Site-Specific Drug Delivery Using Liposomes as Carriers

I. Introduction

- Drug Delivery
  - Multidisciplinary science
    - Biopharmaceutics and pharmacokinetics
    - Physical biochemist, pharmacists and other pharmaceutical research scientists
  - Generic products
    - Old drugs in new forms
    - Novel forms of delivery
  - Drug Price Competition and Patent Term Restoration Act (ANDA-exclusive provisions act)

Area of Research

- Site-specific drug delivery
- Polymers, implantable drug delivery
- Oral drug delivery
- Transdermal, intranasal, ocular and miscellaneous drug delivery
- Regulatory consideration and global outlook

Consideration of DDS

- Routes and target to enhance efficacy through controlled release
- Enhanced bioavailability, improved terapeutic index, and/or improved patient acceptance or compliance
- Definition of Drug Delivery by Flynn
  - The use of whatever means possible, be it chemical, physicochemical or mechanical, to regulate a drug's access rate to the body's central compartment or int some cases directly to the involved tissues.

Site specific delivery by Tomlinson

- Exclusive delivery to specific components
- access to primarily inaccessible sites
- protection of drugs and body from unwanted deposition
- Controlled rate and modality of delivery to pharmacological receptors
- Reduction in the amount of active principle employed
- Properties : biological, drug-related and carrier-related

II. Liposomes in Drug Delivery

- Regional Drug Delivery
- Chemical Characteristics of Liposomes
- Phospholipids

Regional Drug Delivery

- Local or regional injection techniques
  - Intra-arterial or infusions into body cavities such as the peritoneum
  - Reducing systemic toxicity and achieving peak drug levels directly at the target site
  - Problems
    - Drug is cleared from the system so rapidly
    - Localize drugs and affect only the afflicted tissues using drug delivery systems

Chemical characteristics of liposomes

- Utilization of liposome
  - Affinity for various tissue : Modified by synthesizing liposomes containing phospholipids with various fatty acid chain configurations
  - Solid or liquid at defined temperatures
  - Altering the charge on the liposome vesicles influences its distribution in the body.
    - Negatively charged vesicles enter the cell by fusion.
    - Neutral vesicles are incorporated into the cell by phagocytosis.

- Characteristics of liposome
  - Microparticles ranging in size from 0.03 to 10 um
  - Bilayer of phospholipid encapsulating an aqueous space
  - Amphipathic lipid molecules can be used to form the bilayer.

Fig. 1 Schematic of a bilayer vesicle or liposome.

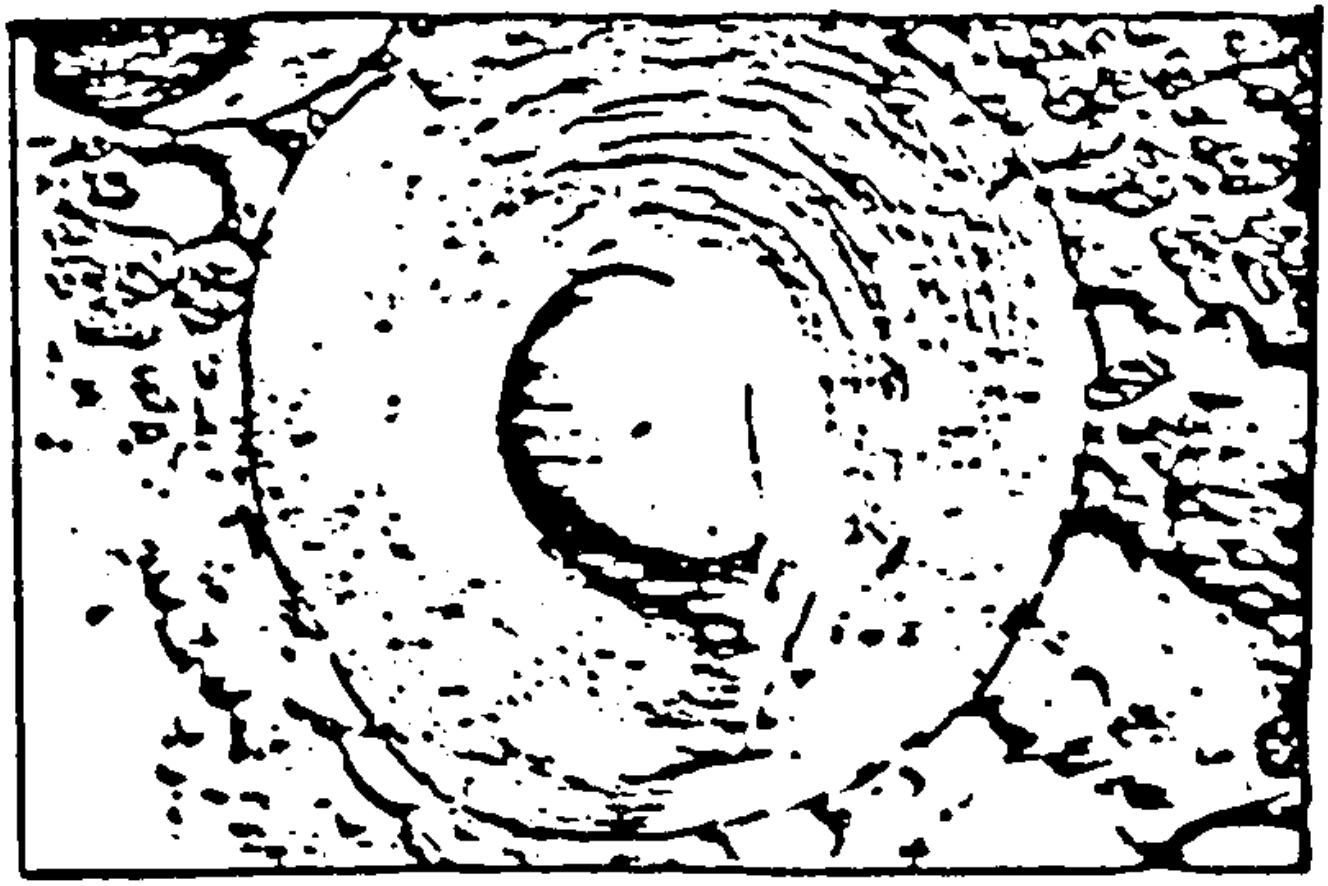
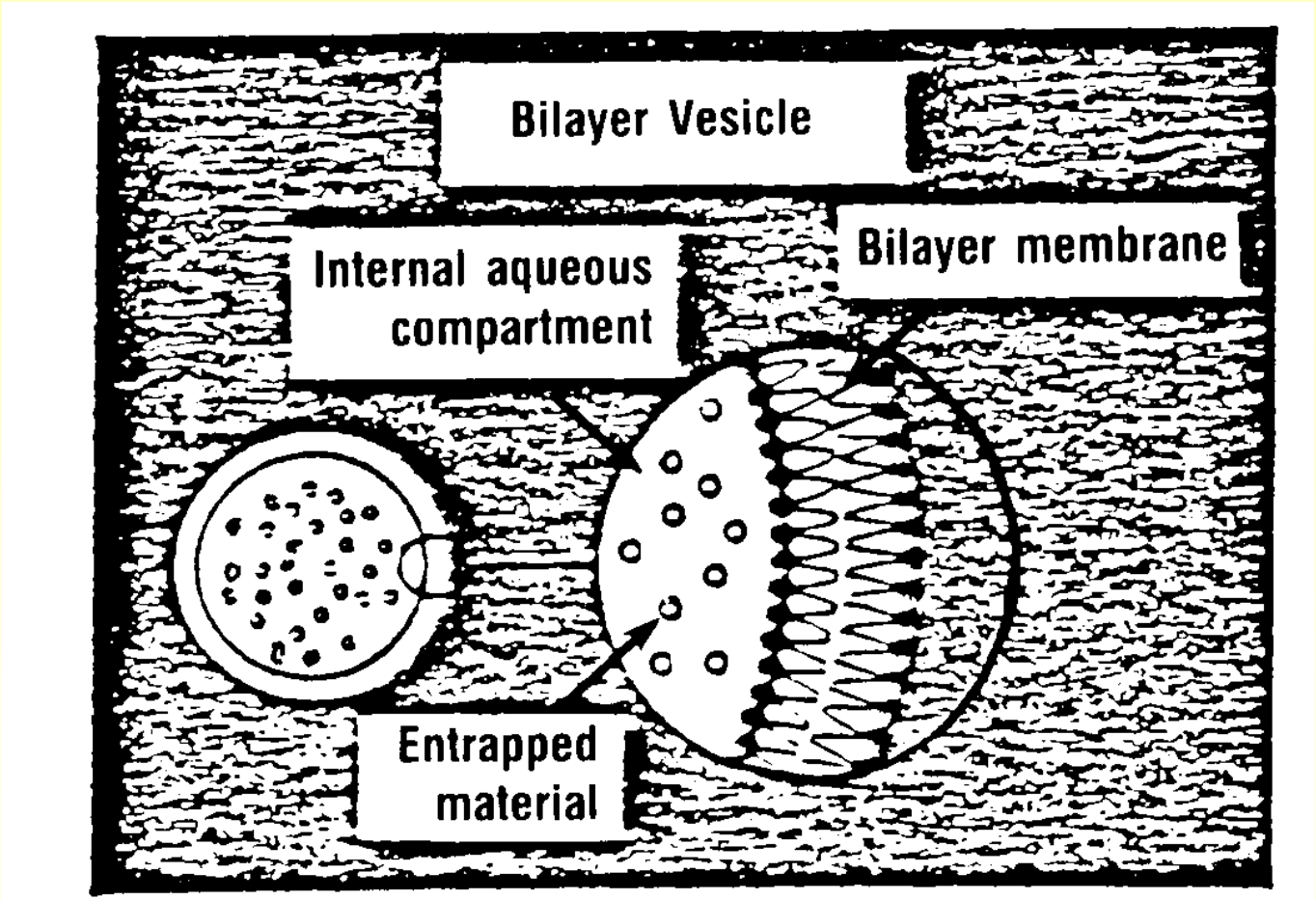


Fig. 2 A micrograph view of a liposome.



- Location of drug depends upon the physicochemical characteristics of the drug and the composition of the constituent lipids.
- Formed at the temperatrue above the gel to liquid-crystalline phase transition temperature (Tc)
  - Represents the melting point of the acyl chain
  - Nature of polar head group and on the length and degree of unsaturation of the acyl chains
  - Above Tc : increased mobility of the acyl chains
  - Below Tc : more rigid gel state

Phospholipids

- Phospholipids : phosphatidylcholine
- Combination with cholesterol
  - Condense the packing of phospholipids in bilayers above the phase transition temperature
  - Reduce the permeability of the bilayers
- Negatively charged lipids such as stearylamine

III. The Liposome-Drug Concept

- Liposome Size
- Targeting Ligands
- Problems

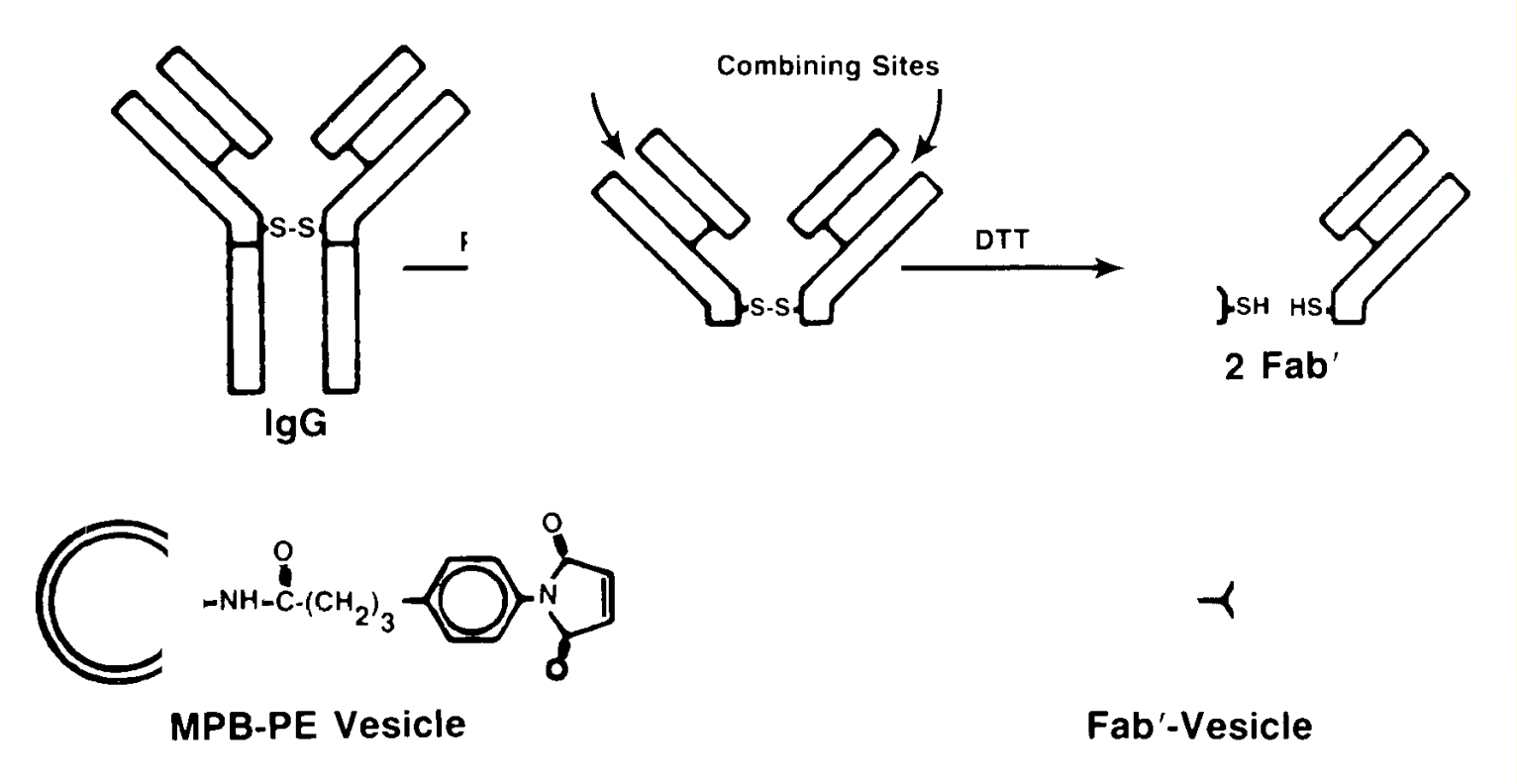
Liposome size

- IV administration
  - 0.1 to 1.0 um : by cells of RES located in the liver and spleen
  - Larger than 3.0 um : deposited in the lung
- Alternative physical approach
  - Heat-sensitive liposome
  - Light-sensitive liposome
  - pH-sensitive liposome

Targeting Ligands

- Antitumor monoclonal antibodies (Mab)
- Carbohydrates
- Vitamins
- Transport proteins

Fig. 3 Chemical coupling methodology for antibody/liposomes



Problems

- Problems In vivo
  - Nonspecific clearance by the RES cells
  - Cross the capillary endothelium and the basement membrane
  - Cell types including tumor cells display a very low endocytotic capacity
    - Endocytosis is the dominant mechanism of liposome-cell interaction.

Fate of liposome

- Small size liposome as carriers of drugs to liver parenchymal cells (penetrate the fenestrated liver endothelium)
  - Degraded in the lysosomal compartment
- Resistant to the intralysosomal enviornment : slowly leak out of the lysosomes into the cytosol
- Release from liposomes phagocytized by macorophages

IV. Liposomes as Carriers of Therapeutic Agents

- Applications
- Manufactures

Application

- 1972 Gregoriadis as carrier of enzymes
- Antineoplastic agents, antimicrobial compounds, immunomodulators
- Increase in the efficacy and reduced toxicity
- Facilitated transfer to fungal cells
- Uptake increase by circulating monocytes and alveolar macrophages

- Activation of macrophages to induce tumor cytotoxicity killing tumor cells.
- Liposomes increase the adjuvant activity of muramyl dipeptide which is nonspecific immune stimulants and activated macrophages.
- Targeting of liposomes to solid tumors
- A sustained- or controlled-release system for cytostatic drugs
- Doxorubicin in liposome-encapsulated form reduces its cardiotoxicity by low uptake by the myocardium or prolonged release from macrophage depots.

Manufactures

- Table 1 Liposome production and indications

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 disorders, 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	Amphosil (Amphotericin B)	Aspergillosis infections
	Plasminogen activators	In canine, encapsulated streptokinase reversed local ischemia

Corporation	Product	Indications
	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)
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* intracellular (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 computed tomography 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

Table 2 Formulation of amphotericin B

Parameter	Formulation	
	Liposomal	Conventional
No. of patients	29	29
Graft losses[a]	6/11 (55%)	14/16 (88%
Mean duration of antifungal treatment	21.3 d	21 d
Adverse reaction reports	3 in 3 patients	'5 in 29 patients
Deaths	9	7
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 and/or pancreas transplant only.

V. Recent Advances

- Highlights of current research
  - Anticancer drug-containing liposomes and heat application
  - Pro-liposome having a significantly stronger membrane
  - Multivesicular liposomes allowing more efficient drug entrapment
  - Dehydrated drug-encapsulated liposomes allowing storage as a stable powder

- Liposomes coupled to monoclonal antibodies
- PGE-PE liposomes prolong the blood circulation time
- Immunoliposomes

VI. Concluding Remarks

- Liposomes posses a number of favorable properties as drug delivery systems
  - Attractive carrier of drugs to macrophages
  - Reduction of toxicity of certain anticancer drugs

Limitations

- Unable to cross the capillary endotherial cells in most organs except the liver
- Many cell types have a limited capacity to phagocytose particles like liposomes

Liposome-based technology

- Large scale production
- Acute and chronic toxicity and immunogenicity