chapter one

*Site-specific drug delivery using liposomes as carriers**

Contents

I. Introduction

Over the past three decades, significant advances have been made in drug delivery technology. This effort, pioneered by Alza Laboratories of Palo Alto, California,[1,2] among others, has been accelerated in recent years due to the substantial decline in the development of new drug entities.

Drug delivery has now become a multidisciplinary science consisting of biopharmaceutics and pharmacokinetics. Great strides have also been made

* Adapted from Ranade, V.V., Drug delivery systems. 1. Site specific drug delivery using liposomes as carriers, *J. Clin. Pharmacol.*, 29, 685, 1989. With permission of the *J. Clin. Pharmacol.*, and J.B. Lippincott Publishing Company, Philadelphia, PA.

by physical biochemists, pharmacists, and other pharmaceutical research scientists working in university and industrial laboratories.[3–6]

The underlying principle that drug delivery technology, per se, can bring both therapeutic and commercial value to health care products has been widely accepted. Recently, large pharmaceutical companies have been losing their market share to generic competitors with increasing rapidity after their patents expire. This has created an intense need for presenting "old" drugs in new forms and utilizing novel forms of delivery. As a result, companies developing new drug delivery systems seem to enjoy a good return on their investment in the form of increased revenues and market share.[7]

In the U.S., the Drug Price Competition and Patent Term Restoration Act (also known as ANDA-Exclusivity Provisions Act) was passed in 1984. This provided new incentives to manufacturers who can distinguish their products from the competition, with features such as longer dosage schedules, improved safety profiles, new indications for existing drugs, and new combinations.[8]

The following chapters, which focus on the area of research and development in the drug delivery field, have been divided into five sections:

1. Site-specific drug delivery
2. Polymers and implantable drug delivery systems
3. Oral drug delivery
4. Transdermal, intranasal, ocular, and miscellaneous drug delivery systems
5. Regulatory considerations and global outlook

Drug delivery, which takes into consideration the carrier, the route, and the target, has evolved into a strategy of processes or devices designed to enhance the efficacy of therapeutic agents through controlled release. This may involve enhanced bioavailability, improved therapeutic index, or improved patient acceptance or compliance. Drug delivery, or controlled release, has been defined by Flynn as "the use of whatever means possible, be it chemical, physiochemical, or mechanical, to regulate a drug's access rate to the body's central compartment, or in some cases, directly to the involved tissues."[9]

Tomlinson[10] has emphasized features such as exclusive delivery to specific components, access to primarily inaccessible sites, protection of body from unwanted deposition, controlled rate and modality of delivery to pharmacological receptors, and reduction in the amount of active principal employed. Tomlinson[10,11] has also described the properties that are needed for site-specific carriers, as well as properties that are biological, drug-related, and carrier-related.

II. Liposomes in drug delivery

A. Regional drug delivery

Most efforts to make drug therapy more efficient by direct delivery of drugs to affected tissues have focused on local or regional injection techniques,

such as intra-arterial or infusions into body cavities, such as the peritoneum. The benefits of regional therapy include reducing systemic toxicity and achieving peak drug levels directly at the target site. However, these methods of administration have met with limited success. For example, although intra-arterial injections effectively concentrate drugs at certain tumor sites, in others the drug is cleared from the system so rapidly that the benefits are not realized. Currently, pharmaceutical researchers are trying to design drug delivery systems that will localize drugs and affect only the afflicted tissues. A carrier system that has received considerable attention in this regard is liposomes.[12–17]

B. Chemical characteristics of liposomes

Liposomal affinity for various tissues can be modified by synthesizing liposomes containing phospholipids with various fatty-acid chain configurations. These microparticles may be either solid or liquid at defined temperatures.[18,19] Altering the charge on the liposome vesicle can greatly influence its distribution in the body. Negatively charged vesicles, for example, enter the cell by fusion. This allows the drug to be discharged into the cell cytoplasm. Neutral vesicles, on the other hand, are incorporated into the cell by phagocytosis. This exposes the drug to the lysosomal hydrolytic system of the cells. Positive- and neutral-liposomal vesicles are cleared more slowly than those negatively charged.

What is a liposome made of and how does it look? The liposome is a microparticulate, ranging in size from 0.03 μm to 10 μm, consisting of a bilayer of phospholipid encapsulating an aqueous space. A variety of amphipathic lipid molecules can be used to form the bilayer.[20] The lipid molecules arrange themselves by exposing their polar head groups towards the water phase. Hydrophobic hydrocarbon moieties adhere together in the bilayer, thus forming close, concentric, bimolecular lipid leaflets separating aqueous compartments.

Drug molecules can either be encapsulated in the aqueous space or intercalated into the bilayer (see Figure 1.1 and Figure 1.2). The exact location of the drug in the liposome depends upon the physiochemical characteristics of the drug and the composition of the constituent lipids.[21] Stable liposomes from phospholipids are formed only at temperatures above the "gel to liquid-crystalline" phase transition temperature (Tc). This represents the melting point of the acyl chains. All phospholipids have a characteristic Tc, which is contingent upon the nature of the polar head group and on the length and degree of unsaturation of the acyl chains.[21,22] Above the transition temperature, phospholipids form a liquid-crystalline phase that constitutes increased mobility of the acyl chains. A reduction in temperature below the Tc creates a transition to a more rigid gel state. This results in restrained mobility of the tightly packed acyl chains. When the liquid molecules arrange themselves to form closed bilayer structures containing water and solutes, drugs are trapped between the adjacent planes of the polar head

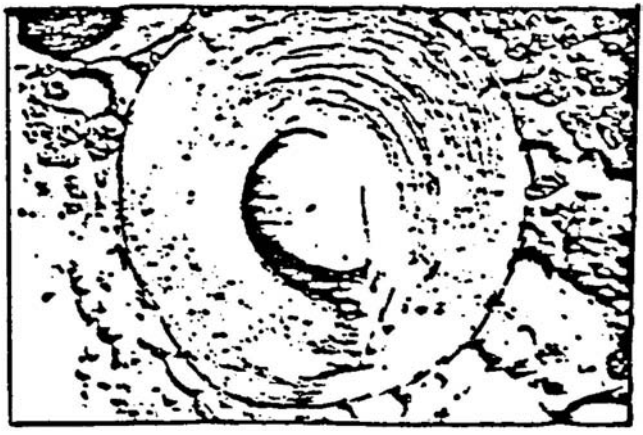

Figure 1.1 Schematic of a bilayer vesicle or liposome. (From *Pharmaceutical Technology*, Conf. Proc., The Latest Developments in Drug Delivery Systems, Oct. 1985. With permission.)

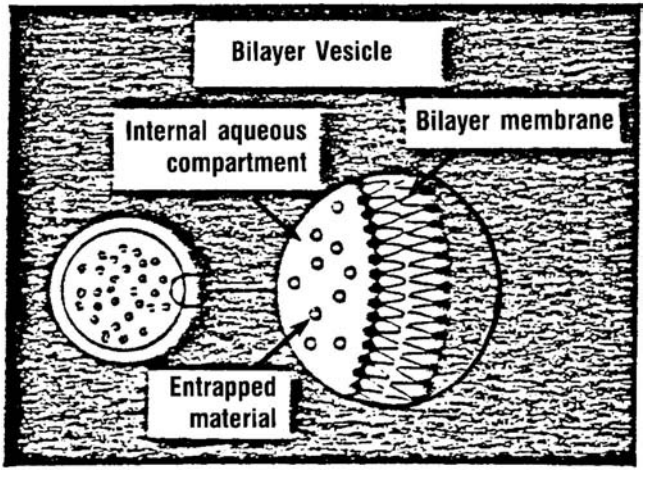

Figure 1.2 A micrograph view of a liposome. (Reprinted by permission of The Liposome Company, Inc., Princeton, NJ.)

groups. This compartmentalization has been discussed in detail by Roerdink et al.[14]

C. Phospholipids

A variety of phospholipids can be used to prepare liposomes. The lipid most widely used is phosphatidylcholine,[23,24] which has been used individually or in combination with cholesterol. Cholesterol is known to condense the

packing of phospholipids in bilayers above the Tc. Cholesterol also reduces the permeability of the bilayers to encapsulated compounds.

Negatively charged lipids, such as stearylamine, are usually used in order to provide a surface charge to the liposomes. For drug molecules encapsulated in the aqueous space, the bilayer serves as a diffusion barrier, permitting the liposomes to serve as a rate-controlling input device. Papahadjopoulos and co-workers have done pioneering research in trying to establish and develop the liposomal delivery system from experimental therapeutics to clinical applications.[25–29] The introduction of this delivery system directly to the target site (such as the eye, lung, or bladder) is a well-established approach for treating local diseases, and liposomes have been shown to play a beneficial role when applied in this way.

III. The liposome-drug concept

A. Liposome size

Liposomes have been used via a variety of administration routes, including intravenous, intramuscular, intraperitoneal, and oral.[30–32] However, IV injection is the most widely utilized route. The half-life of liposomes in the vascular system can range from a few minutes to many hours, depending on the size and lipid composition of the vesicles.

Following IV administration, small liposomes (0.1 to 1.0 μm) are taken up preferentially by cells of the reticuloendothelial system (RES), located principally in the liver and spleen,[33] whereas liposomes larger than 3.0 μm are deposited in the lungs.[34] This preferential uptake of smaller-size liposomes by cells of the RES system has been utilized to deliver chemotherapeutic agents to macrophages and tumors of the liver.[14]

Alternative physical approaches based upon the ability to destabilize the liposome bilayer have led to the design of heat-sensitive, light-sensitive, and pH-sensitive liposomes.[35–37]

B. Targeting ligands

The chemical approach to achieving site-specific delivery requires that the liposome has a targeting ligand bound to its surface, thereby enabling it to attach preferentially to the target site. A variety of targeting ligands have been proposed for this purpose, including antitumor monoclonal antibodies (MAb), carbohydrates, vitamins, and transport proteins.[38] Only carbohydrate and MAb-modified liposomes have thus far shown promise in achieving targeting specificity.

Successful targeting of liposomes to cells other than those belonging to the RES is fairly restricted, but appears to include hepatocytes and circulating red blood cells.[39] A high degree of specific liposome-cell association has been obtained *in vitro* by coating the vesicles with cell-specific ligands, such as MAbs or $F(ab^1)_2$ fragments (see Figure 1.3).[40–42]

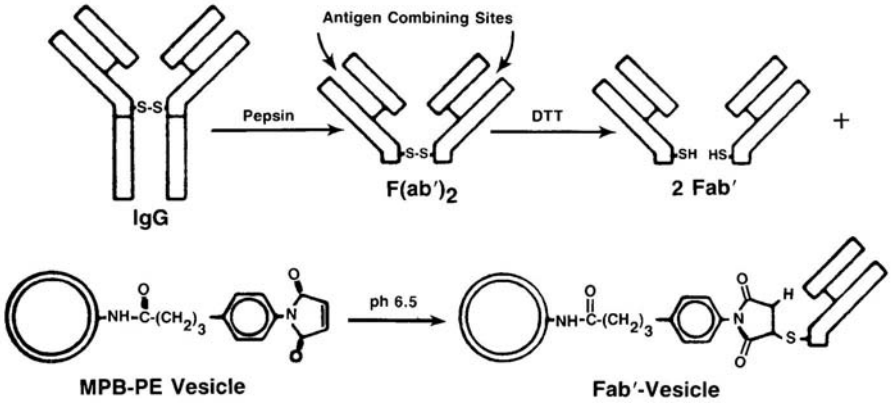

Figure 1.3 Illustration of the chemical-coupling methodology for antibody/liposomes. (From *Pharmaceutical Technology,* Conf. Proc., The Latest Developments in Drug Delivery Systems, Oct. 1985. With permission.)

C. Problems

In vivo, the obstacles to successful targeting that have to be overcome are substantial. First, the liposomes have to escape nonspecific clearance by the RES cells. Second, the vesicles have to cross the capillary endothelium and the basement membrane. Third, many cell types, including most tumor cells, display a low endocytotic capacity. Since it has been found that endocytosis is the dominant mechanism of liposome-cell interaction, this is a serious limitation to the successful application of liposomes as a drug delivery system.[14]

Small-size liposomes may serve as drug carriers to liver parenchymal cells by virtue of their capacity to penetrate the liver's fenestrated endothelium. Once taken up by the cells, liposomes may be degraded in the lysosomal compartment. Liposome-encapsulated drugs, when resistant to the intralysosomal environment, may slowly leak out of the lysosomes into the cytosol and may become available to exert their therapeutic action. Drugs may also be released from liposomes phagocytized by macrophages.

Another important aspect of the liposome-drug relationship involves reducing toxicity of the liposome-encapsulated agent. This is particularly important for antineoplastic agents with low therapeutic indices, such as adriamycin or antimicrobial drugs like amphotericin B.[43–45]

D. Manufacturing issues

Liposomes are phospholipid vesicles composed of one or more phospholipid bilayer membranes and they carry aqueous or lipid drugs. The lipids are both hydrophobic and lipophilic in aqueous media, and their hydrophobic regions sequestrate into spherical bilayers. These layers are referred to as lamellae. Liposomes vary in charge and their size, depending on the method of preparation and the lipids used.

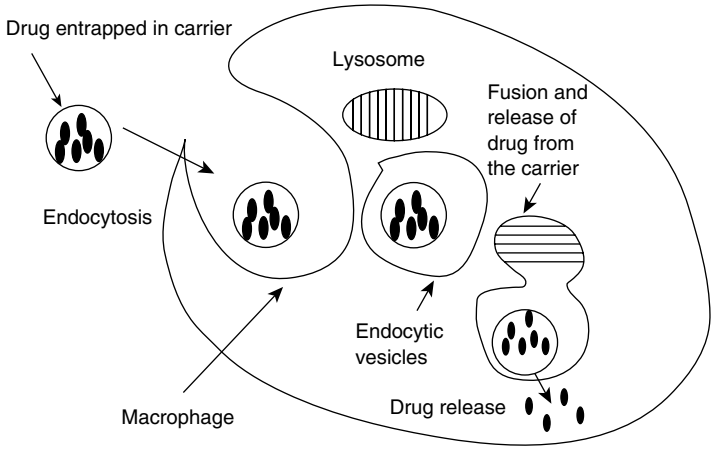

Figure 1.4 Schematic of phagocytosis of particulate carriers by macrophages. Macrophages take up the carriers by the process of endocytosis. Drugs are released from the carriers following intralysosomal degradation of the carriers. (With permission, Elsevier, *J. Control. Rel.*, 79, 29–40, 2002.)

Two major methods are usually used to make liposomal systems for drug incorporation. The first method deals with hydration of a lipid followed by high-intensity agitation using sonication or a high-shear propeller. Liposomes are subsequently sized by filtration or extrusion. In the second method, a phospholipid is first dissolved in an organic solvent and then added to an aqueous medium by vigorous agitation. The organic solvent is removed under vacuum, and the resulting liposomal dispersion or emulsion is sized by filtration or extrusion. Generally, the first method yields multiple lamellae (see Figure 1.4).

Liposomes produced by the high-encapsulation injection process are found to exhibit a broad size distribution in the range of 0.2 to 1.5 μm; downsizing such liposomes results in a loss of encapsulated materials. An alternative method involves the extrusion of a heterogeneous population of fairly large liposomes through polycarbonate membranes under moderate pressures. This technique can reduce a heterogeneous population to a suspension of vesicles that exhibit a mean particle size near that of the pores through which they are extruded (see Figure 1.5).

Incorporation of drugs into liposomes is achieved by using one of the three primary mechanisms: encapsulation, partitioning, and reverse loading. Encapsulation is useful for water-soluble drugs, and it involves hydration of a lipid with an aqueous solution of a drug. The dissolved drug remains in the intralamellar spaces. In the process of partitioning, the drug is dissolved along with the phospholipids in a suitable organic solvent. It is then dried first or added directly to the aqueous phase. The residual solvent is removed in a vacuum. The reverse-loading process is used for weak acidic drugs that exist in both charged and uncharged forms, depending on the

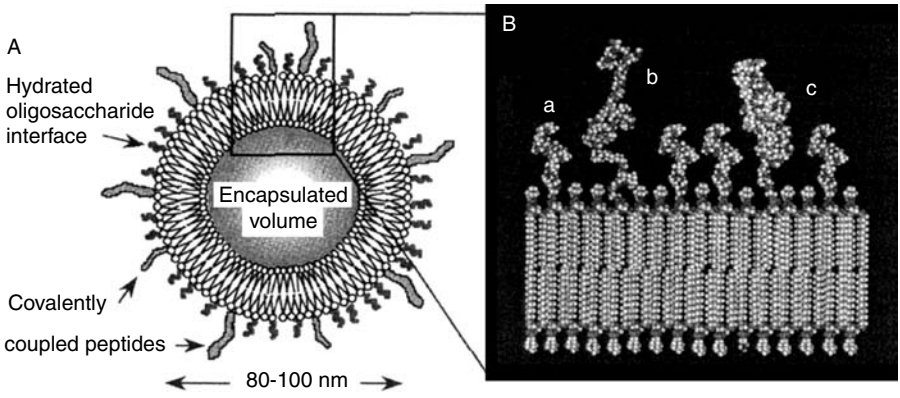

Figure 1.5 Molecular schematic of a surface-modified liposomal drug delivery vehicle for intravascular targeting. (A) The liposome surface consists of a glycocalyx-like oligosaccharide layer to minimize nonspecific interactions and peptide ligands to mediate selective receptive targeting. (B) Composite molecular model showing glycolipids hydrating the surface of the phospholipid bilayer (a), an RGD peptide coupled to the liposome through a poly(ethylene oxide) spacer (b), and a hypothetical coagulation factor VII peptide for targeting endothelial TF (c). (With permission, Elsevier, *J. Control. Rel.*, 78, 235–247, 2002.)

pH of the environment. Such drug molecules are added to an aqueous phase in the uncharged state to permeate into liposomes. The pH is then adjusted to create a charge on the drug molecule. The charged drug molecule is not lipophilic enough to pass through the lipid bilayer and return to the external medium (see Figure 1.6).

Concentrations of the drug and lipids in the vesicles, measurements of captured volume, size distribution, and lamellarity characterize lipid vesicles. The size of liposomes is an important aspect in measuring liposome-complement interactions. The complement system is not known to discriminate according to the liposomal size. Mean vesicle size and size distribution are important parameters for the physical properties and biological fate of liposomes and their entrapped substances *in vivo*. One of the most commonly used methods to determine size and size distribution is light-scattering analysis. Newer methods use laser light scattering. If the liposomes are monodisperse, light-scattering analysis is used. For heterogeneous liposomes, accurate estimate of their size–frequency distribution is necessary. Other systems, such as dispersion, emulsions, and suspensions, are used frequently (see Figure 1.7).

By utilizing a dehydration–rehydration process, a number of molecules can be quantitatively entrapped into the aqueous phase of liposomes. Small, unilamellar vesicles are mixed with a solution of the drug and used for entrapment. The mixture is dehydrated by freeze drying, and the powder thus obtained is rehydrated under controlled conditions. Microfluidization of the drug containing dehydration–rehydration vesicles in the presence of

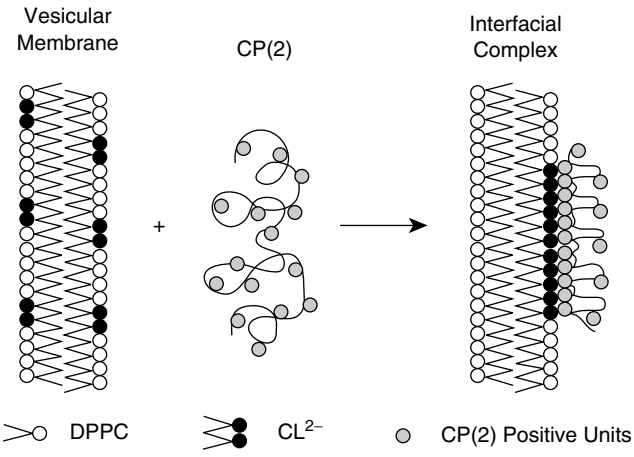

Figure 1.6 Adsorption of CP(2) on the membrane of liquid mixed-negative vesicles (schematic presentation). (With permission, Elsevier, *J. Control. Rel.*, 78, 267–271, 2002.)

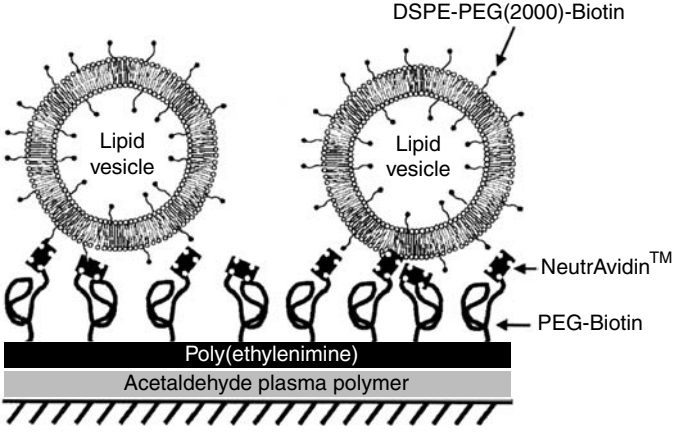

Figure 1.7 Schematic drawing (not to scale) of the multilayer construct used for immobilizing PEG-biotinylated liposomes onto solid polymeric carrier materials via NeutrAvidin™ binding. (With permission, Elsevier, *J. Control. Rel.*, 80, 179–195, 2002.)

nonentrapped solute generates smaller vesicles. In gene delivery, cationic liposomes that interact with negatively charged nucleic acid polymers are used. Relatively homogenous and physically stable suspensions can be obtained by carefully controlling the complex conditions.

Liposome stability is determined by using controlled systems, which are stabilized electrostatically, sterically, or electrosterically. Besides normal colloids, self-assembling colloids can undergo fusion or phase change after aggregation. Liposome dispersions exhibit both physical and chemical stability. Physically stable formulations preserve both liposomal size distribution and the quantity of the material entrapped. Stability depends on the

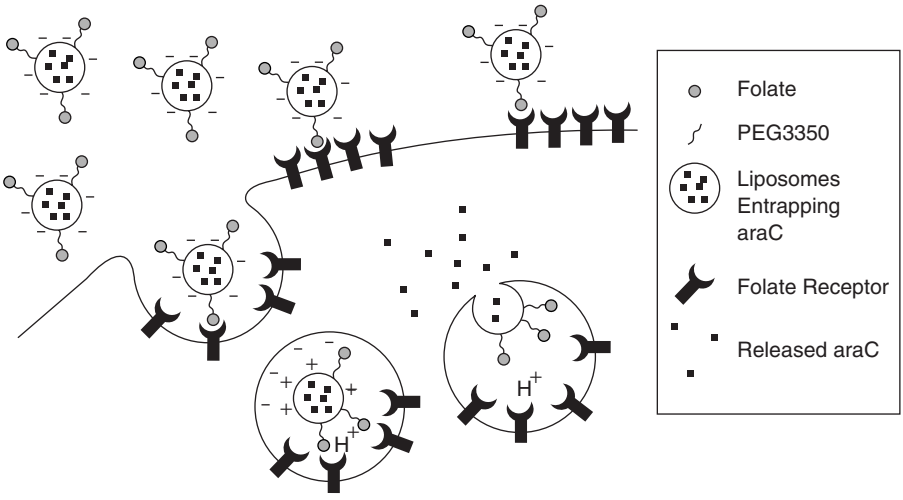

Figure 1.8 Possible mechanism of intracellular araC delivery by FR-targeted, cationic, lipid-based, pH-sensitive liposomes. At first, the folate-derivatized liposomes are taken into the cell by binding to the FRs on the plasma membrane and FR-mediated endocytosis. This is followed by acidification of the endosome, which results in protonation of the anionic lipid component and generation of a net positive surface charge on the liposomes. Finally, the electrostatic interactions between the liposomal and endosomal membranes result in bilayer fusion and the cytosolic delivery of the encapsulated araC. (With permission, Elsevier, *J. Control. Rel.*, 80, 309–319, 2002.)

mechanical properties of the liposomal membranes, their thermodynamics, and colloidal properties of the system. Often, stability tests stress a system to limits beyond those to which the product will ever be subjected.

High-temperature testing (greater than 25°C; see Figure 1.8) is frequently used for heterogeneous products. Phase-transition temperatures for a liposomal system are critical, but the changes, should this occur, are reversible. On the other hand, under frozen conditions, ice crystals are formed. Certain polymers are known to retard or suppress ice crystal growth. Aging studies involving determinations of zeta potential and dielectric constants are usually performed. These analyses determine the status of structural alterations in liposomal vesicles. In summary, in liposomal preparations, each test condition indicating stability of the vesicles should express conditions for microscopic observation (e.g., flocculation), particle-size profiles, rheological profiles, extent of leakage, and chemical and physical stability.

IV. Liposomes as carriers of therapeutic agents

A. Application

Since 1972, when Gregoriadis proposed the use of liposomes as carriers of enzymes in the treatment of lysosomal storage diseases, the application of

liposomes has been extended to a variety of drugs, such as antineoplastic agents,[16,46,47] antimicrobial compounds,[42,48] and immunomodulators.[49–52] In addition to utilizing liposomes as drug carriers in the treatment of intracellular infections,[53] liposomes have also been used as carriers of amphotericin B in the treatment of mycotic infections, such as histoplasmosis,[54] cryptococcosis, and candidiasis.[54] Lopez-Berestein et al.[55] reported that liposomal amphotericin B is effective in the treatment of candida and aspergillus infections in leukemia patients who have not responded to the nonencapsulated drug. The increase in amphotericin's efficacy by encapsulation in liposomes is associated with reduced toxicity.[56]

Incorporation of lipophilic amphotericin within liposomes might result in a facilitated transfer of the drug to fungal cells. In turn, this selective transfer of amphotericin from liposomes to fungal cells may form the molecular basis of the reduced toxicity. Other factors, such as altered kinetics or tissue distribution, may also play an important role.[57]

Antibacterial activity with liposome encapsulation has been reported by Sunamoto and co-workers (in experimental Legionnaires' disease).[58] They showed that uptake of IV-injected liposomes by circulating monocytes and alveolar macrophages can be increased by coating the vesicles with a palmitoyl derivative of amylopectin. After IV injection, the amylopectin-modified liposomes were found to preferentially distribute in the lungs.

Macrophages have an affinity for liposomes, and this property has been utilized in the use of these vesicles as carriers of immunomodulators to create macrophages cytotoxic to metastatic tumor cells. As a result, macrophages serve as an important barrier against the proliferation and metastatic spread of tumor cells. Activation of macrophages to induce tumor cytotoxicity occurs as a result of exposure to a variety of immunomodulating substances, such as lymphokines,[59] γ-interferon, and muramyl dipeptide (MDP).[60–63]

Liposomes are known to increase the adjuvant activity of MDP. Adjuvants are nonspecific immune stimulants that boost immunoresponses to weak antigenic molecules. MDP micelles, for example, are highly potent adjuvants in tests for vaccination against bovine viral diarrhea. Although it is unknown how this process occurs, activated macrophages can selectively kill tumor cells. Activated macrophages have been considered in the management of metastatic cancer, which is often seriously hampered by the biological heterogeneity of tumor cells with respect to growth rate and sensitivity to various cytotoxic drugs.

Although preliminary results with liposome-encapsulated immunomodulators are encouraging, successful application in the treatment of patients with liver metastasis may be hampered by unfavorable macrophage-to-tumor cell ratios in many metastatic tumors.[64] Therefore, it would appear that therapeutic regimens designed to stimulate macrophage-mediated tumor cytotoxicity will have to be used in combination with other treatment modalities.[65–67]

Successful targeting of liposomes, at least to solid tumors located outside the main circulatory system, faces numerous challenges. As described by

Roerdink et al.,[14] selective introduction of antineoplastic drugs into tumor cells *in vivo* by means of liposomes is currently a difficult task. However, application of liposomes as a drug delivery system for antitumor drugs may be of great benefit in diminishing toxicity of encapsulated compounds by altering their pharmacokinetics or tissue distribution. In addition, liposomes can serve as a sustained- or controlled-release system for cytostatic drugs, such as cytosine arabinoside. The therapeutic effect of this cell-cycle-specific drug is enhanced by liposomal encapsulation, possibly by maintaining therapeutically favorable drug levels for a prolonged period of time following leakage from the liposomes, or, alternatively, from macrophages that have phagocytosed the drug-loaded liposomes.

A promising example of a liposomal delivery system for an antitumor drug has been the use of doxorubicin in liposome-encapsulated form.[14] Doxorubicin, an anthracycline antibiotic, is useful in the treatment of a variety of solid neoplasms, lymphomas, and leukemias. Its clinical use, however, is limited by its cardiotoxicity. Several investigators have shown that entrapment of doxorubicin within liposomes greatly reduces its cardiotoxicity without loss of antitumor activity.[68–71] The mechanism responsible for doxorubicin's increased therapeutic index is not fully understood, but may involve low uptake of the liposomal drug by the myocardium[14] or prolonged release of the drug from macrophage depots.[72]

While in the bloodstream, liposomes may be susceptible to destabilizing effects of serum proteins, resulting in the escape of encapsulated water-soluble compounds. In addition, high-density lipoproteins (HDL) have been found to penetrate liposomal bilayers. This process is accompanied by loss of phosphatidylcholine from the liposomes to the HDL.[73,74]

High susceptibility to phosphapidylcholine loss was found at the gel-to-liquid phase-transition temperatures of the liposomal lipids, while both above and below those temperatures the liposomes were relatively stable.[75] Net loss of phospholipid can be prevented by incorporation of cholesterol into the liposomal membranes, thereby causing obstruction to penetration of serum lipoproteins. This may result in an increased stability of liposomes.[76]

B. Manufacturers

Table 1.1 presents a list of liposome technology-based research and development firms, their products, and indications for their usage.[77]

In addition to those presented in the table, the following industrial establishments are also involved in liposome delivery research and development: American Bioproducts, American Lecithin Co., Applied Genetics, Argus Pharmaceuticals, Becton Dickinson & Co., Bristol-Myers Squibb, Brocades Pharma ESCA Agenetics Corp., Fountain Pharmaceuticals, Genzyme Corp., IGI, Inc., Nichiyu Liposome Co., Pharmos Ltd., RibiImmunochem Research, Inc., Schering AG, Schering-Plough Corp., Somatogen, Inc., Structure Probe, and Vical, Inc. According to FIND/SVP's report on the total

Table 1.1 Summary of activity in liposome usage

Corporation	Product	Indications
Fujisawa	Vestar's liposomal formulation of amphotericin B (AmBisome)	Systemic fungal infections
Vestar	MiKasome — aminoglycoside antibiotic, amikacin	Drug-resistant tuberculosis
	Hemoglobin	Blood substitute
	Cyclosporine	Multidrug resistance to cancer chemotherapy
	Liposomes linked to specific proteins	Affinity for sites on diseased cells
	Liposomes coated with a specific viral receptor protein	AIDS and other viral diseases
	Boron isotope of mass 10	Cancer therapy
	Vescan	MRI enhancer in animal tumors
Teijin-Taisho	Epoprostanol derivatives	Myocardial infarction
	Isocarbacyclin	Cerebrovascular orders, chronic arterial obstruction in rats
ImmunoTherapeutics	Glucosamyl muramyl analog	Delivery to the monocyte/macrophage system in cancer chemotherapy
Ciba-Geigy	Muramyl tripeptide	Cancer therapy Metastatic melanoma
Genset	Development of liposomes	For antisense delivery
Liposome Technology Activators	Amphosil (amphotericin B)	aspergillosis infections
	Plasminogen streptokinase reversed	In canines, encapsulated local ischemia
	Amphotericin B cholesterol sulfate-based delivery system (known as ABCD), amphotericin B colloidal dispersion	Leishmaniasis
	(5,12-naphthacenedione) doxorubicin (Lip-Dox)	Advanced cancer patients
	Stealth liposomes (Doxil)	Kaposi's sarcoma
	Metered dose technology	Respiratory and systemic diseases
	Liposome inhalation products	
	Albuterol, Salbutamol (inhaled liposomal formulations)	Beta 2 adrenoreceptor agonist (asthma)

Table 1.1 Summary of activity in liposome usage (Continued)

Corporation	Product	Indications
Technology Unlimited	Development of liposomes	Delivery of water and lipid-soluble material to skin, oral cavity, lungs, digestive tract, vagina, urinary, bladder, liver solid tumors, and HIV-infected cells
The Liposome Co.	Defensins, potent antifungal and antiviral peptides isolated from human neutrophils	Cryptococcal infections in AIDS patients
	Gentamicin — (aminoglycoside antibiotic) TLC G-65	*Mycobacterium avium* intracellulare (MAI) infections in AIDS patients
	Amphotericin B (AB lipid complex) ABLC	Fungal infections in AIDS and cancer patients
The Liposome Co.	TLC I-16, nonionic, iodinated contrast agent	Liver imaging in CT scans, potential in the detection of liver metastases in patients with advanced breast, colon, and lung cancer
Univax & Micro Vesicular Systems	Novasome liposome technology for vaccines	Bacterial and viral vaccines (e.g., for pseudomonas, HIV)
	Liposomal adjuvant system using TLC A-60	Human influenza vaccine
	TLC C-53 (prostaglandin E)	Acute inflammatory and veso-occlusive conditions

market for liposomal pharmaceuticals and diagnostics for 1995, anti-infectives will occupy 75.2% of the market, followed by anticancers (18.8%), diagnostics (5.0%), and respiratory (1.0%). The total market for pharmaceutical and diagnostic liposomes reached an estimated $18 million in 1991, and this is expected to grow dramatically as new products gain regulatory approval. The overall market for drug delivery systems is observed to reach $399 million in 1995.

In Table 1.2, liposomal and conventional formulations of amphotericin B are compared in transplant recipients with systemic fungal infections.[78]

V. Recent advances

Recent studies and examples of liposomal formulations containing various entrapped ingredients are as follows:[79–81]

Table 1.2 Formulation

Parameter	Liposomal	Conventional
Number of Patients	29	29
Graft losses[a]	6/11 (55%)	14/16 (88%)
Mean duration of antifungal treatment	21.3 days	21 days
Adverse reaction reports	3 in 3 patients	
Deaths	9	17
Survival rates		
Liver transplant	71.4% (n = 7)	20.0% (n = 5)
Kidney transplant	72.7% (n = 11)	62.5% (n = 16)
Bone marrow transplant	63.6% (n = 11)	12.5% (n = 8)

[a] Kidney or pancreas transplant only.

L-NDDP (cis-bis-neodecanoato-*trans*-R,4–1, 2-Daminocyclohexane platinum), vincristine (in stealth liposome), cytochalasin B, vaginal antifungal agents, such as miconazole, steroidal liposomes from sterols, such as cholesterol, vitamin D, steroid hormones and fluorinated steroids, benzylpenicillin, topical and in-aerosol devices for anti-inflammatories, beclomethasone dipropionate, dexamethasone palmitate, bronchodilators, such as metaproterenol (Metasome) and terbutaline, indomethacin, daunorubicin (Cerbudine), radiopharmaceutical VS-101, cisplatin (Platinol), minoxidil, calcitonin, camptothecin,[111] indium, cephalothin, nystatin, α-tocopherol, α-tocopherol nicotinate, vitamins A and E, tin mesoporphyrin, cyclosporin A (aerosol formulation), apolipoprotein B,5-fluoro-2′-deoxyuridine and its pro-drug, 3–5′-*O*-dipalmitoyl derivative, (dpFUdR) corticosteroids, 14-*O*-palmitoyl-hydroxyrubicin, plasmid DNA, glutathione, idarubicin, dideoxyinosine triphosphate, bovine somatotropin, antimonial drug meglumine antimonate for leishmaniasis, hamycin, Novasome vaccines, 2–133-Interleukin 2, imidazolidines, and dyphylline.[77]

A. Highlights of current research

- Liposome products to deliver medication to the eye (e.g., dry eye syndrome)[82,83] or gastrointestinal tract have been described.[77]
- Animal studies have been carried out using liposomes and heat application to deliver anticancer drugs. Anticancer drug-containing liposomes are injected into the bloodstream. At temperatures a few degrees above normal, the liposomes melt, allowing drugs to leak out.[85]
- Primaquin (an antimalarial agent) has been coupled to a liver cell-targeting peptide to form a complex that can be encapsulated.[86,87]
- A new liposomal product known as pro-liposome has been developed that has a significantly stronger membrane.[82]
- Multivesicular liposomes for the administration of anticancer agents have been developed. These liposomes are composed of multiple,

nonconcentric, aqueous chambers, allowing more efficient drug entrapment and improved incorporation of drugs, including cytarabine and bleomycin.[14]

- Battelle has developed a process to dehydrate drug-encapsulated liposomes, allowing storage as a stable powder that can be reconstituted when required for drug delivery.[77]
- Macromolecular carriers and liposomes have been covalently coupled to monoclonal antibodies targeted against cardiac myosin heavy chain. Deferoxamine-modified polymers were bound tightly with ^{67}Ga and ^{68}Ga radioisotopes.[88]
- The penetration behavior of liposomes (prepared from NAT 106) incorporated with proteins has been investigated *in vivo* utilizing MAbs. Within 20 minutes of topical application to young pig skin, an even distribution through all skin layers was demonstrated.[89]
- Dioleoyl-N-(monoethoxy polyethyleneglycol succinyl)-phosphatidylethanolamine (PGE-PE) (mol wt of PEG = 5000), an amphipathic polymer, can be incorporated into the liposome membrane and significantly prolong the blood circulation time of the liposome.[90]
- Two rat MAbs, 34A and 201B, that specifically bind to a surface glycoprotein (gp112) of the pulmonary endothelial cell surface, have been coupled to unilamellar liposomes (immunoliposomes) of approximately 0.25 μm in diameter. Time-course studies reveal that 34A liposomes bind to lung antigens within 1 min after injection, indicating that binding takes place during the first few passages through the lung capillary bed.[91]
- The MAb DAL K29 against a human renal cell carcinoma associated cell surface antigen has been covalently linked to small unilamellar lipid vesicles (SUV) containing the antifolate methotrexate, with full retention of antibody activity.[92]
- Two T lymphocyte cell surface molecules, CD4 and CD7, have been studied as targets for the specific delivery of drugs from antibody-directed liposomes. The efficiency of uptake by peripheral lymphocytes, thymocytes, and two CEM sublines (CEM.MRS and CEM-T4) of anti-CD4 and anti-CD7 liposomes containing methotrexate have been evaluated by methotrexate-mediated inhibition of the incorporation of d-[3H]Urd into DNA. This was compared with similar liposomes targeted to MHC-encoded HLA class I molecules, which are known to be taken up efficiently by T cells.[93]
- Generation of cytotoxic T lymphocytes (CTL) *in vitro* and tumor-rejection responses by sensitization of semisyngenic mice with tumor antigen reconstituted liposomes has been investigated. Liposomes were prepared from a crude butanol extract of BALBRVD leukemia cells and egg phosphatidylcholine (PC): 1,2-Dimyristoylamido-1,2-Deoxyphosphatidylcholine (DDPC) (3:2), or dimyristoylphosphatidylcholine (DMPC):DDPC (1:4).[94]

- A new method for the elimination of mononuclear phagocytic cells from cell suspensions has been described. By making use of liposome-encapsulated dichloromethylene diphosphonate, macrophages were effectively removed from spleen cell suspensions. This effect was not observed when using the free-drug or control liposomes.[95]
- Comparative studies of the preparation of immunoliposomes with the use of two bifunctional coupling agents and investigation of *in vitro* immunoliposome target cell binding by cytofluorometry and electron microscopy have been carried out. The specificity of the binding of B8–24.3-liposomes to EL4 target cells was visualized by scanning electron microscopy. Antibody-mediated endocytic uptake of immunoliposomes was demonstrated.[96]
- The potential of small unilamellar liposomes coupled to antitumor MAbs to accumulate in solid tumor tissue has been tested in two systems: a human malignant melanoma xenografted into nude mice and a syngeneic murine lymphoma ESb.Mp exhibiting spontaneous metastasis to the liver. Both MAbs tested were partly released from immunoliposomes within a few hours and produced a constant level of circulating antibody.[97]
- A differentiation inducer, sodium butyrate (SB), encapsulated in liposomes conjugated covalently to an MAb directed to CD19 antigen has been successfully targeted to human lymphoma cell lines SKLY-18 and Ramos grown *in vitro* and *in vivo* in nude mice. Various control liposomes (lacking SB or antibody, or coupled to an irrelevant antibody) are not effective targeters. In view of the limited number of tumor-specific antigens, targeting of differentiation inducers rather than cytotoxic drugs to tumor cells may provide a useful approach for conversion of highly malignant to less malignant tumors.[98]
- A technique for loading certain types of drug molecules into preformed liposomes has been described. The drug must be amphiphatic and exist in a charged, protonated form. Liposome suspensions are formed with a higher concentration of ammonium ions within the liposome than in the external aqueous phase, thus establishing a pH gradient across the liposome boundary. The amphiphatic drug molecule is added to the suspension and, because of the pH gradient, is preferentially loaded in the core of the liposome.[99]
- Liposomes targeted to the liver have been prepared with a high content of a nonionic surfactant. This formulation was prepared by mixing soybean phosphatidylcholine, α-tocopherol, and ethoxylated hydrogenated castor oil (HCO-60) in methanol, concentrating the mixture under vacuum, and then shaking it with water. Transport to rat liver after IV administration was claimed to be approximately twice as high for liposomes containing castor oil ethoxylate as for controls without surfactant.[100]
- The extent to which liposomes promote the permeability of insulin through the nasal mucosa with or without pretreatment with sodium

glycocholate (an enhancer of nasal absorption) has been studied. Results indicate that sodium glycocholate remaining in the nasal mucosa causes the lysis of liposomes that come to the surface of the nasal mucosa and the subsequent release of insulin from the liposome. The relatively high level of insulin in the mucosal surface caused permeation of insulin through the mucosa.[101]

- The release of 5(6)-carboxyfluorescein (CF) from liposomes and phospholipid peroxidation against time in the presence of different concentrations of collagen, albumin, and γ-globulin has been studied. Results indicate that collagen decreases liposome permeability by an antioxidant effect and also by a specific interaction with phospholipids. Collagen provides nonspecific protection against the detergent-induced release of CF from liposomes. Thus, a liposome-collagen complex may provide an improved drug delivery system.[102]
- Removal of intravenously injected liposomes from the circulation has been achieved by cells of the mononuclear phagocyte system (i.e., RES). On exposure to blood, liposomes become coated with plasma proteins. Some of these proteins (opsonins) are thought to become determinants for subsequent recognition by mononuclear phagocytes. The review by Patel provides a critical discussion of factors that control opsonization of liposomes and their phagocytosis *in vivo* and *in vitro*.[103]
- The distribution of 2-imidazolines in neutral dimyristoylphoaphatidylcholine (DMPC) liposomes in negatively charged liposomes containing dicetylphosphate (DCP) or phosphatidylserine (PS) and in positively charged liposomes containing stearylamine (STA) has been investigated. Electrophoretic mobilities of multilamellar liposomes were measured as a function of drug concentration. The results indicate the relative importance of the membrane surface characteristics on partitioning behavior and the membrane transport behavior of the 2-imidazoline drugs.[104]
- The effect of P_o (a cell-adhesion molecule from avian peripheral nerve myelin) on the rate of interaction of liposomes with human M21 melanoma cells has been studied. Liposome uptake by the cells was quantitated using radioactive lipids and liposome entrapped drugs under various conditions. The results suggest that the attachment of liposomes to the cell surface can increase their drug delivery potential. This may be the result of triggering binding endocytic processes or a temporary permeability increase of liposome and cellular membrane, thereby leading to enhanced uptake.[105]
- Liposomes have been prepared containing two lipophilic drugs, dl-α-tocopherol nicotinate (TN) and the anti-inflammatory substance, L440. In the case of TN liposomes, oleoyl-hydrolyzed animal protein (OHAP) was added in order to control the vesicle size. The skin penetration ability of both drugs from liposomal gels into human stratum corneum was determined *in vivo* by a stripping method and

compared with conventional galenical formulations. The anti-inflammatory effect of the released L440 was examined in the ear edema model in mice. The penetration tests showed significantly higher absorption rates for both drugs after application of the liposomal preparations in comparison to other topical formulations. However, only a slight relationship between drug permeation into the stratum corneum and liposome diameter was observed.[106]

- The pulmonary delivery of liposomes has recently been reviewed. The technological aspects of delivering liposomes to the lungs are discussed, including the characterization of liposome-containing aerosols and the potential advantages and disadvantages of the various methods that have been employed for their generation. Studies indicate that liposomes can be effectively deposited in the human respiratory tract, wherein they may remain for prolonged periods. Prolonged retention in the airways may markedly alter the pharmacokinetics of liposome-associated materials, thereby increasing local concentrations while decreasing levels at sites distant from the lung. The future potential for such systems, including the possibilities for selective drug delivery to specific cell populations within the lung, has yet to be determined.[107]
- Treatment of pulmonary diseases using the immunosuppressive drug cyclosporin A (CsA) is limited, in part, by poor penetration into the lungs following oral or intravenous administration and by the development of limiting renal, hepatic, and other toxicity following prolonged administration. CsA aerosol delivery may provide an alternate route of local administration that could improve treatment and reduce systemic toxicity. The total CsA dosage administered via aerosol is expected to be less than the conventional oral or intravenous dosage routes. Lipophilic CsA prepared with different phosphatidylcholine liposome formulations has been studied in this regard.[108]
- Liposomes composed of dioleolylphoaphatidylethanolamine, 1,2-Dipalmitoylsuccinylglycerol with polyethylene glycol are pH-sensitive, plasma-stable and have a long circulation time in the blood. The complete destabilization of these liposomes might be useful for the targeted delivery of drugs such as anticancer agents.[124]
- Polyethylene glycol-coated liposomes containing 3,5-dipentadecyloxybenzamide hydrochloride strongly and selectively bind to subendothelial cells via certain kinds of chondroitin sulfate proteoglycans and have an advantage for use as a specific drug delivery system. Fusogenic liposomes composed of the UV-inactivated Sendai virus effectively and directly deliver their encapsulated contents into the cytoplasm using a fusion mechanism of the Sendai virus, whereas conventional liposomes are taken up by endocytosis.[125]
- To enhance the efficiency of gene delivery by the introduction of molecules directly into the cells, virosomes have been developed by combining liposomes with fusogenic viral envelope proteins.[126,127]

- The liposomal gel-controlled ibuprofen release and dural permeation *in vitro* showed a permeation pattern favorable for maintaining constant drug levels. Therefore, this liposomal polaxamer gel represents a new formulation approach to increase the local epidural availability of ibuprofen.[128]
- A significant breakthrough was achieved in the effectiveness of adriamycin by using its liposomal formulation coated with polyethylele glycol (PEGylation).[129]
- The positively charged liposomal formulation of tropicamide, a mydriatic cycloplegic drug, and liposomes dispersed in polycarbophil were found more effective than those with neutral liposomal dispersion.[130]
- Antitumor activity of mitoxantrone was enhanced by using controlled destabilization of a liposomal drug delivery system. For this drug, programmable fusogenic vehicles with PEG with an 18-carbon acyl chain length were used.[131]
- The ophthalmic drug delivery system consisting of oligolamellar system made up of dipalmitoylphosphatidylcholine-cholesterol-dimethyldioctadecyl glycerol bromide in 7:4:1 molar ratio presented the highest encapsulation capacity and delivered greater amounts of the drug acyclovir into the aqueous humor than saline acyclovir or a physical liposome/drug blend.[132]
- The use of magnetoliposomes (liposomes with entrapped magnetic particles in their bilayers), which are magnetosensitive, may be maneuvered to a given site in the organism. Magnetoliposomes that are strong microwave absorbers can be heated to higher temperatures, which may subsequently lead to a leakage of the entrapped drug. The beneficial effects of glucocorticoids in treating pulmonary inflammatory disorders are complicated by systemic adverse effects. The administration of liposome-entrapped dexamethasone has distinct advantages of enhancing the anti-inflammatory activity of the drug.[133, 134]
- Liposome-infused doxorubicin hydrochloride (DXR), an anthracycline anticancer antibiotic, can be effectively used on murine neuroblastoma and may reduce the incidence of cardiac toxicity as compared to DXR alone.[135,136]
- Since it is known that liposomes are naturally taken up by cells of the mononuclear phagocytic system, liposome-based therapy represents a convenient approach to improving the delivery of anti-HIV agents into infected cells, thereby improving the efficacy of drugs and reducing their adverse side effects. A more specific targeting of HIV-infected cells could be obtained using liposomes bearing surface-attached antibiotics.[137]
- A proniosome-based transdermal drug delivery system of levonorgestrel has been developed, and this system is effective in preventing contraception.[138]

- The analysis of *in vitro* antiproliferative activity on cultured human leukemic K562 cells demonstrated that ionic and neutral liposomes containing chromomycin were 1.5- and 7-fold more effective, respectively, as compared with the free drug.[139]
- Liposomes encapsulating adenosine triphosphate were prepared by sonication, and the liposomes have been evaluated for treatment of ischemic retina.[140]
- The intrahepatic distribution of liposomes containing glycolipid derivatives showed that these were suitable for the selective delivery of liposomes to hepatic parenchymal cells. The authors synthesized branched-type galactoyllipid derivatives for liposome modification for the targeting of asialoglycoprotein receptors on the surface of liver cells. Galactose was coupled to the α- and γ-carboxyl groups of glutamic acid via a triethyleleglycol spacer, and the glutamic moiety bound to the lipid anchor.[141]
- Liver accumulation of liposomes depends on the galactosyl residues. The number of galactosyl residues was more effective for accumulation in the liver than for branching. Niosome-encapsulated ciprofloxacin and norfloxacins were studied, and it was found that intestinal, but not nasal, absorption was significantly higher in comparison with that of nonliposomal parent drugs.[142]
- It is known that a single liposome may carry greater than or equal to 10,000 drug molecules and that the use of PEG-conjugated immunoliposomes increases the drug-carrying capacity of the monoclonal antibody by up to four logarithmic orders in magnitude. Specific OX-26 monoclonal antibody-mediated targeting of daunomycin to the rat brain was achieved by this immunoliposome-based drug delivery system.[143]
- The use of liposome can be helpful in optimizing sustained delivery of glucocorticoids to the lungs via topical administration. The use of triamcinolone acetonide phosphate liposomes as a pulmonary-targeted drug delivery system has been explored.[144]
- Different sugar-coated liposomes were prepared and tested against experimental leishmaniasis *in vivo* using pentamidine isethionate and its methoxy derivative. Both drugs, when encapsulated in sugar-grafted liposomes, were found to be more potent in comparison to normal drug delivery.[145]
- A significant reduction in tumor volume and increased survival time were observed in tumor-bearing mice treated with a combination of hyperthermia and thermosensitive liposome-encapsulated melphalan compared with animals treated with an equivalent dose of free melphalan, with or without hyperthermia. These results suggest that hyperthermia in combination with temperature-sensitive liposome-encapsulated melphalan may serve as a useful targeted drug delivery system for more effective management of melanoma.[146]

- Using virosomes prepared from the P3HR1 strain of Epstein–Barr virus, the authors demonstrated that these particles fused with human hepatocarcinoma cell line Li7A might be used as a drug delivery system to enhance specific macrophagic functions.[147]
- Use of radioprotective drugs in radiotherapy is desirable to protect normal tissues. 2-Mercaptopropionylglycine (MPG) showed promising results in experimental radioprotection. A statistically significant, dose-dependent enhancement of protection by liposome-encapsulated MPG was observed. Liposome-encapsulated MPG, as compared to free MPG, improved the viabilities of spleen and bone marrow cells for different doses of radiation.[148]
- The nuclear enzyme, topoisomerase I, was recently recognized as the target for the anticancer drug, camptothecin (CPT), and its derivatives. This drug was reported to display effective antitumor effects on a variety of human tumor models xenografted in nude mice. The intramuscular administration of liposome-incorporated CPT had considerable potential for the treatment of human neoplastic diseases, especially lymph node metastases.[149]
- The authors developed a lipid-based drug delivery system to provide prolonged levels of gentamicin in local tissues after local administration. Local injection of multivesicular liposome/gentamicin provided sustained drug concentrations in regional tissues, which protected against a massive bacterial challenge for at least 4 days.[150]
- Cytosine arabinoside (Ara-C) was contained in polymer-coated liposomes. Polymers such as dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine, and dicetyl phosphate were used and coated with a derivatized polysaccharide. These liposomes were found stable in harsh environments, such as those encountered after oral administration.[151]
- The authors investigated the *in vivo* characteristics of liposomes coated with a polyvinylalcohol having a long alkyl chain as a hydrophobic moiety at the end of the molecule. This moiety reduced uptake in the reticuloendothelial system.[152]
- In the search for an effective immunization against diseases such as cancer, parasitic disease, AIDS, and other viral infections, several peptides and recombinant proteins were synthesized, examined for the ability to induce antibodies and cytotoxic T lymphocytes (CTLs), and tested for binding capability and therapeutic or prophylactic efficacy against the original target cell of the organism. The data suggest that small synthetic peptides, synthesized with or without a lipid tail, or chemically conjugated to the surface liposomes, might serve as effective antigenic epitopes in combination with liposomal lipid A for induction of antibodies and CTLs.[153]
- After intravenous injection in rats, the pharmacokinetics and biodistribution of methotrexate were significantly changed by liposomal incorporation and also by the composition of liposomes. Liposomes

containing a 2:1 ratio of phosphatidylcholine and cholesterol and distereoylphosphatidyl-ethanolamine-N-poly(ethylene glycol) 2000 most effectively prolonged blood circulation and reduced hepatosplenic and kidney uptake of methotrexate.[154]

- Boronphenylalanine-loaded conventional and stabilized liposomes were prepared by the reversed-phase evaporation method to treat liver metastases by boron neutron capture therapy. Conventional vesicles were composed of phosphatidylcholine and cholesterol in a molar ratio of 1:1. To obtain stealth liposomes, polyethylene glycol was included in the lipidic bilayer. Tissue boron concentrations were determined by using inductively coupled plasma-mass spectroscopy. Results indicated that PEG-modified liposomes accumulated boron in therapeutic concentrations in metastatic tissue.[155]
- Spherulites (noncationic multilamellar vectors) composed of phosphatidylcholine, cholesterol, and polyethylene alcohol, entrapping 125-I protein A, were prepared and biodistribution was studied after IV injection in Wistar rats. Approximately 70% of the radioactivity was found in the liver and about 35% in the spleen. Oral administration of III-In-NTA in fasting rats showed a significant increase of radioactivity in the blood. This formulation did not show cytotoxicity to human cells and could be used as a drug delivery system.[156]
- Proliposomes in the form of enteric-coated beads using glyburide were prepared. The beads were enteric-coated with Eudragit L-100 by a fluidized bed-coating process using triethyl citrate as a plasticizer. The dissolution study of enteric-coated beads exhibited enhanced dissolution as compared with the pure drug.[157]

VI. Concluding remarks

Liposomes possess a number of favorable properties which, theoretically, may enable them to function as drug delivery systems.[109–113] However, limitations exist. First, they are unable to cross the capillary endothelial cells in most organs except the liver,[114] and second, many cell types have a limited capacity to phagocytize particles like liposomes.

Liposomes may seem to be attractive carriers of drugs to macrophages, as has been demonstrated by the successful application of liposomes in the treatment of certain forms of cancer. The use of liposomes also appears to be of significant benefit in the reduction of toxicity of certain anticancer drugs. Potentially important results have also been obtained with the application of liposomes for the delivery of other drugs.[85,115–119]

Problems concerning large-scale production of liposomes as pharmaceutical products, acute and chronic toxicity, and immunogenicity of liposome preparations are certainly important aspects of liposome-based technology and they require careful attention before the clinical application of liposomes as a drug delivery system can be contemplated.[82,120–122] Recently, Klimchak and Lenk have addressed some of these topics.[123] Finally, Roerdink et al.[14] in

their review article point out that "despite some early remarkable successes, liposomes are by no means a panacea for pharmacotherapy in general." Their observation still seems to be aptly justified.

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