CHAPTER 1
INTRODUCTION AND LITERATURE SURVEY

A. Introduction

1.1 Statement and significance of the problem

Liposomes have been widely investigated for their properties as model membrances and as a potential drug delivery system (Gregoriadis, 1988 and Lasic, 1993). They have become a valuable experimental and commercially important drug delivery system because of their biodegradability, biocompatibility, low toxicity and their ability to entrap both lipophilic and hydrophilic drug. Unfavorable pharmacokinetic profiles of certain drug can be altered by entrapping of the drug in liposomes. The tissue distribution of the liposomes themselves can be influenced by varying the particle size, compositions and modifying the surface charges. The application of liposomes have been extended to drug efficacy and potency (Mayer, 1993 ; Mayer, 1995 ; Gabizon, 1996), reduction of toxicity of the encapsulated drugs (Forssen, and Tokes, 1981 ; Bally, 1994 ; Rahman, 1990), targeting to specific tissue sites (Margolis, and Lishko, 1992), control the timing and the amount of drug released (Soltan, 2000), enhancement of the penetration of drug into the skin with the slow release and moisturizer effect(Manosroi, 1995 ; Mezei, 1991). Thus, liposome is an excellent novel formulation as drug and cosmetic carrier. In sciniigraphic studies, liposomes which carry radionuclides within the aqueous space, have potential diagnostic imaging application (Lasic, 1992). Gramma-emitting radionuclides, such as ^{99m}Tc,^{111}In and ^{67} Ga, have been encapsulated in liposomes for imaging agents (Gregoriadis, 1997 ; Oku, 1993 and Ogihara-Umeda, 1996). Some clinical trials for tumor imaging have already been started using labeled liposomes (Presant, 1988 ; Presant, 1990 and Kubo, 1993).

Tranexamic acid (TA) is an antifibrinolytic agent. It has been claimed to have antiinflammatory (Kathlem, 1999) and whitening effects for topical use (Maeda, and Naganuma, 1998). The current commercial available preparation of TA in the

market are tablets and injections (British Pharmacopoeia,1995). But, no preparation in liposome formulations. It has been evidence that TA can cause irritation and allersy (Kathlem, 1999). TA entrapped in liposome has been investigated because multilamellar liposomes can reduce irritation of the drug and improve moisturizer effect from the lipid concentrations of liposomes with prolong action. The liposomal TA will be a novel formulation. In this study multilamellar liposomes was prepared by a chloroform film method with sonication. TA which is a water soluble compound will be incorporated in the aqueous layers of the liposomal membrane. The release study of the entrapped TA from liposomes, physico-chemical properties as well as the stability properties when kept at various temperature for three mounths were investigated. The result from this study can be used for future development of liposomal TA in pharmceutical and cosmetic use.

1.2 Objective

The objectives of this study are the following :

1. To compare the effects of entrapment of TA in various liposome formulations.
2. To study the physico-chemical properties and the stability of TA from various liposome formulations.
3. To study the releasing of TA from various liposome formulations compared with the drug in solution.

1.3 Scope of study

This study is divided into 7 steps which are the followings :

1. Preparation of nine liposome formulations which are :
   - 1.1 Blank without charged liposomes 7:2
   - 1.2 5% of TA entrapped in without charged liposomes 7:2 (5%TA)
   - 1.3 10% of TA entrapped in without charged liposomes 7:2 (10%TA)

1.4 Blank positively charged liposomes 7:2:1 (+)

1.5 Blank negatively charged liposomes 7:2:1 (-)

1.6 5% of TA entrapped in positively charged liposomes 7:2:1(5%TA,+)

1.4 10% of TA entrapped in positively charged liposomes 7:2:1 (10%TA,+)

1.5 5% of TA entrapped in negatively charged liposomes 7:2:1 (5%TA,-)

1.6 10% of TA entrapped in negatively charged liposomes 7:2:1 (10%TA,-)

2. Characterization of the liposome formulations, which are size and size distribution by a light scattering particle analyzer, lamellarity by a transmission electron microscope, transition temperatures and the enthalpy of transition by a differential scanning calorimeter.
3. Quantitative analysis of TA entrapped in liposome formulations by forming a derivative with 2,4,6 trinitrosulfonic acid and measuring the absorbance by a spectrophotometer at wave length 415 nm.
4. Determination of the percentage of entrapment and the loading of TA in liposome formulations.
5. Physical and chemical stability study of TA entrapped in liposomal formulations when kept at 4, 30 and 45 ^{0}C for three months.
6. Releasing study of TA from liposome formulations comparing with TA in solution by a vertical Franz diffusion cell apparatus.
7. Collecting, assessment, evaluation and discussion of the collected data.

B. Literature survey

1.1 Tranexamic acid (TA)

1.1.1 Research on TA

The current commercial available preparation of TA in the market are tablets and injections (Kathlem, 1999). TA in the form of liposomes are not available in pharmaceuticals or cosmetics. No research group is studying about liposomal TA (I.P.A., 1970 to 1998 and http:// www.Yahoo.com). Currently the medical application of TA is as an antifribinolytic and anti-imflamatory agent. The new application research of TA has been reviewed as below.

A double-blind randomized study was carried out to evaluate the clinical hemostatic effect of TA mouth wash after dental extraction in patients who received anticoagulant agents. Surgery was performed with a reduction in the level of anticoagulant therapy in the control group and with no change in the level of anticoagulant therapy in the group who received the TA. After the extraction, the surgical field was irrigated with a 5% solution of TA in the group of patients whose anticoagulant treatment had not been discontinued and with a placebo solution in the group of patients for whom the anticoagulant therapy was reduced. Patients were instructed to rinse their mouths with 10 ml of the assigned solution for 2 mins four times a day for 7 days. There was no significant difference between the two treatment groups in the bleeding incidence after oral surgery. It has been concluded that the anticoagulant treatment does not need to be withdrawn before oral surgery provided that local antifibrinolytic therapy is instituted (Borea, 1993).

The incidence of post-extraction bleeding and the amount of replacement therapy needed to control bleeding in hemophilic children following the local use of TA mouth wash. The results of the study showed that 91.6% of patients who used TA mouthwash as a supplement to systemic therapy, did not develop post-extraction bleeding; while in 25% of the control patients who received only systemic TA, postoperative bleeding was not observed (Waly, 1995).

In patients with nose bleedings, the hemostatic effect of local application of TA gel or placebo was compared in a randomized, double-blind, multicenter clinical trial with parallel groups. The times needed to arrest the initial bleeding were recorded, as well as any rebleedings within 10 days. The results showed no significant differences in any of the efficacy variables. TA was no better than placebo in the early treatment of nose bleedings, but the gel itself seemed to have a beneficial effect. The gel preparations were easy to insert into the nasal cavity and caused no discomfort to the patients (Tibbelin, 1995).

The effect of a single application of 5% TA on the time course of barrier recovery and on the development of epidermal hyperplasia induced by injury produced by tape stripping, acetone treatment or detergent treatment in hairless mice and in healthy male volunteers was investigated. The results showed that TA accelerated barrier recovery in both hairless mice and human skin. The effects of daily topical application of TA on epidermal hyperplasia, induced by repeated tape stripping or acetone treatment for 7 days,

were also evaluated. The degree of hyperplasia was reduced in both models by repeated applications of TA. Also, proteolytic activity in both human and mouse epidermis increased 1-2 hr after epidermal injuries that disrupted the barrier. It was concluded that these results demonstrate that the inhibition of plasmin accelerates barrier recovery and inhibits the epidermal hyperplasia induced by repeated barrier disruption (Denda, 1997).

The effect of TA on skin pigmentation induced by ultraviolet (UV) exposure in Weiser-Maples guinea pigs has been studied. When guinea pigs were exposed to UV radiation (840 mJ cm-2), skin pigmentation was clearly observed from seven days after exposure and continued to increase to 29 days. Post-exposure applications of 2 and 3% solutions of TA to the exposed regions prevent or inhibit the pigmentation process. When the skin is removed and stained by the Fontana-Masson method, melanin content in the basal layer of UV-exposed epidermis is significantly reduced in the regions to which 2 and 3% TA solutions have been applied, compared with the vehicle control. As plasmin is known to contribute to the release of arachidonic acid (AA) and the production of prostaglandins (PGs), has been examined the effects of TA on AA-induced pigmentation in guinea pig skin. Topical application of TA cause a dose-dependent decrease in AA-induced pigmentation. These results suggest that TA reduces melanocyte tyrosinase activity by suppressing the production of PGs, UV-induced melanogens, through the suppression of the UV-induced increase in epidermal plasmin activity (Maeda, and Naganuma, 1998).

1.1.2 General information about TA

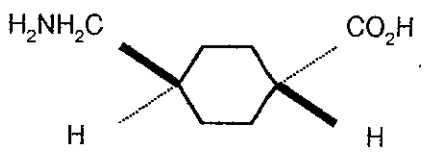

Figure 1.1 : Structure of Tranexamic acid (TA) (Kathlem, 1999)

1.1.2.1 Physical and chemical properties of TA

TA is a trans-4-aminomethyl cyclohexane carboxylic acid. It has a molecular formula of $C_8H_{15}NO_2$, with molecular weight of 157.21 and melting point of 386-390 ^{0}C (decomposition). It occurs as white crystals or powder, odorless and has bitter taste. It is freely soluble in water or glacial acetic acid, very slightly soluble in ethanol, practically insoluble in ether and soluble in sodium hydroxide TS (Kathlem, 1999).

1.1.2.2 Pharmacokinetic of TA (Drug Facts and Comparison, 1999)

Absorption of TA after oral use is approximately 30% to 50%. Its bioavailability is not affected by foods. The peak plasma level 3 hrs after 1 g orally is 8 mg/L and after 2 g is15 mg/L. An antifibrinolytic concentration of the drug remains in different tissues for about 17 hrs and in serum up to 7 or 8 hrs.

TA diffuses rapidly into joint fluid and the synovial membrane. In the joint fluid, the same concentration is obtained as in the serum. The biological half-life in the joint fluid is about 3 hrs.

The concentration of TA in a number of other tissues is lower than in the blood. TA concentrations in the cerebrospinal fluid is about 10% that of plasma concentration.

TA has been detected in serum where it inhibits fibrinolytic activity but does not influence sperm migration. The protein binding of TA to plasminogen is approximately 3% at the therapeutic plasma level.It does not bind to serum albumin. After an IV dose of 1 gm, the plasma concentration time curve shows a triexponential decay with a half life about 2 hrs.for the terminal elimination phase. The initial volume of distribution is approximately 9 to12 L. Urinary excretion is the main route of elimination via glomerular filtration. Overall renal clearance is equal to overall plasma clearance (110 to 116 ml/min). More than 95% of the dose is excreted unchanged in the urine. Excretion of TA is approximately 90% at 24 hrs after IV administration of 10mg/kg. After oral administration of 10 to 15mg/kg, the cumulative urinary excretion at 24 and 48 hrs is 39% and 41% of the ingested dose, respectively, or 78% and 82% of the absorbed material, respectively. Only a small fraction is metabolized. After oral administration, 15 of the dicarboxyic acid and 0.5% of the acetylated compound are excreted approximately.

1.1.2.3 Pharmacology of TA (Pascual, 1998)

Fibrinolysis is a phenomenon involving the degradation of fibrin in the body either physiologically or pathologically. It plays a role in increasing the vascular permeability. It is also related to the onset, development and cure of various types of hemorrhagic diseases and allergies including in *vivo* reactions caused by plasmin. TA inhibits the action of plasmin and shows antihemorrhagic, antiallergic and antiinflammatory effects. TA binds strongly to the lysine bonding site (LBS), the site of fibrin affinity of plasmin and plasminogen and inhibits the binding of plasmin and plasminogen to fibrin. Therefore, the degradation of fibrin by plasmin is strongly inhibited. In the presence of antiplasmin, eg α_2-macroglobulin, in the plasma, the antifibrinolytic agent action of TA is even further strengthened. Abnormally exacerbated plasmin causes inhibition of platelet aggregation, decomposition of coagulation factors, etc. but, even mild exacerbation to occur first. Therefore, in case of ordinary hemorrhages, TA appears to cause hemostasis by suppressing this fibrin degradation. TA inhibits the production of kinin and other active peptides due to plasmin which causes increase of vascular permeability, allergic and inflammatory lesions.

1.1.2.4 Indication and new application research of TA

TA has been used for short-term use (2 to 8 days) in hemophillia patients to reduce or prevent hemorrhage and to reduce the need for replacement therapy during and following tooth extraction (Borea, 1993). It also has been used for many hemostatic purposes including prevention of bleeding after surgery or trauma (eg.tonsillectomy and adenoidectomy, prostatic surgery and cervical conization), and to prevent rebleeding of subarachnoid hemorrhage. It has also been used to treat primary or IUD-induced hemorrhagia, gastric and intestinal hemorrhage (Drug Facts and Comparison, 1999), recurrent epitaxis (Tibbelin, 1995) and hereditary angioneurotic edema.TA has been used with systemic therapy topically as a mouth wash to reduce bleeding after oral surgery in patients on anticoagulant therapy (Waly, 1995).The drug also inhibits induced hyperfibrinolysis during thrombolytic treatment with plasminogen activator.A solution of TA has been used to prevent ultraviolet radiation-induced pigmentation (Maeda, and Naganuma

1998) and accelerate barrier recovery and prevent the epidermal hyperplasia induced by epidermal injury in hairless mice and human (Mitsuhiro, 1997).

1.1.2.5 Dosage and Administration of TA (Kathlem, 1999)

TA is given by mouth and by slow intravenos injection or continuous infusion.Administration by injection is usually changed to oral administration after a few days. Alternatively, an initial intravenous injection may be followed by continuous infussion. Oral doses are 1 to 1.5 gm (or 15 to 25 mg per kg body-weight) 2 to 4 times daily. When given by slow intravenous injection, doses are 0.5 to 1 g (or 1 to 15 mg per kg body-weight) 3 times daily. TA is administered by continuous infusion at a rate of 25 to 50 mg per kg body-weight daily. These doses are used in the short term for hemorrhage. TA is given for prolonged periods in hereditary angioedema in doses of 1 to 1.5 g by mouth 2 or 3 times a day. Children may be given doses of 25 mg per kg body-weight by mouth or 10 mg per kg body-weight intravenosly usually administered 2 or 3 times daily. The following dosages are recommended for impaired renal function (moderate to severe) patients in (Table 1.1).

Table 1.1 : The following dosages are recommended for impaired renal function (moderate to severe) patients (Drug Facts and Comparison, 1999)

Serum creatinine (μmol/L)	IV Doses	Tablet Doses
120-250 (1.36-2.83 mg/dl)	10 mg/kg bid	15 mg/kg bid
250-500 (2.83-5.66 mg/dl)	10 mg/kg/day	15 mg/kg/day
>500 (>5.66mg/dl)	10 mg/kg every 48 hrs or 5 mg/kg every 24 hrs	15 mg/kg every 48 hrs or 7.5 mg/kg every 24 hrs

1.1.2.6 Adverse effects of TA

TA appears to be well tolerated. It can produce dose-related gastro- intestinal disturbances. Hypotension has occourred, particularly after rapid intravenous administration. Thrombotic complications have been reported in patients receiving TA. But, these are

usually a consequence of its inappropriate use. A patient undergoing regular peritoneal dialysis for Epstein's syndrome developed ligncous conjunctivis, gingival hyperplasia and peritoneal protein loss associated with the use of TA (Kathlem, 1999).

For effects on skin, TA gives wide spread, patchy rash with associated blisters. It is considered on skin biopsy to be a fixed-drug eruption, which can be occurred in a 33-years old woman (Kathlem, 1999). TA that the patient had taken for eight years well tolerated was identified as the causative agent. Desensitisation was attempted for sensitization of TA but was unsuccessful. Thus, TA was also suspected as being the cause of a fixed-drug eruption in a 36-year-old woman (Kathlem, 1999). Pruritic, vesicle-bullous appeared within a few hrs of commencing treatment with TA and the lesions resolved completely three days after discontinuing therapy even though other drug treatment was continued.

1.1.2.7 Precautions for application of TA (Kathlem, 1999)

TA should not be used in patients with active intravascular clotting because of risk of thrombosis. Patient with a predisposittion to thrombosis are also risk if given antifrinolytic therapy. Hemorrahage due to disseminated intravascular coagulation should therefore not be treated with antifribinolytic compounds unless the condition is predominantly due to disturbance in fribrinolytic mechanism. TA has been used when the latter conditions are met but with careful monitoring and anticoagulant cover.

Lysis of existing extravascular clots may be inhibited in patients receiving TA. Clots in the renal system can lead to intrarenal obstruction. So, caution is required in the treatment of patients with haematuria. Doses of TA should be reduced in patients with renal impairment. Some studies have suggested that TA when given to patients following a subarachnoid haemorrhage increases the incidence of cerebral ischaemia complications. Rapid intravenous administration may be associated with adverse effects.

1.1.2.8 Interactions of TA with other drugs (Kathlem, 1999)

Drugs with actions on haemostasis must be given with caution to patients on antifribrinolytic with TA therapy. The potential for thrombus formation of TA may be increased by oestrogens. The action of the antifribrinolytic can be antagonised by compounds such as the thrombolytics.

1.2 Liposomes

1.2.1 Introduction of liposome

Lposomes are sealed sacs in micron or submicron range dispersed in an aqueous environments. The wall of the sacs consists of bilayers composed of suitable amphiphiles. The nature of the bilayers ensures the formation of the internal aqueous compartments which can differ from outside medium. The present of two different environments in the carrier, the aqueous and the membrane, makes liposomes a unique model for studying biological membranes and a versatile carrier for a broad spectrum of hydrophobic, amphipatic, and hydrophillic agents (Gregoriadis, 1988).

Liposomes are composed of phospholipids, cholesterol, charge or the other lipid. They have the ability to encapsulate selected molecules and enhance their safety and efficacy of selected molecules. They do this by beneficially altering the distribution and kinetics of the molecule in the body following its administration through various routes (topical, intravenous, inhalation, subcutaneous, intravenous) of delivery. Liposomes have been extensively studies and aggressively pursued as drug vehicles for several years since Bangham and co-workers at Babraham in England found liposomes in mild-1960s (Bangham, 1974).

The list of some liposomal products for therapeautic use now available on the market is shown in Table 1.2. It is possible that the liposome as a dosage form has had more intensive and extensive scrutiny than other delivery system. Now liposomal products are advertised in the medical and pharmaceutical press and compete in the commercial arena. Liposomal carriers for a given drug, vaccine or gene must be proven to be not only of high quality, safe and efficacious, but perhaps also superior to conventional formulations.

1.2.2 Physicochemical characteristics

Liposomes are closed vesicles consisting of one or more concentric spheres of lipid bilayers (or lamellae) enclosing an equal number of aqueous compartments. Many lipids are natural components of biological membranes can be used in the formation of liposomes. Phosphatidylcholine and cholesterol are the most commonly used lipids but various others are frequently used depending on the application. Because of their structure, liposomes can

Table 1.2 : Liposome-based products or under development (Andrew, 1999)

Company	Product	Drug	Indications	Status
NeXstar Pharmaceutical , Inc.,(formerly, Vestar Inc.), Boulder, CO	AmBisome® (Intravenous)	Amphotericin B	Systemic fungal infections; visceral leishmaniasis	Approved in the U.S. and 29 other countries
	DaunoXome ® (Intravenous)	Daunorubicin	First line treatment for advanced Kaposi 's sarcoma	Approved in the U.S. and 21 other countries
	MiKasome® (Intravenous)	Amikacin	Serious bacterial and mycobacterial infections	Phase II
	VincaXome™ (Intravenous)	Vincristine	Solid tumors	Preclinical development
SEQUUS Pharmaceutical , Inc.,(formerly Liposome Technology Inc.), Menlo Park, CA	Doxil® (Intravenous)	Doxorubicin	Kaposi 's sarcoma	Approved in the U.S. and 18 other countries
	Amphotec™ (Intravenous)	Amphotericin B	Systemic fungal infections	Approved in the U.S. and 20 other countries
	SPI-77	Cisplatin	Advanced forms of cancer	Phase I
	SPI-119	CD_4	HIV infection	Preclinical development
The Liposome Company, Inc.,Princeton, NJ	Abelcet® (Intravenous)	Amphotericin B	Systemic fungal infections	Approved in the U.S. and 19 other countries
	Evacet® (Intravenous	Doxorubicin	First line therapy for metastatic breast cancer	Phase III

Table 1.2 : Liposome-based products or under development (Andrew S. Janoff, 1999) (continue)

Company	Product	Drug	Indications	Status
The Liposome Company, Inc., Princeton, NJ	TLC ELL-12	Ether lipid	Cancer	Preclinical development
Aronex Pharmaceutical, Inc., The Woodlands, Texas	Nyotran™ (Intravenous)	Nystatin	Candidemia Systemic fungal infections	Phase II Phase III
	Atragen™ (Intravenous)	All-trans retinoic acid	Leukemia Kaposi 's sarcoma	Phase II Phase III
	Liposomal Annamycin (Intravenous)	Annamycin	Refractory breast cancer	Phase I/II
IGI Inc.,Vineland Laboratories, Vineland, NJ	Newcastle Disease vaccine (intramuscular)	Newcastle disease virus (killed)	Newcastle disease (chickens)	Licensd by U.S.D.A
	Avian Reovirus Vaccine (intramuscular)	Avian Reovirus (killed)	For vaccination of breeder chickens; for Passive protection of baby chicks against reovirus infection	Licensd by U.S.D.A
Novovax Inc., Rockville, MD	*E.coli* 0157:H7 vaccine(oral)	*E.coli* 0157:H7 (killed)	*E.coli* 0157 infection	Phase I
	Shigella flexneri 2A vaccine (oral)	*S. flexneri* 2A (killed)	*S. flexneri* 2A infection	Phase I

Table 1.2 : Liposome-based products or under development (Andrew S. Janoff, 1999) (continue)

Company	Product	Drug	Indications	Status
Swiss Serum and Vaccine Institute Berne, Berne, Switzerland	Epaxal-Berna vaccine (intramuscular)	Inactived hepatitis A virions (HAV) (Antigen: RG-SB strain)	Hepatitis A	Approved in Switzerland
	Trivalent influenza vaccine (intramuscular)	Hemagglutinin and neurominadase from H_1N_1, H_3N_2 and B strains	Influenza	Phase III

entrap lipophilic substances in the lipid bilayers and hydrophilic substances within the aqueous compartment (Figure 1.2).

The permeability of liposomes to entrapped agents is dependent in large part on the partition characteristics of the compound. Agents that are very hydrophilic will be very slowly released because of their insolubility in the lipid phase. Similarly, very hydrophobic substances will also be very slowly released because of their insolubility in the aqueous phases. These types of compounds will only show significant release when the liposomal structure is disrupted. If gradual release is required, then a drug (or modified derivative) must be chosen that had a partition coeffient somewhere between these two extremes.

Liposomes permeability is also strongly influenced by the lipid makeup of the bilayers. Both headgroup interactions and acyl chain packing are important. The permeability of vesicles prepared from various phosphatidylcholines increases with increasing temperature, decreasing chain length and increasing unsaturation (R.A.Demel, 1972). Cholesterol is frequently added to liposomal formulations to decrease permeability and increase stability.

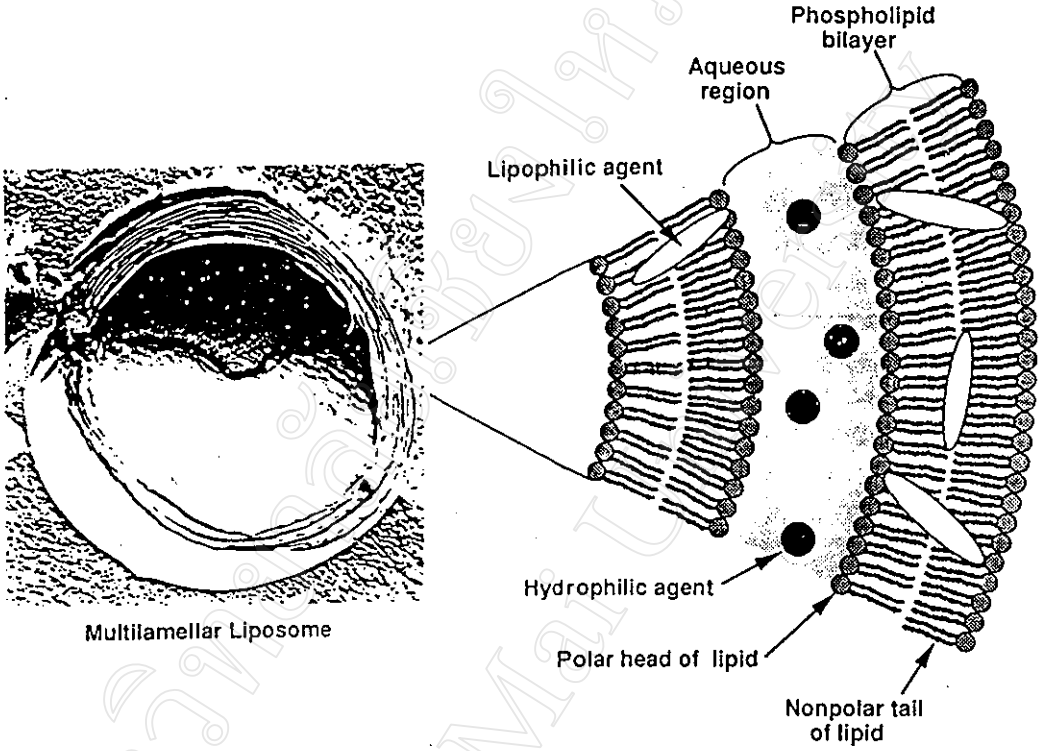

Figure 1.2 : The multiple concentric lipid bilayers of a multilamellar liposome are clearly indicated by the freeze-fracture electron micrograph. The arrangement of the amphipathic phospholipid molecules is shown in the schematic. The polar headgroups situate themselves on the bilayer surface with nonpolar fatty acid side chains extending into the bilayer core. Amphipathic and nonpolar drug penetrate the lipid matrix to an extent determined by their inherent hydrophobicity. Water-soluble drugs can be entrapped in the aqueous compartment (Shek, 1986).

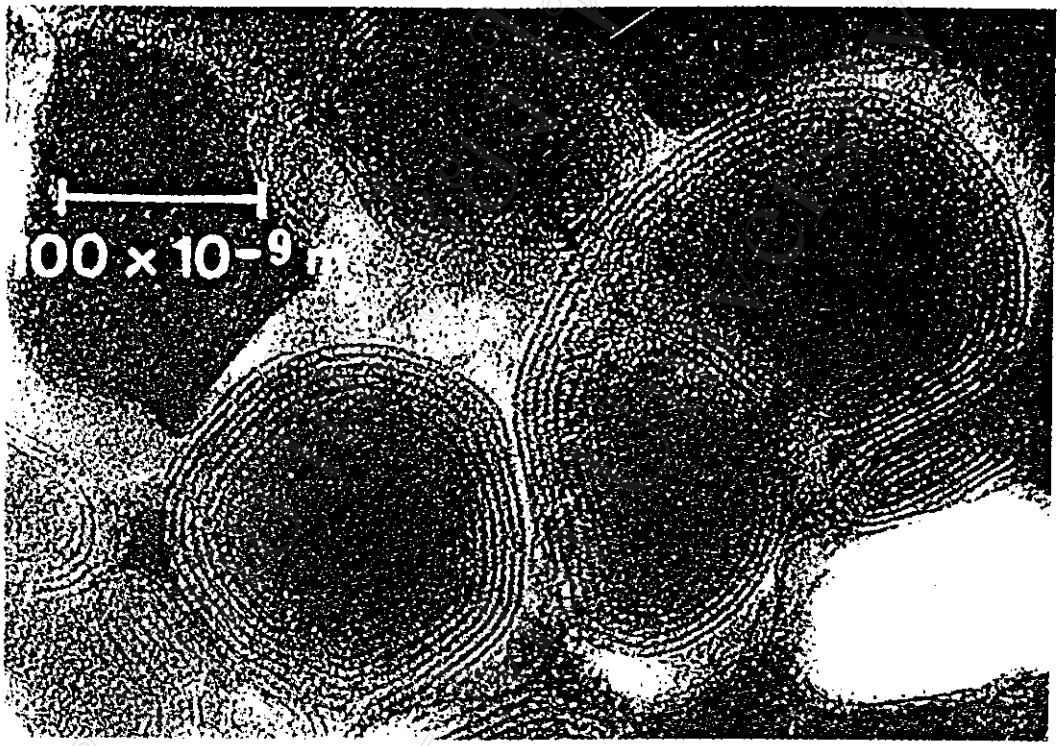

Figure 1.3 : TEM photomicrograph of multilamellar liposomes following negative staining with phosphotungstic acid (Benita, 1996)

1.2.3 Classification of liposomes

Liposomes can be classified either on the basic of their structural properties or on the basic of the preparation method used. These two classification systems are in principle independent of each other. The different vesicles with the regularly used acronyms are listed in Table 1.3. Figure 1.4 : presents a schematic view of the major liposome types.

Table 1.3 : Liposome classifications (Talsm, 1994)

A. Based on the structural parameter	
MLV	Multilamellar large vesicles, > 0.5 μm
OLV	Oligolamellar vesicles, 0.1-1 μm
UV	Unilamellar vesicles (all sizes)
SUV	Small unilamellar vesicles, 20-100 mm
MUV	Medium sized unilamellar vesicles
LUV	Large unilamellar vesicles, > 100 mm
GUV	Giant unilamellar vesicles, (vesicle with diameters > 1 μm)
MUV	Multivesicular vesicles, (usually large > 1 μm)
A. Base on the method of liposome preparation	
REV	Single or oligolamellar vesicles made by the reverse-phase Evaporation method
MLV-REV	Multilamellar vesicles made by the reverse-phase evaporation method
SPLV	Stable plurilamellar vesicles
FATMLV	Frozen and thawed MLV
VET	Vesicles prepared by extrusion methods
FPV	Vesicles prepared by French Press
FUV	Vesicles prepared by fusion
DRV	Dehydration-rehydration vesicles
BSV	Bubblesomes

There are various terms and methods used in the literature to classify the different morphological categories of vesicles or liposomes. Multilamellar vesicles (MLV), in which a multiple " onionlike " bilayer structure surrounds a relatively small internal core, as defined and produced by Bangham (Travers, 1978). Oligolamellar vesicles, (OLVs), in which the large central aqueous compartment is surrounded by two to ten bilayer structures, also on some rare ocassions called paucilamellar vesicles (PLVs) (Goodhardt, 1973). Unilamellar vesicles, (ULVs), in which there is only a single bilayer structure surrounding the internal aqueous core. This particular category has several subcategories based on their size. Small unilamellar vesicles (SUVs), with the size range of 20-40 nm, with little use in drug delivery. Medium unilamellar vesicles (MUVs), with the size range of 40-80 nm. Large unilamellar vesicles (LUVs), with the large internal aqueous core having the size range of 10-1000 nm. Giant unilamellar vesicles (GUVs), which have the size larger than 1000 nm probably are most unstable from physicomechanical consideration. Multivesicular vesicles (MVVs), in which a large vesicle contains smaller and usually, unilamellar vesicles.

1.2.4 Compositions of liposomes

The chemical composition particularly the choice of the phospholipid used, is an essential step in the development of liposomes. Natural (yolk or soya) or synthetic and semisynthetic phosphatidylcholins are mostly used. Apart from this phospholipid, it can be useful to add sterol (cholesterol, beta-sitosterol) to modulate the "transition temperature" and the lipidic bilayer microviscosity (Benita, 1996). Electrocharged components such as dicetyl phosphate or stearylamine can also be added to provide the liposomes with a negative or positive charge. These charges generate repulsions among sheets and among vesicles and improve encapsulation capacity and stability.

1.2.5 Liposome preparation

There are several methods to prepare liposomes. They are depending on the types of liposome desired (SUVs, MLVs, LUVs or other). Some commonly used procedures are the following:

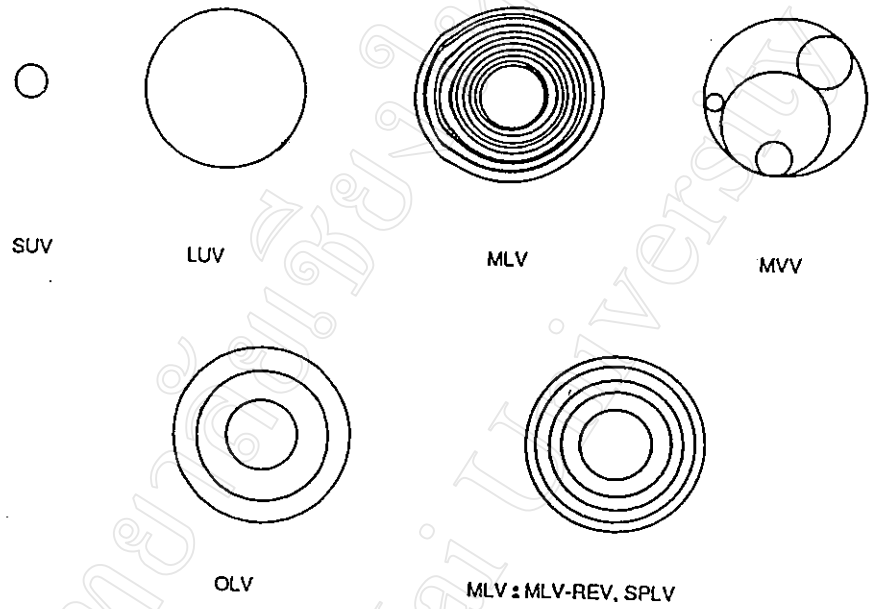

Figure 1.4 : Morphology of different liposome structures (Barenholz, 1994)

1.2.5.1 Solvent evaporation method (Esmail Tabibi, and Christopher, 1996)

In this method, the MLVs, the bilayer-forming formulation will be produced. They are composed of phospholipids and cholesterol along with lipophilic active ingradient. All lipid mixture is dissolved in a suitable organic solvent, such as chloroform, carbon tetrachloride or ether. The solvent is then evaporated in a rotavapor under reduced pressure to create a thin layer of lipidic film deposited on the inner wall of the round bottom flask. The produced film is then hydrated with either distilled water or a proper solution of drug depending on the nature and concentration of the lipidic ampliphiles, rate of hydration and so on. The advantages of this method of liposomal preparation are ease of production (scale-up possibility), relative storage stability and suitability for encapsulation of large molecules. However, the liposome prepared by this method will be suffer from size heterogeneity, low encapsulation capacity, and risk of material degradation by solvent or heat.

1.2.5.2 Sonication method (Guru, 1993)

In this method, SUV or MLV are sonicated either with a bath-type sonicator or probe sonicator, under an inert atmosphere (usually nitrogen or argon). Although probe sonication leads to more rapid size reduction, problems such as heat production, degration of lipids, metal particles shredding from the probe tip and aerosol generation may be present. Although temperature can be accurately regulated in bath-type sonicators and the tube containing the specimen is sealed (allowing for aseptic operations with little likehood of personnel exposure to aerosols), these instruments are difficult to tune (i.e., regulate power output in a reproducible way) and are available only in small sizes. Flow-through sonic devices are available that are capable of processing larger volumes. But, they have yet to be proven in liposome manufacturing. It is likely that the same disadvantages will apply including particle shredding, difficulty with energy regulation and heat-exchange problems.

1.2.5.3 Reverse-phase evaporation method (Francis J. Martin., 1990)

LUV can also be prepared by forming an water –in-oil emulsion of phospholipids and buffer in excess organic phase followed by removal of the organic phase under reduced pressure. The two phases are usually emulsified by sonication. But, other mechanical means have also been successfully employed. Removal of the organic solvent under

vacuum causes the phospholipid-coated droplets of water to coalesce and eventually form viscous gel. Removal of the final trace of solvent under high vacuum or mechanical disruption (such as vortexing) results in the collapse of the gel into a smooth suspension of LUVs. This general sequence is shown in Figure 1.5. With some lipid compositions, the transition from emulsion to LUV suspension is so rapid that the intermediate gel phase appears not to form. The method has gained wide spread use for applications that require high encapsulation of a water soluble drug. Entrapment efficiencies up to 65% can be obtained with this method.

To prepare liposomes by the reverse-phase evaporation method, the phospholipids are first dissolved in an organic solent such as diethyl ether, isopropyl ether, or mixtures of two solvents such as isopropyl ether and chloroform. Emulsification is most easily accomplished when the density of the organic phase matchs that of the buffer (i.e., about 1). The aqueous phase containing the material to be entrapped is added directly to the phospholipid-solvent mixture, forming a two-phase system. The two phases are sonicated for a few minutes, forming a water-in-oil emulsion. The organic phase is then carefully remove under a partial vacuum produced by a water aspirator on rotary evaporator. Removal of the last traces of solvent transforms the gel into LUVs which have been encapsulated both small and large molecules. The principal disadvantage of the method is the exposure of the material to encapsulated to organic solvents and mechanical agitation conditions that can lead to the loose stability of some materials. An advantage of the method is high encapsulation.

1.2.5.4 Ethanol injection method (Guru, 1993)

An alternative method for producing small liposomes that avoid both sonication and exposure to high pressure is the ethanol injection method. Lipids dissolved in ethanol are rapidly injected through a fine needle into an excess of buffer solution, where SUV forms instantaneously. The force of the injection is usually sufficient to achieve complete mixing so that the ethanol is diluted almost instantaneously in water and phospholipid molecules are dispersed evenly throughout the medium. The procedure is simple, rapid and gentle to both lipids and materials to be entrapped. Unfortunately, the method is restricted to the production of relatively dilute SUV suspensions. The final concentration of ethanol can not

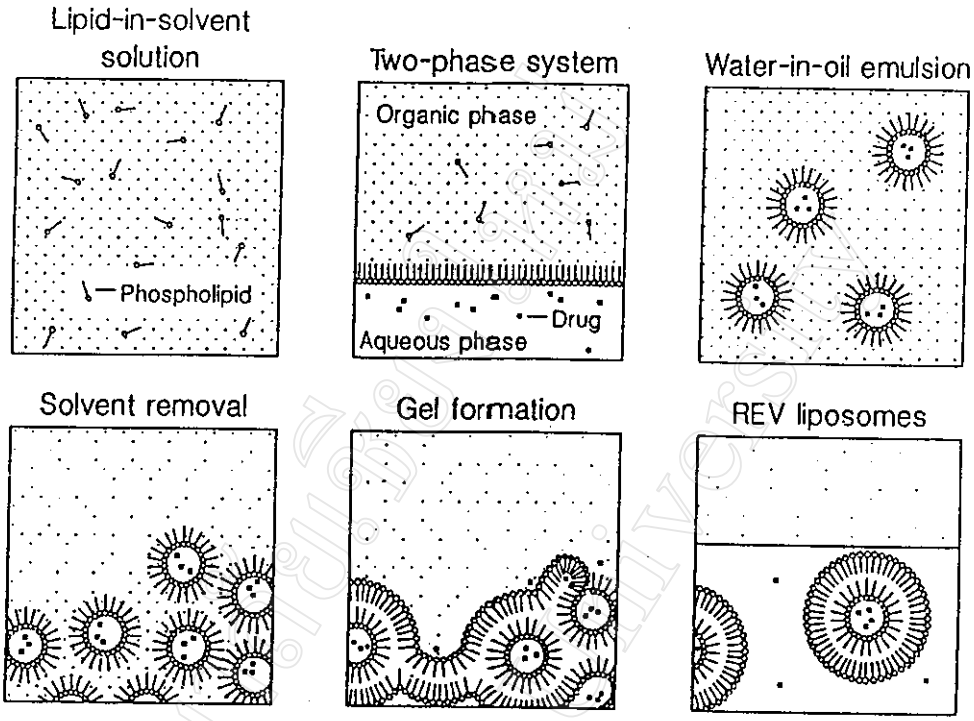

Figure 1.5 : Diagram showing the key steps in liposome formulation by the REV (reverse-phase evaporation) technique. First bilayer-forming phospholipid are dissolved in ether (or another suitable water-immisicible organic solvent) to form a lipid-in-solvent solution. The aqueous phase containing the drug (squares) is added to form a two-phase system that is subsequently emulsified by mechanical means to form a water-in-oil emulsion. As the organic solvent is removed, the preparation becames viscous and usually form an intermediate gel. As the last traces of solvent are removed, the gel collapses into smooth suspension of single-layered REV liposomes (Martin., 1990)

exceed about 10-20% by volume or the SUV either will not form, or they will grow in size soon after formation. Removal of residual ethanol by vacuum distillation can also present a problem since its partial vapor pressure at low residual concentrations is small compared to that of water. Ultrafiltration represents a promising means of both removing ethanol and concentrating the suspension if desirable (Guru, 1993). Still another disadvantage is that some biologically active macromolecules tend to become inactive in the presence of even low amounts of ethanol.

1.2.5.4 French pressure method (Guru, 1993)

Dispersions of MLVs can be converted to SUVs by passage through a small orifice under high pressure. A French pressure cell was used by Hamilton and Guo for this purpose (Bangham, 1974). MLVs dispersion is placed in the French press and extruded at about 20,000 psi at 4 ^{0}C. One pass through the cell produces a heterogeneous population of vesicles ranging from several micrometers in diameter to SUVs size. Multiple extrusions result in a progressive decrease in the mean particle diameter. Following abot four or five passes, approximately 95% of the vesicles have conversted to SUVs as juded by size exclusion chromatography. The resulting vesicles are somewhat larger than sonicated SUV, ranging in size from 30 to 50 nm. The methoh is simple, reproducible and nondestructive. However, temperature control is difficult. The body of the pressure cell must be allow to cool between extrusion or the temperature rise may lead to damage to the lipids or drug. The working volumes are relatively small (about 50 ml maximum) (Guru, 1993).

However, in many methods, drug encapsulation is not complete. Nonassociated or nonencapsulated in the case of bilayer or nonbilayer interacting drugs respectively, drug is present. In some method, the free drug can be left in the formulation. In others, it has to be removed because of its toxicity, as with many antineoplastic drugs (doxorubicin), antibiotics (aminoglycosides) or biological response modifiers suuch as IL-2 or TNF. It is possible also that free drug interferes with the liposome stability (Nicolay, 1985). For removal of the nonencapsulated drug, the following techniques such as dialysis and ultracentrifugation, ultracentrifugation, gel permeation chromatography and ion exchange reactions have been used. The technique was used depend on physico-chemical properties of the drug and types of liposome.

1.2.6 **Characterization and quality control of liposomes** (Janoff, 1999 ; Swarbrick, ; Boylan, 1994)

1.2.6.1 Characterization of liposomes

Liposome characterization should be performed immediately after preparation. One should also ensure that no major changes occur on storage. Chemical and physical characterization are very important for a meaning comparison of different liposome preparation or different batches prepared according to the same protocol. The chemical

characterization focuses on the quality of liposome constituents (raw material) and the prepared liposomes. Both chemical and physical characteristics of liposomes influence their behaviors in *vivo* and in *vitro*. Several more examples demonstrating the importance of proper selection of liposome structures to obtain optimum and reproducible therapeutic effects have been published (Goren, 1990). Therefore, it is essential to characterize liposomes properly. Different techniques are used based on chemical, enzymatic or chromatographic principles as shown in Table 1.4.

1.2.6.2 Liposome quality control

There is different between quality control strategies to characterize vesicles during the development of a liposome dosage form and the quality control assay used to routinely characterize batchs for clinical applications. During the development of invasive methods, labels or agents irrelevant for the formulation itself can be used. This cannot be done with batches employed in the clinical or in final stages of animal toxicity or efficacy testing. The quality control assays of liposomal formulation are shown in Table 1.5.

1.2.7 Advantages and therapeutic application of liposomes

Advantages and therapeutic application of liposomes will be reviewed in liposomes as a parenteral drug delivery system and liposomes as a topical drug delivery system.

1.2.7.1 liposomes as a parenteral drug delivery system

Liposomes provide a range of advantages in drug delivery. When administered parenterally, either by the intravenous, subcutaneous, intramuscular and intraperitoneal route, liposome can protect an encapsulated drug from degradation and provide controlled "depot" release over an extended period of time. This feature can reduce the side effect of the drug by controlling the rate at which free drug becomes available in the blood stream. Liposomes can also alter the tissue distribution of an uptake of a certain drug, especially in a therapeutically favorable way (such as targeting to elements of the reticuloendothelial system RES)). This can also increase the convenience of therapy by allowing less frequency drug administration.

Table 1.4 : Techniques used for characterization of liposomes (Janoff, 1999 ; Swarbrick, ; Boylan, 1994)

Parameters	Techniques used
Mean size and size distribution	Electron microscope Light scattering Coulter counter Polarization intensity differential scattering
Number of lamellae	NMR spectroscopy Small angle x-ray scattering Electron microscope Colored or fluorescent probes
Electrical surface potential, charge	Micro-electrophoresis or zeta potential probes
Encapsulated volume	Encapsulation and assay of water-soluble material
Structure and motional behavior of the lipids in the liposomes	Fluorescent probes NMR spectroscopy Differential scanning calorimetry
Distribution of lipid between the faces of the lipid bilayer with special emphasis on the components exposed to the external medium	NMR spectroscopy Chemical labeling
Distribution of drug between liposome membranes and its internal aqueous compartment	Comparison with encapsulated volume Zeta potential Fluorescent

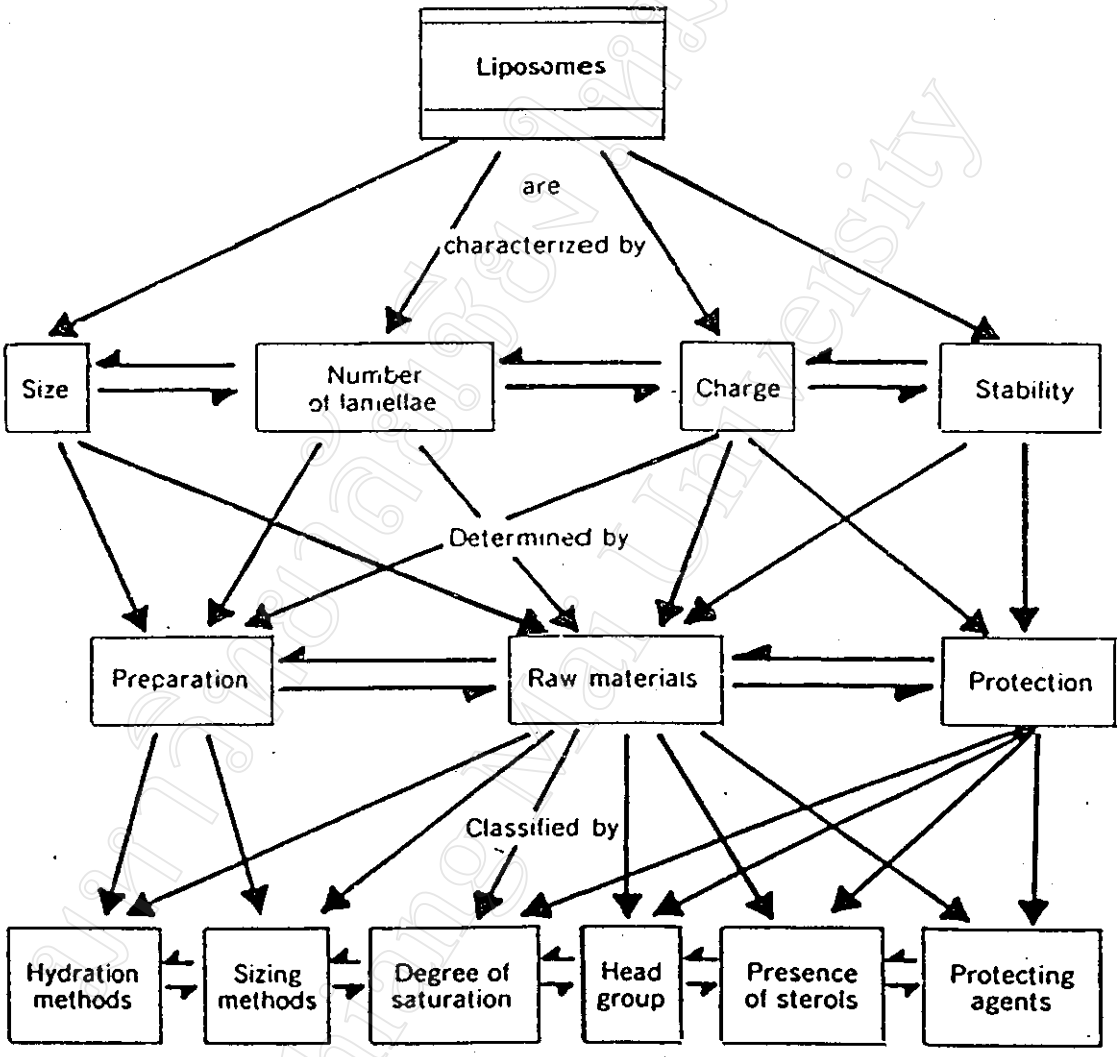

Figure 1.6 : Interrelationships of factors to consider in the design of pharmaceutical liposome preparations (Lichtenberg, 1988)

Table 1.5 : Quality control assays of liposomal formulation (Janoff, 1999 ; Swarbrick, ; Boylan,1994)

Assays	Techniques used
Characterization	
PH	pH meter
Osmolarity	Osmometer
Phospholipid (PL) concentration	Lipid phosphorus content (PL) (Bartlett method)
Phospholipid composition	TLC (combined with Bartlett method) Cholesterol oxidase assay, HPLC
Cholesterol concentration	
Drug concentration	Spectrophotometry, HPLC, or other chromatographic procedure
Chemical stability	
PH	pH meter
Phospholipid peroxidation	Conjugated dienes, lipid peroxides, TBARS and fatty acid composition (GC)
Phospholipid hydrolysis	TLC, HPLC, freefatty acid level
Cholesterol autooxidation	TLC, HPLC
Antioxidant degradation	TLC, HPLC
Drug degradation	TLC, HPLC, spectroscopy
Physical stability	
Vesicle size distribution :	Photon correlation spectroscopy (PCS)
- Submicron range	Gel exclusion chromatography and specific turbidity,
- Micron range	Coulter counter, lasser diffraction, and light microscopy
Electrical surface potential, surface pH	Zeta potential measurements, use of electrical field or pH-sensitive probes.
Percentage of free drug	Gel exclusion chromatography, ion-exchange chromatography
Dilution-dependent drug release	Dilution effect (up to 10,000 folds) on liposomal drug / PL ratio

Table 1.5 : Quality control assays of liposomal formulation (Janoff, 1999 ; Swarbrick, ; Boylan,1994) (continue)

Assays	Techniques used
Drug / phospholipid ratio	Determination of drug and phospholipid contents
Relevant body fluid, induced leakage	Gel exclusion chromatography, ion-exchange chromatography
Biological characterization	
Sterility	Aerobic and anarobic bottle cultures
Pyrogenicity	Rabbit or LAL tests
Animal toxicity	Monitor survival, histology, pathology

- Intravenous

The intravenous is widely regarded as the most promising route of administration. The majority of reported studies on liposome utilized the intravenous route. Upon intravenous administration, liposome are normally removed from the circulation rapidly by the RES, especially the fixed macropharges present in the liver and spleen. This action by the macropharges can be exploited for passive target of these cells, but it is normally considered an obstacle to over come (Patel, 1981). The use of liposome and poly-L-lactic acid microsphere carriers to deliver tobramycin via intravenous and endotracheal routes to the lungs in rats was investigated by monitoring pulmonary, renal and vascular distribution. There was a significant difference between free drug which was rapidly disseminated to other organs. Liposomal and microcapsular tobramycin was primarily retained in the lungs. Renal drug levels of intravenously delivered microcapsular tobramycin were significantly higher than those produced by the liposomal administration at 6 and 24 hrs. The drug entrapped in liposomes produced pulmonary levels 3 times higher than those of the free drug at both 6 and 24 hrs. It was concluded that tobramycin can be retained in the lung by means of liposomal and microencapsulated delivery after endotracheal delivery (Poyner, 1995).

- Subcutaneous

Subcutaneous injection of liposome products can provide local action and/or serve as a drug reservoir for prolonged release. SUVs with a positive or neutral net charge are taken up by the regional lymph nodes more readily than negatively charge liposomes (Garbizon, 1988). The terminal lymphatics have gaps greater than 0.1 μm which are enlarged dramatically by inflammation. These gaps allow the penetration of MLVs, large unillamellar vesicles (LUVs) and SUVs into the lymphatic system. Thus, it is possible to delivery high concentrations of cytotoxic drugs or radioImaging agents to lymphomas or solid tumors that have metastasized to the regional lymph nodes (Poste, 1984). After uptaken by the lymphatic system, liposomes may enter the systemic circulation. Very large liposomes are not taken up by the lymphatic system there by resulting the disappearance of liposomes very slowly from the site of injection. MLVs which were recovered from the injection site 5 hrs post-injection were smaller than before injection. This was perhaps due to a slow layer-by-layer disintegration by neutrophils. Subcutaneous administration provided a prolonged physiological effect from liposomal-encapsulated insulin (Patel, 1981).

- Intramuscular

Intramuscular route of the negative charged liposomes are dispersed from intramuscular injection site more slowly than positively charged or neutral liposomes (Betagen., 1993). Liposomes may release drug at the injection site, but evidence suggests that positive charged SUVs may be taken up by muscular fiber possibly by liposome-cell fusion. Increasing the cholesterol content of liposomes prolongs the release of the entrapped drug (Betagen., 1993).

- Intraperitoneal

The intraperitoneal route is similar in many respects to intravenous administration. But, the associated pain makes it less desirable. Several studies demonstrated the possible effectiveness of intraperitoneal administration of liposomes. Doxorubicin (I), cisplatin (cis-diamminedichloroplatinum II; II) and chloroquine (III) were encapsulated in liposomes and studied to assess their therapeutic activity in animals. Liposome encapsulation increased the therapeutic index for I in tumor-bearing rats. However, liposome encapsulation of II

decreased the antitumor effect. Both the therapeutic and prophylactic activity of III were improved after intraperitoneal injection of III liposomes in mice with malaria, compared with free drug. These effects may be due to the sustained release of III from liposomes. Examples of the successful drug targeting with III liposomes in a murine malaria model are presented (Crommelin. DJ., 1990). The fate of liposomes after intraperitoneal administration is primarily controlled by the liposome size. Small unilamellar vesicles (SUVs) are rapidly removed by the lymphatic system and releases into the general circulation. Hence, they are subject to be removed by the RES just as intravenous injected liposomes. Multilamellar vesicles (MLVs) are too large to enter the lymphatics and are retained in the peritoneal cavity. They can, therefore, function as a depot for a drug (Patel, 1981). In general, the percentages of drug found in the RES cells after intraperitoneal injection is 2-3 times less than when the same dose given intravenous (Patel, 1981). The exact mechanism of liposome uptake after intraperitoneal administration is not understood (Patel, 1981).

1.2.7.2 **Liposomes as a topical drug delivery system**

Topical application of liposomes includes eyes, lung and skin as target sites and oral application. Topical administration may be used for either local or systemic delivery of drugs. Liposomes may be preferred for topical drug administration due to their ability to contain drug at the site of application. This could increase the duration of action while reducing or eliminating the systemic effects. However, topical target sites are more unique with respect to the pathological or physiologic conditions encountered, the nature of uptake, and the metabolic fate of drug and carrier material (Crommelin, and Schreier, 1994).

- Ocular

The use of liposomes as an ocular drug delivery system has been proposed to achieve the sustained release of drugs applied to the cornea and corneal lesions or to be administered by subconjuncctival injection and prolonged retention or targeting of drug to selected intraocular cell populations in lens and vitreous. Since acyclovir cannot be given as eyedrops (Law, and Hung, 1998), attempts to use liposomes to entrap acyclovir as an ophthalmic dosage form have been a great interest in drug delivery research. Acyclovir was

encapsulated in target-sensitive immunoliposomes composed of antibody (palmitoyl conjugated IgG against Herprs simplex virus) and egg phosphatidylcholine or transphosphatidylated phosphatidylethanolamine. An *in vitro* virus inhibitory effect with less cell cytotoxicity was evident. An enhancement of antivirus activity of a lipophollic prodrug of acyclovir in egg phosphatidylcholine was found in cell cultures. Liposomes can thus be used as an efficient carrier for the delivery of acyclovir in ocular drug delivery system (Law, 1998). The other used of liposomes for ocular drug delivery system was enhanced efficacy of liposome-encapsulated idoxuridine in herpes simplex infected cornea lesions in rabbits (Smolin, 1981). The ocular controlled release drug delivery systems is presented including descriptions of hydrogel-type contact lenses, micropump devices and liposome encapsulated drug delivery systems for continuous and consistent ocular drug delivery. Examples of these systems include an ocular therapeutic system prepared with polymer matrices for the slow release of pilocarpine or epinephrine, hydrophilic contact lenses for the controlled release of antibiotics and polypeptide matrices for the ocular controlled administration of steroids (Udupa. 1993). Early work until 1985 included the study of corneal accumulation and transcorneal flux of liposome-associated penicillin G (Shaeffer, 1982), triamcinolone acetonide (Singh, 1983), dihydrostreptomycin sulfate (Singh, 1984) , epinephin (Stratford, 1983), pilocarpine (Benita, 1984) and gentamicin by subconjunctival injection (Barza, 1984). These compounds were incorporated in a greate variety of liposomes, both with respect to size and structure as well as lipid composition (Crommelin, and Schreier, 1994).

- Skin

Liposomal carriers have been successful in enhancing the clinical efficacy of a number of drugs. These have included tretinoin for the treatment of acne (Frosch et al., 1985), glucocorticoids for the treatment of atopic eczema (Korting et al., 1991), lignocaine and tetracaine as anesthetics (Gesztes, and Mezei, 1988; Planas et al., 1992) and others as reviewed in 1995 (Imbert, and Wickett, 1995). The first marketed topical liposomal drug has been Pevaryl Lipogel®,produced by Cilag AG which became available in Switzerland in 1988. The product contains 1% econazole in a liposomal gel form. Some drugs applied to the skin in conventional dosage forms which are irritants, e.g. imidazole type of topical

antifungals like econazole can be advantageous when entrapped in liposomes. If they are encapsulated in liposomes, they have no direct contact with nerve endings. Only the "free" form of the drug can produce irritation. A large portion of the drug when entrapped in liposomes; only a small portion is in the "free" form. As it is releasd from the liposomes due to the enzymes (present in the epidermis and dermis) that hydrolyze or oxidize the lipids, this causes the breakdown of liposomal membranes. Consequently, the "free" form of the "irritant" drug is always lower in the liposomal products than in the conventional vesicles, and less irritation is expected. This was confirmed during the clinical investigations with econazole and retinoids. The reduced, compared to vitamin A acid cream or gel irritation definitely can enhance patient compliance. The first liposomal drug product, Pevaryl lipogel® marketed by CILAG AG in Switzerland since 1988, contains 1.0% econazole in a liposomal gel dosage form. Comprehensive biodisposition studies with seven different types of liposome encapsulated econazole products clearly indicated the superiority of the liposomal form over the commercial (Pevaryl®) cream, gel and lotion form (Mezei, M., 1985., Mezei, 1980 ; Mezei, 1990). Treatment with three liposomal products resulted in an increase (7-9 folds) in drug concentration in the epidermis, which is the site of actions, while the drug concentration in internal organ was less or similar to that of the treatment with the commercial preparation, both in the skin and internal organs than the conventional or commercial products. The advantage of the liposomal form over the conventional dermatological form was particularly striking when the activity of the local anesthetic agents was evaluated in the cream, ointment or lotion form in comparing to the liposomal form. A liposomal product contatning 0.5% tetracain produced more intensive activity (6-8 folds) than 1.0% tetracain in cream, ointment or lotion which was a commercial preparation e.g., Pontocain®. Similar results were obtained when the cream or ointment form were compared with the liposomal forms with other local anesthetic agents, e. g. lidocain, benzocain. The 0.5% liposomal form was more effective than the 1.0% cream form (Touitou, 1994). Several studied have been performed on the dermal and transdermal delivery of substances encapsulated within liposomes including the mechanism of dermal and transdermal delivery, potential drug products and the safety of topical liposomes. Investigators have mostly focused on dermal corticosteroid liposome products. However, localized effects of liposome-associated proteins such as superoxide dismutase, tissue growth factors and interferons

appear to be enhanced. The delivery of liposome-encapsulated proteins and enzymes into the deeper skin layers has been reported, although the mechanism of delivery remains to be elucidated. An objective assessment of the performance of topical liposome formulations in comparing to conventional dosage forms is frequently obscured by investigators comparing equal concentrations rather than equivalent thermodynamic activities for their respective formulations. It was concluded that liposomes may become a useful dosage form for a variety of dermally active compounds, specifically due to their ability to modulate drug transfer and serve as nontoxic penetration enhancers (Schreier. 1994). Other research focused on the use of liposomes as carriers for targeted drug delivery into the pilosebaceous structures. It has indicated that liposomal encapsulation could be beneficial for treating hair follice-associated disorders such as acne, alopecias and various cancers, as well mediating accelerated systemic delivery via transport through the shunt pathway (Lauer et al., 1995). Experiments with the Syrian hamster ear model have demonstrated that carboxyfluorescein-loaded liposomes delivered much higher drug concentrations into the sebaceous glands than conventional carboxyfluorescein formulations (Lieb et al., 1992). Li and co-workers found that liposomal entrapment of calcein (Li et al., 1992), melanin (Li et al., 1993a) and DNA (Li et al., 1993b) resulted in specific delivery into the hair follicles of histocultured mouse skin while aqueous control solutions of these molecules showed no drug localization within the follicle. This indicated that liposomes depending on their nature (size, surface charge and chemical composition of the product) can be useful for dermal or transdermal drug delivery.

- Oral , nasal mucosa and intranasal

Normally, drugs applied to the oral or nasal tissue penetrate readily into the mucosa leading to a very short duration of action. In addition, the vehicle used is often irritating to the mucosa. Liposomes may be useful for delivery drugs to the mucosa since they slowly release drug at the site of action and decrease the systemic drug concentration. Triamcinolone acetonide palmitate incorperated in liposomes to treat ulcerated oral mucosa in hamsters was successfully delivered to the site of action. The liposome did not cause mucosa irritation (Mezei, 1988). To develop nifedipine multilamellar liposomes for intranasal administration, liposomes were prepared using a conventional cast film method. The

liposomes were evaluated for stability, release, bioadhesion and bioavailability in rabbits. Nasal administration demonstrated effective drug blood levels for prolonged periods with improved bioavailability and elimination of hepatic first-pass metabolism. It was concluded that liposome systems can be effective in the control of the release of nifedipine (Vyas. 1995).

In conclusion, liposomes are ideally suited for drug delivery by virtue of following properties.

- Acommodate both water and oil-soluble compound
- Biocompatible and biodegradable.
- Protect the encapsulated drug from metabolic degradation
- Act as depot, releasing the contents slowly and gradually
- Provide selective passive targeting to tumor tissues
- Increase efficacy and therapeutic index
- Reduce toxicity of the encapsulated drug
- Improve pharmacokinetic effects (reduce elimination, increase circulation life time)
- When coupled with antibodies, serves as means to confer active targeting
- Various possible routes of administration

1.3 Tranexamic acid (TA) entrapped in liposomes

Drugs encapsulated in liposomes exhibit pharmacokinetic and pharmacodynamic properties markely different from those of free drugs (Lopez-Bersetin, 1984 and Weissmann, 1977). For certain drugs, an enhancement of therapeutic efficacy and/or a drastic reduction of toxicity has been demonstrated (Jin-Chul, 1997). The improvement of diagnostic localization or therapeutic efficacy attributed to liposomes results from their ability to transport entrapped drug in the circulation, to the target relative to other tissues. Pharmacokinetic parameters such as the rate of clearance from blood, the rate of accumulative in various tissues, the rate of release of encapsulated content defined, and control strategies developed in order to realize the clinical potential of liposomes must be defined. TA

entrapped in liposomes is a novel formulation. No research has been performed on TA entrapped in liposomes. TA is a hydrophilic drug which can cause irritation when used topically. It is also a small with short half life. The entrapment of TA in liposme will be containing advantageous. TA in liposomes can not only be served as a depot system for TA but can reduce the irritation of the drug for topical use as well.