accumulated (in vivo) evidences suggest that some liposome-entrapped drugs and vaccines exhibit superior pharmacological properties to those observed with conventional formulations especially in cancer chemotherapy, antimicrobial therapy, vaccines, diagnostic imaging and the in treatment of ophthalmic disorders. Liposome technology has progressed *from conventional liposomes to the current generation STEALTH liposomes, and to the future ligand-targeting STEALTH liposomes (fig.1).*

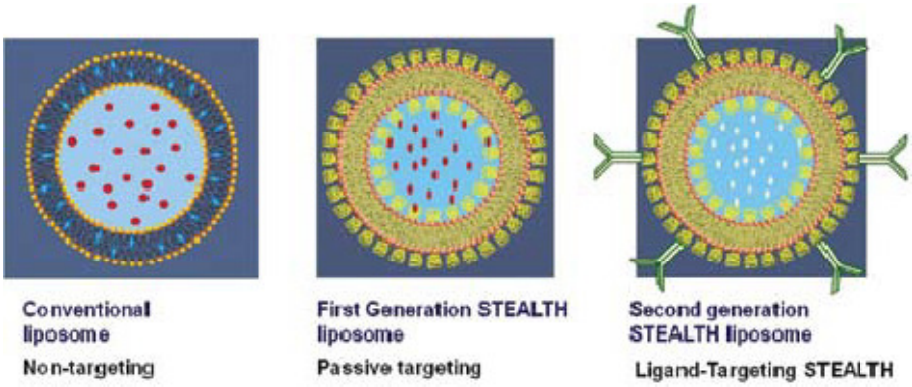


Fig. 1 Schematic Presentation of the Advancement of Liposomes Technologies from Conventional Liposomes to the Current Generation STEALTH Liposomes, and to the Future Ligand-Targeting STEALTH Liposomes.

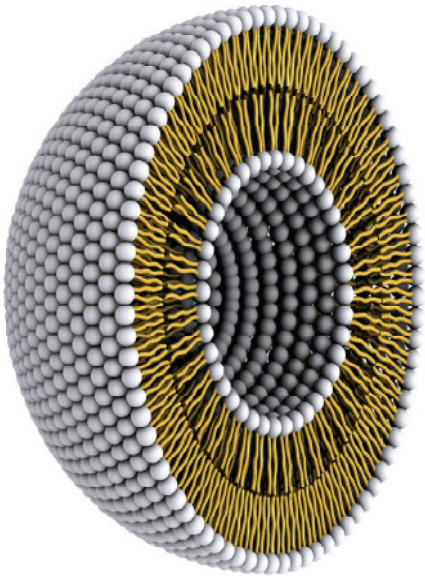


Fig.2 Liposome Structure

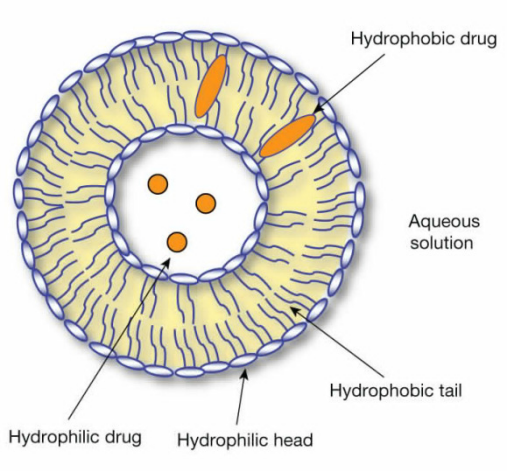


Fig.3 Structure of Liposome with Drug Entrapment

Following intravenous injection, liposomes and nanoparticles are cleared rapidly from the blood (usually within minutes) by elements of the RES, particularly the hepatic Kupffer cells of liver and fixed macrophages of spleen[1]. The rate of liposome uptake by RES is believed to be related to the process of opsonization or dysopsonization of liposomes. There are two different ways with which liposomes may interact with macrophages. Liposomes may directly interact with the surface receptor(s) of macrophages (fig.4) or indirectly via certain serum proteins. Liposomes can be assumed to be effective "Therapeutic System" only when we can keep them out of RES, allowing them to be directed to other sites *in vivo.* Except for the cases where the drugs have to be directed to RE system.

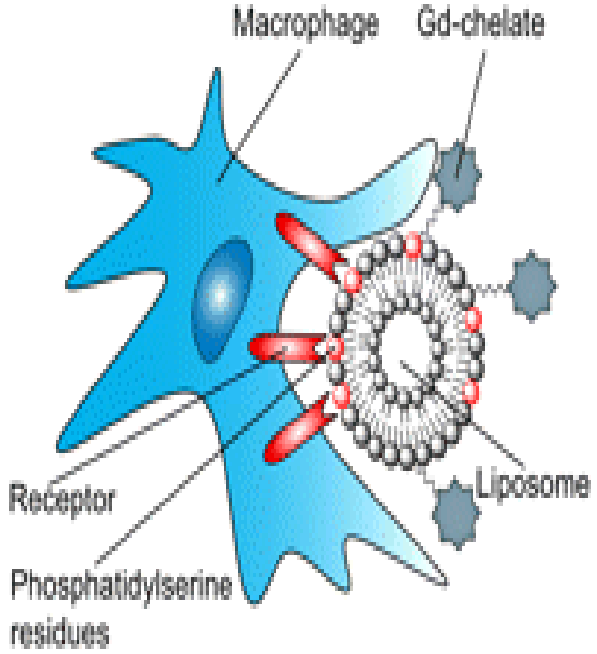


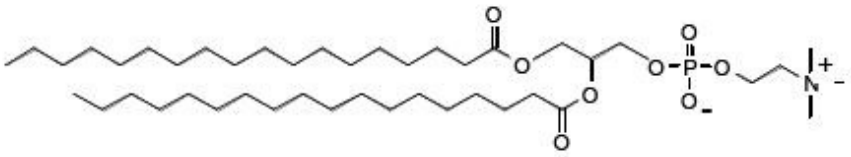
Fig.4 Liposome Interaction with Macrophage Receptors

RES uptake presents some major problems with regard to therapeutic applications of conventional liposomes.

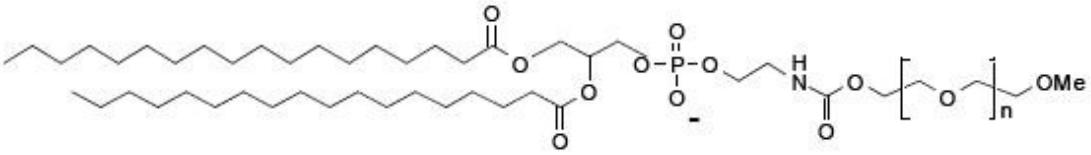
The problems associated with the conventional liposomes are,

1. The pronounced tendency of liposomes to localize in RES raises concerns about RES impairment and its consequences particularly during continued liposomes administration.
2. Short circulation times severely limit the use of liposomes as micro-reservoir systems for the slow release of biologically active molecules which are normally degraded rapidly within the vasculature.
3. Rapid uptake of liposomes into liver and spleen greatly reduces the possibility of extra vascularization of liposomes and substantially prevents targeting of liposomes to the cells within the vasculature or targeting to non-RE tissues[2-4].

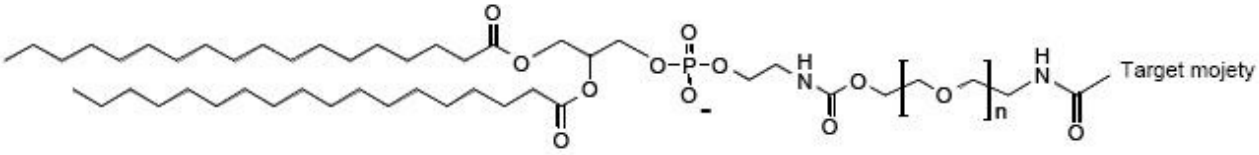
Liposomes that are formulated to "escape" from being recognized by RES can remain in circulation for prolonged periods and may serve as micro-reservoir system and may minimize the problems associated with conventional liposomes. The term "Stealth" liposomes was coined to describe this evasive property (stealth is a registered Trademark of Liposome Technology Inc, Menlo park, CA (USA) and polyethylene glycol lipids (PEG-lipids) are commonly referred to as Stealth Lipids. Among the different polymers investigated in the attempt to improve the blood circulation time of liposomes, poly-(ethylene glycol) (PEG) has been widely used as polymeric steric stabilizer. It can be incorporated on the liposomal surface in different ways, but the most widely used method at present is to anchor the polymer in the liposomal membrane via a cross-linked lipid (i.e.PEG-distearoyl phosphatidylethanolamine [DSPE] as schematized below)[5,6].



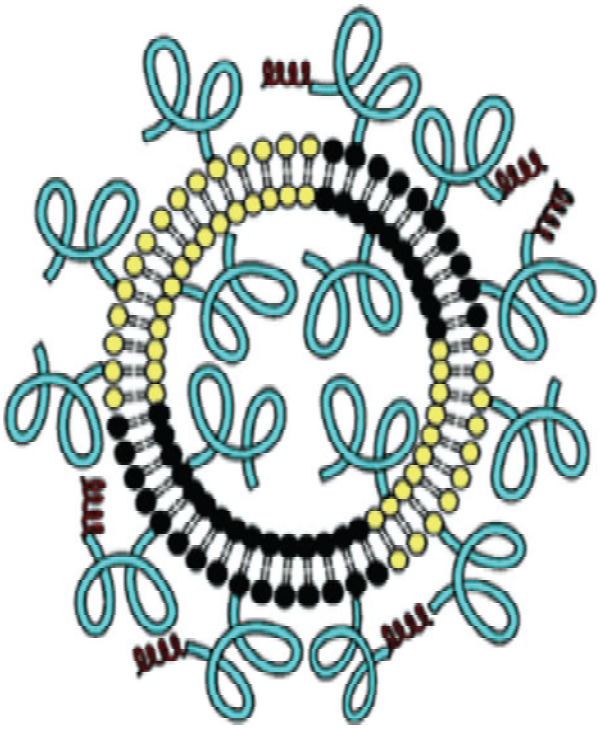
DSPC



DSPE-PEG



DSPE-PEG- Target moiety



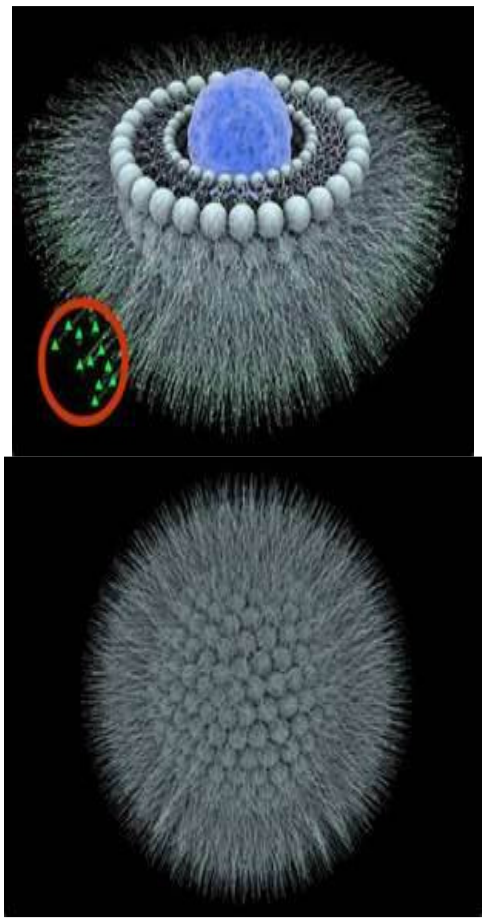


Fig.5 Schematic Depictation of Stelth PEGylated Liposome

Table 1: Composition of Few Stealth Liposomal Systems and Their Performance In *Vivo*[7-10]

Sr. No.	Composition of the system	Performance in vivo
Polyethylene glycols		
1.	Hydrogenated SOY Phosphotidylcholine/ cholesterol/polyethylene glycol-distearoyl phosphotidylethenolamine.	Stealth liposome formulation was significantly more effective than conventional liposome formulation (Egg phosphotidylglycerol/ Egg phosphotidylcholine/ cholesterol/ dI-alpha tocopherol) in reducing the incidence of metastases from intra mammory implants of tumor MC2A, tumor MC2B and tumor MC65.
2.	Phosphotidylcholine liposomes casted with upto 10 mol% PE with a covalently attached PEG 5000 head group.	Circulation time of stealth liposomes within first 24 hrs exceeded 8000% over pure phosphotidylcholine liposomes.
3.	DSPE/ cholesterol (2:1) liposomes were grafted with PEG 1900 mg/ml.	Membrane bound PEGs can exert a significant inter bilayer repulsion thereby polymer chain extends to

		50°A from lipid bilayer surface, which may reduce interactions with plasma proteins and phagocytic cells.
Gangliosides and glycolipids		
1.	Liposomes were prepared with saturated phospholipids and sphingomyelin.	These liposomes attracted serum dysopsonins which inhibit their uptake by liver cells. Inclusion of cholesterol in these liposome preparations enhanced this uptake in splenic cells but not liver cells.
2.	Egg PC/ sphingomyelin/ cholesterol/ ganglioside GM1 in molar ratio 1:1:1:0.14	The ability of GM1 to reduce leakage of aqueous contents from liposomes there by reducing opsonization was due to (i) Molecular confirmation. (ii) Location of negative charge relative to phospholipid bilayer and carbohydrate back bone (iii) Packing characteristics of GM1 in phospholipid bilayers.
Synthetic phospholipids		
1.	Sphingomyelin/ cholesterol/ egg PC (1 :l :l) liposomes were prepared and a carramate derivative of PEG 1900 with distearoyl phosphotidylethanolamine (DSPE) was incorporated into these liposomes.	(PEG-DSPE) liposomes had the greatest ability to decrease the uptake by mononuclear phagocyte system.

ADVANTAGES OF PEG[11,12]:

1. PEG is a linear polyether diol
2. Biocompatibility
3. Solubility in aqueous and organic media
4. Lack of toxicity
5. Very low immunogenicity and antigenicity and
6. Good excretion kinetics

These properties allow its use in a variety of applications, including the biomedical field.

METHODS OF PREPARATION:

Liposome membranes containing bilayer-compatible species such as poly (ethylene glycol)-linked lipids (PEG-lipid) or the gangliosides are being used to prepare stealth liposomes. These, so called “Stealth” liposomes have a relatively longer half-life approximately 1 day (whereas the conventional liposomes have only minutes) in blood circulation and show an altered biodistribution in vivo. Vaage et al prepared stealth liposomes of doxorubicin and used to treat recently implanted and well established, growing primary mouse carcinomas, and to inhibit the development of spontaneous metastases from intra-mammary tumor implants and concluded that long circulation time of the stealth liposomes of doxorubicin formulation accounts for its superior therapeutic effectiveness. A brief review of different approaches undertaken for the preparation and characterization of stealth liposomes are discussed[13-17].

1. Polymer grafted lipid membranes:

Several workers have recently tried to prolong the circulation times of the liposomes by grafting the polymers like PEGs and gangliosides into the lipid vesicles. In addition, the attachments of similar polymers are being investigated in order to minimize or prevent the adsorption of proteins and cells from the blood stream and as a model polymer covered surfaces. A brief account of the composition and performance of few stealth liposomal systems is given in Table 1[18].

2. Polyethylene glycols:

Hershfield *et al.* prepared stealth liposomes of doxorubicin composed of hydrogenated SOY phosphotidylcholine/ cholesterol/ polyethylene glycol-distearoyl phosphotidylethanolamine and the therapeutic efficacy was compared in mice with conventional liposomes composed of egg phosphotidylglycerol or egg phosphotidylcholine or cholesterol or dl-alpha tocopherol. Stealth Liposome formulation was significantly more effective than the conventional liposome formulation in reducing the incidence of metastases from intra-mammary implants of tumor MC19 and tumor MC65 in curing mice with recent implants of tumor MC2A, tumor MC2B and tumor MC65 and in increasing the 8-week survival of mice with well established implants of tumor MC2B.

Senior *et al.* prepared liposomes which quantitatively retain aqueous markers, were covalently coupled *via* dipalmitoyl phosphotidylethanolamine (DPPE), to the hydrophilic polymer, monoethoxy poly (ethylene glycol) (MPEG 5000). These liposomes retained the coating in the presence of plasma, and appeared to adsorb plasma components more slowly than liposomes without the polymer. MPEG- coupled liposomes were cleared from the blood circulation up to 30% more slowly than liposomes without MPEG after i.v. administration in mice. New lipid carriers consisting of phosphotidylcholine (PC) liposomes casted up to 10 mol% phosphotidylethanolamine (PE) with a covalently attached PEG 5000 head group PE-PEG were prepared by Blume *et al* and in vivo studies were performed in mice. Vesicles exhibited very long circulation time after an i.v. administration in mice. The improvement over pure PC liposomes within the first 24 hours exceeded 8000%, at this point nearly 25% of the applied PE-PEG liposome being still in the circulation. Needham *et al* have performed X-ray diffraction studies to characterize the surface structures that promote steric stability of PEG (1900 mg/ml, degree of polymerization - 43 mers) grafted lipid vesicles [DSPE/cholesterol (2:1)] and concluded that membrane-bound PEGs can exert a significant inter bilayer repulsion thereby polymer chain extends a distance of 50^{o}A from the lipid bilayer surface, which inhibits mutual aggregation and likely reduces interactions with plasma proteins and phagocytic cells that normally lead to conventional liposome disintegration and uptake. The stealth property was not related to any augmentation of mechanical stability due to incorporation of PEG lipid[19-22].

3. Gangliosides and glycolipids:

Moghimi *et al* prepared liposomes from sphingomyelin and saturated phospholipids and their affinity to different serum opsonins (liver and spleen) was characterized neither liver nor spleen specific opsonins have affinity for sphingomyelin saturated phospholipid liposomes since serum fails to enhance their uptake.

On the contrary, these liposomes attract serum dysopsonins which inhibit their uptake by liver cells. Inclusion of cholesterol in these liposome preparations enhanced their uptake in spleenic cells but not liver cells. It was concluded that the fluidity and hydrophobicity of liposomal membranes play an important role in attracting the right opsonins which determine their phagocytic fate. Allen *et al* and Gabizons used gangliosides like GMI, GMz, GMs, G&s, GDla and glucolipids like sulfatides, globosides, glycosylceramide and several others for the preparation of stealth liposomes. But GMI, only has shown the ability to prolong circulation half-life and other negatively charged glycolipids with bulky head groups i.e., sulfatides and phosphotidylinositol, had some effect in prolonging circulation half-life. Bilayer rigidity, imparted by sphingomyelin or other high phase transition lipids acted synergistically with

negatively charged components especially GM1 in extending circulation time. This exclusive ability of GM1 may be accounted for different reasons;

(a) Presence of screened negative charge may contribute to RE avoidance, perhaps by decreasing or preventing opsonization of the bilayers. The negative charge of GM1 is shielded from the surface by the presence of two neutral sugars, while with GD, GT, GM2, GM3 this is not the case;

(b) It has been predicted that the surface hydrophobicity may be a key factor in the phagocytosis of particulate matter. At the critical concentration of GM1 (7mol %) used, it imparts minimum charge density and sufficient surface hydrophilicity to the liposomes to prevent opsonization[23,24].

4. Synthetic phospholipids:

Park *et al* worked on dioleoyl phosphotidylethanolamine (DOPE) derivatives (negatively charged phospholipids). A series of negatively charged phospholipid derivatives has been synthesized by coupling aliphatic dicarboxylic acids to DOPE. The individual derivatives were incorporated into egg PC/cholesterol liposomes and injected into mice to test its effect on liposome circulation in vivo.

(I)Liposomes containing negatively charged phospholipids are more rapidly removed from circulation and localized in the RES cells of liver, spleen and bone marrow than the neutral or positively charged liposomes.

(II) DOPE derivatives with n= 1 or 2 accelerated the clearance of liposomes from circulation while those with n=3 or 4 delayed the clearance. Derivatives with a longer hydrocarbon chain than n= 1 appeared not to effect the liposome clearance in either way. Allen *et al* incorporated a carbamate derivative of PEG 1900 with distearoyl phosphotidylethanolamine (DSPE) (PEG-DSPE) into liposomes (sphingomyelin/ cholesterol/ egg PC 1:1:1) in concentrations of 5-7mol% and compared for circulation half life with liposomes bearing 10% mono siaganglioside GM~, (PEG-DSPE) liposomes had the greatest ability to decrease the mononuclear phagocyte system uptake of liposomes. Altering vesicle size for liposomes containing PEG-DSPE resulted in only minor changes in blood levels of liposomes[25].

Gabizons and papahadjopoulos have divided various negatively charged lipids into two categories.

(I) A diacetyl phosphate type of lipid has negatively charged groups which are exposed to the aqueous environment. The exposed negative charge promotes opsonization of liposomes *via* charge-mediated interaction with certain proteins in serum.

(II) The other type of lipid is some glycolipids such as GMT, phosphotidylinositol or sulfatides, which have a negative charge shielded by surrounding bulky, neutral, hydrophilic groups[26].

It is suggested that this “Shielded negative charge” was responsible for prolonged circulation of liposomes. But Park *et al* reported that, negatively charged phospholipids with the exposed and unshielded carboxylic group such as N-glutaryl DOPE (NGPE) and N-adipyl DOPE (NAPE) show considerable activity to prolong the circulation time of liposomes.

For the derivatives with a short hydrocarbon chain i.e., N-malonyl DOPE (NMPE) and N-succinyl DOPE (NSPE) the position of the carboxyl groups is close to the interfacial surface of liposome. Nonspecific adsorption of opsonins responding to the surface negative charges may be responsible for the increased RES uptake of liposomes containing negatively charged phospholipids such as phosphotidylserine (PS) and phosphotidylglucine (PG). Chann *et al* have reported that liposome adsorption of the activated complement component Cs, a liposome opsonin, is significantly enhanced with the presence of negatively charged phospholipid. Such Non-specific adsorption of the opsonin(s) would be completely inhibited when the length of the hydrocarbon chain increases. In addition, the adsorption of dysopsonin to the liposome surface, which might require the terminal carboxyl group, should be located at a certain distance from the liposome surface. The activity of the dysopsonin is to decrease the uptake of liposomes by RES[27-28].

CHARACTERIZATION OF STEALTH LIPOSOMES:

A detailed characterization of structure of stealth liposomes including particle size distribution, lamellarity, and bilayer repeat distance and encapsulated volume has to be performed, since it gives information about differences in structure caused by changes in method of preparation and lipid composition. These differences in structure affect the behavior of the vesicles in vitro (stability) as well as in vivo (disposition)[29].

1. Morphology[30,31]:

A. Small angle X-ray scattering (SAXS):

Small-angle X-ray scattering (SAXS) is a small-angle scattering (SAS) technique where the elastic scattering of X-rays (wavelength 0.1 to 0.2 nm) by a sample which has in homogeneities in the nm-range, is recorded at very low angles (typically 0.1 - 10°). This angular range contains information about the shape and size of macromolecules, characteristic distances of partially ordered materials, pore sizes, and other data. SAXS is capable of delivering structural information of macromolecules between 5 and 25 nm, of repeat distances in partially ordered systems of up to 150 nm.

In a SAXS instrument a monochromatic beam of X-rays is brought to a sample from which some of the X-rays scatter, while most simply go through the sample without interacting with it. The scattered X-rays form a scattering pattern which is then detected at a detector which is typically a 2-dimensional flat X-ray detector situated behind the sample perpendicular to the direction of the primary beam that initially hit the sample.

B. ^{31}P-NMR:

^{31}P-NMR has been one of the most accurate and straightforward technique that determines lamellarity of the liposomes. The technique exploits ^{31}P-NMR to monitor to phospholipid phosphorous signal intensity. In particular, adding an impermeable paramagnetic shift or non-permeable broadening agent to the external medium will decrease the intensity of the external ^{31}P-NMR signal by an amount proportional to the fraction of lipid exposed to the external medium. Mn^{2+} ions interact with the outer leaflet of the outermost bilayer. Thus, 50% reduction in the signal indicates unilamellar vesicles whereas subsequent reduction indicates multilamellar vesicles.

C. Freeze fracture electron microscopy (FFEM):

Freeze fracture electron microscopy can be used not only to assess the shape of the liposomes but also the topology of liposomes. In this technique the fracture plan passes through the vesicles, which are randomly positioned in the freezed position. The Fracture plane may not necessarily pass through the mid-plane thus non-mid-plane may result in erroneous readings. The observed distribution profile thus depends on the distances of the vesicles center from the plane of fracture. Furthermore, heterogeneous population requires a careful monitoring before analyzing the final results. However, quick freeze and deep etching techniques give much better lamellarity evaluation. It is reported that etching of freeze fractured specimen can provide information about fractures of vesicles that are unilamellar in a given population. After 5mins of etching, cross-fractured vesicles are clearly seen and the numbers of the lamellae can be readily determined.

2. Leakage study[32]:

Allen and Clelanda developed a technique to study the leakage rates of the drugs or entrapped substances (fluorescent) from liposomes.

Fluorescence increase accompanying leakage was studied using Perkin Elmer MPF-A spectrofluorimeter at 37^{o}C in 25% human plasma. Exchange/Transfer with High density lipoproteins (HDL): The radiolabel led phospholipids are used to prepare the stealth liposomes. Liposomes are incubated for 2-16 hrs at 37^{o}C with HDL followed by chromatography over a Sepharose CL-4B column and quantization of the radiolabel associated with the HDL peak.

3. Localization/Targeting[33]:

Stealth liposomes prepared using radiolabelled phospholipids are injected (known quantity) into mice and after approximate 7 hrs, anaesthetized and radioactivity of each internal organ is counted in a gamma counter.

APPLICATION OF STEALTH LIPOSOMES[34-36]:

1. Targeting of anticancer drugs to tumor sites.

2. Targeting of drugs to non-RE tissues which has not been possible with conventional liposomes.
3. Stealth liposomes may be used in depot applications for slow release of contents for prolonged periods.
4. Stealth liposomes can be used for controlled release within the vasculature by manipulating the phospholipid composition of bilayers.
5. For the diseases of vasculature origin, stealth liposomes provide the best therapeutic effect over conventional drug delivery system

In addition to the common applications possible with conventional liposomes stealth liposomes broaden the area of liposome usage as a drug delivery system by their ability to retain in blood circulation relatively for longer periods.

Ahmed et al reported that PEG-liposomes, containing entrapped doxorubicin, targeted to KNL-205 squamous cell carcinoma of the lung by means of specific antibodies attached at the liposome surface were capable of reducing tumor burden to a high degree and eradicating tumor in a significant percentage in mice.

PEGylated liposomal doxorubicin (PLD) (DOXIL/ Caelyx) was the first and is still the only stealth liposome formulation to be approved in both the USA and Europe for treatment of Kaposi's sarcoma and recurrent ovarian cancer.

DOXIL®/ Caelyx is now undergoing trials for treatment of other malignancies such as multiple myeloma, breast cancer, and recurrent high-grade glioma. Several studies are under way to investigate the anticancer activities of PLD in combination with other therapeutics, including the taxanes (paclitaxel or docetaxel), temozolomide (Temodal® Schering-Plough, Kenilworth, NJ, USA) and vinorelbine.

Nearly 100% of the drug detected in the plasma after PLD injection was in liposome-encapsulated form; plasma clearance is clearly slow (0.1 L/hour) and the distribution volume small (4 L). The rigid bilayer of PLD is composed of HSPC, CHOL, and mPEG-DSPE (molecular weight 2000) at a molar ratio of 55:40:5. Liposomes with a mean diameter of 85 nm are able to incorporate doxorubicin at a concentration of 2 mg/ml. The pharmacokinetics is very slow: plasma elimination follows a biexponential curve, with half-lives of 1.5 and 45 hours (median values); in comparison, plasma half-lives are 0.2 hours for free drug, 2–3 hours for Myocet and 5 hours for Daunoxome.

Due to its pharmacokinetic behavior, cardiotoxicity, myelosuppression, alopecia and nausea are significantly decreased with PLD compared with an equal effective dose of conventional doxorubicin. These bio-distribution characteristics also make skin treatment of localized cancers such as Kaposi's sarcoma possible; on the other hand, due to its reduced clearance, the palmar-plantar skin reaction and stomatitis / mucositis are the chief dose-related toxicities of PLD.

Gabizon and Papahadjopoulos have reported that when small liposomes of size 0.1 mm were administered by i.v./ i.p. routes elevated tumor levels were achieved compared to conventional liposomes. This suggests the use of liposomes for targeting the drugs to non-RE tissues which has not been feasible with conventional liposomes.

When stealth liposomes were administered by subcutaneous route, they were observed to remain at the site of injection in significant quantities for several days. It is therefore possible that these formulations may be useful in depot applications where slow release of contents is desired over a long period of time. Allen *et al* developed a new liposome system of 1-beta-D-arabinofuranosylcytosine with prolonged circulation half-life and dose-independent pharmacokinetics for the treatment L121 O/C2 leukemia in mice.

Another stealth liposome formulation is SPI-077™ (Alza Corporation, Mountain View, CA, USA), in which cisplatin is encapsulated in the aqueous core of sterically stabilized liposomes (fully hydrogenated soy HSPC, CHOL, and DSPE-PEG). The stealth behavior of these compounds is evident from their apparent half-life of approximately 60–100 hours. Phase I/II clinical trials have been run to treat head and neck cancer and lung cancer. Although the toxicity profile was promising, the therapeutic efficacy requires improvement. To obtain the desired balance between encapsulation

and release of cisplatin from liposomes, another formulation was evaluated by Alza Corporation (SPI-077 B103); they chose B103, in which HSPC is replaced by unsaturated phospholipids, because of its greater theoretical propensity to release cisplatin (Alza Corporation, data on file). However, Zamboni et al (2004) were not able to detect released cisplatin in vitro systems, plasma, or tumor extracellular fluid after administration of either stealth formulation of liposomal cisplatin.

Recently, S-CKD602 (Alza Corporation), a PEGylated stealth liposomal formulation of CKD-602, which is a semi-synthetic analog of camptothecin, was submitted for a Phase I trial. After administration of S-CKD602 at doses of 0.5 mg/m2, the plasma AUC was 50-fold that of non-liposomal CKD-602; S-CKD602 showed minimal toxicity and interesting activity.

Lipoplatin™ (Regulon Inc. Mountain View, CA, USA) is another liposomal cisplatin formulation composed of dipalmitoyl phosphatidylglycerol (DPPG), soy PC, CHOL, and mPEG2000-DSPE. Its reported half-life is 60–117 hours, depending on the dose. The study found that Lipoplatin™ has no nephrotoxicity up to a dose of 125 mg/ml every 14 days without the serious side effects of cisplatin.

Stealth liposomes with a large range of contents leakage rates can be formulated and can be used for controlled release within the vasculature. E.g. in atherosclerosis, stealth liposomes may provide the best therapeutic effect over the conventional drug delivery systems.

CONCLUSION:

The development of liposomes as carriers for therapeutic molecules is an ever-growing research area. With the recent developments achieved in the field of liposome technology especially stealth liposomes, a number of therapeutic applications are possible which have not previously been possible. However the mechanisms involved in reducing opsonization of liposomes by grafting the vesicle membranes with suitable polymers has not yet been clearly understood. Although much clinical and laboratory research is required to ascertain for practical utility of stealth liposomes, the likely directions appear to be established. The possibility of modulating the technological characteristics of the vesicles makes them highly versatile both as carriers of several types of drug (from conventional chemotherapeutics to proteins and peptides) and in therapeutic applications (from cancer therapy to vaccination). In recent years, several important formulations for the treatment of different diseases have been developed. Among these, PEG-coated liposomes are becoming increasingly important, giving technological and biological stability to liposomal systems. At present, few PEGylated liposomal formulations have been approved or are in advanced trials (DOXIL®, Lipoplatin™) but stealth technology for different applications is destined to continue developing. PEG-derivatized liposomes with increased stability can easily be modified using a wide array of targeting moieties (MAb, ligands) to deliver the drug specifically to the target tissues with increasing accuracy. Moreover, PEG grafted onto the liposome surface can guide the liposome to a specific intracellular target, using for example cell-penetrating proteins and peptides as targeting agents. The development of liposome delivery to particular subcellular compartments is a field of great interest in different fields, such as gene therapy and vaccination. The interaction of stealth liposomes with cell membranes, and release of the drug in the neighborhood of target tissues are still under investigation, but some recent studies indicate that the use of detachable PEG may facilitate cell penetration and/or intracellular delivery of vesicles. Taking into account these considerations and the great advantages of PEGylated liposomes in decreasing specific drug toxicity and in passively targeting the incorporated molecules to the site of action, new and “improved” stealth liposomal formulations designed for different therapeutic and diagnostic areas may soon be expected to arrive on the market.

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