WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 1, 408-425. Review Article ISSN 2277–7105

A COMPREHENSIVE REVIEW: LIPOSOMAL DRUG DELIVERY SYSTEM

Ruchika Kaushal*, Shalu Kumari, Mukul Gautam and Alka Sharma

Baddi University of Emerging Sciences and Technology, Makhnumajra, Distt, Baddi, Himachal Pradesh, India.

Article Received on
28 Oct. 2021,

Revised on 18 Nov. 2021,
Accepted on 08 Dec. 2021

DOI: 10.20959/wjpr20221-22570

***Corresponding Author**
Ruchika Kaushal
Baddi University of Emerging Sciences and Technology, Makhnumajra, Distt, Baddi, Himachal Pradesh, India.
ruchika9696ap@gmail.com

ABSTRACT

The ability of bilayer vesicles to transport medications, vaccines, diagnostic agents, and other bioactive molecules has accelerated the development of the liposomal drug delivery system. Furthermore, a liposomal product could be used to achieve site avoidance and site-specific medication targeting therapy, reducing the cytotoxicity of many powerful therapeutic drugs. The purpose of this article is to give an overview of the liposomal drug delivery technology. It has concentrated on the elements that influence liposome behaviour in the biological environment. The study also covered a number of topics such as the mechanism of liposome synthesis, as well as the characterisation and stability of liposomal medicinal products. Tumor targeting, genetic transfer, immunomodulation, skin and topical therapy are all areas where liposomes can be employed as a therapeutic tool.

KEYWORDS: Bilayered vesicles, % drug encapsulation, cytotoxic formulation.

INTRODUCTION

Combinatorial chemistry breakthroughs have resulted in the identification of a slew of novel chemical entities (NCEs) with potential therapeutic effects on biological systems. However, because of their physicochemical features such as poor solubility and permeability, the majority of the NCEs found provide a challenge to formulation scientists. Despite the fact that the aforementioned issues might be solved, the majority of molecules fail to demonstrate their desired therapeutic action in vivo, resulting in a lack of in vitro – in vivo connection.[1,2]

The majority of anti-neoplastic drugs that are highly cytotoxic to tumour cells in vitro also have an effect on normal cells. This is because they have a low therapeutic index (TI), which means that the dose required to generate an anti-tumor effect is toxic to normal cells. To decrease their hazardous effects on normal tissues, such medications must be targeted to a specific place (diseased site).[3] As a result, an effective drug delivery system is needed to ensure that the maximum fraction of the supplied dose reaches the target site. To target the medicine to a specific place, several carriers such as nanoparticles, microparticles, polysaccharides, lectins, and liposomes can be utilized.[4-9]

Liposomal drug delivery is gaining popularity as a result of its contributions to a variety of fields, including medication transport, cosmetics, and biological membrane structure.[10] Liposomes can operate as a carrier for a wide range of medicines, with therapeutic potential. Liposomes are colloidal carriers with diameters ranging from 0.01 to 5.0μm. Indeed, when phospholipids are hydrated in excess of aqueous media, bilayered vesicles are generated[11,12] Liposomes have the benefit of being able to encapsulate both hydrophilic and hydrophobic medicines and deliver them to the sick spot in the body.[10] The structure of a liposome (bilayered vesicle) and phospholipid.

Anticancer medicines, vaccines, antimicrobials, genetic materials, proteins, and macromolecules can all be encapsulated in bilayered vesicles.[13] Paclitaxel[14], acyclovir[15], tropicamaide[16], arteether[17], chloroquine diphosphate[18], cyclosporine[19], and dithranol[20] have all been successfully encapsulated using liposomal technology. The list of few liposomal products that have been approved for human use is shown in Table 1.[3]

Table 1: List of liposomal products approved for commercial use.

Drug	Product	Indication
Ambisome™	Amphoteracin B	Fungal infection
DaounoXome™	Daunorubicin	Kaposi's sarcoma
Doxil™	Doxorubicin	Refractory Kaposi's sarcoma, recurrent breast cancer and ovarian cancer
Visudyne®	Verteporfin	Age-related macular degeneration, pathologic myopia and Ocular histoplasmosis
Myocet®	Doxorubicin	Recurrent breast cancer
DepoCyt®	Cytarabine	Neoplastic meningitis and lymphomatous meningitis
Lipoplatin®	Cisplatin	Epithelial malignancies
DepoDur®	Morphine	Sulfate Postoperative pain following major surgery

Mechanism of Liposome Formation

Phospholipids, which are amphiphilic molecules, make up the core of a liposome (having a hydrophilic head and hydrophobic tail). The hydrophilic part consists primarily of phosphoric acid coupled to a water soluble molecule, whereas the hydrophobic part is made up of two fatty acid chains each with 10–24 carbon atoms and 0–6 double bonds.[21]

When these phospholipids are disseminated in aqueous medium, they organise into lamellar sheets with the polar head group facing outwards to the aqueous region and the fatty acid groups facing each other, resulting in spherical/vesicle-like structures known as liposomes. The polar portion remains in touch with the aqueous environment, while the non-polar section is shielded (which is oriented at an angle to the membrane surface).[22]

When phospholipids are hydrated in water, the hydrophilic/ hydrophobic interactions between lipid – lipid and lipid – water molecules result in the formation of bilayered vesicles, allowing the aqueous phase to establish thermodynamic equilibrium.[23]

Some of the elements that influence bilayer formation include.

• Folding into tight concentric vesicles reduces the unfavourable interactions between the hydrophilic and hydrophobic phases.

• By generating large bilayered vesicles, the high free energy difference between the hydrophilic and hydrophobic environments may be reduced, and maximum stability to supramolecular self-assembled structures can be achieved.

Classification of Liposome

Liposome's come in a variety of shapes and sizes, according to the literature. Their size, number of bilayers, composition, and technique of manufacture are all used to classify them. Liposomes are categorized as multilamellar vesicles (MLV), large unilamellar vesicles (LUV), and small unilamellar vesicles (SUV) based on the size and quantity of bilayers, as shown in Fig. 2. Conventional liposomes (CL), pH-sensitive liposomes, cationic liposomes, long circulating liposomes (LCL), and immuno-liposomes are the different types based on their composition. They are divided into three types based on how they are made: reverse phase evaporation vesicles (REV), French press vesicles (FPV), and ether injection vesicles (EIV).The classification based on the size and number of bilayers.

Multilamellar vesicles (MLV)

MLVs have a diameter of more than 0.1 m and are made up of two or more bilayers. Their preparation process is straightforward, using thin-film hydration or lipid hydration in excess of organic solvent. They are mechanically stable when stored for a long time. Because of their enormous size, they are quickly removed by reticulo-endithelial system (RES) cells and so can be used to target RES organs.[3] MLV have a moderate trapped volume, defined as the ratio of aqueous to lipid volume. Slower hydration and careful mixing can help to increase drug entrapment in the vesicles.[24] Encapsulation efficiency can be improved by hydrating thin films of dry lipids.[25] MLV with a 40% encapsulation efficiency can be achieved by lyophilization and rehydration with the aqueous phase (containing the drug).[26, 27]

Large unilamellar vesicles (LUV)

Liposomes in this category are made up of a single bilayer and are larger than 0.1μm in diameter. Because they can store a large volume of solution in their cavity, they have a better encapsulation efficiency.[28] They can be used to encapsulate hydrophilic medicines because of their large trapped volume. The advantage of LUV is that it requires less lipid to encapsulate a significant amount of medication. Due to their bigger size, they are removed by RES cells more quickly than MLV.[3,8] Various procedures, including as ether injection, detergent dialysis, and reverse phase evaporation processes, can be used to make LUV. Apart from these procedures, slow swelling of lipids in non-electrolyte solution[32], freezethawing of liposomes[29,30], dehydration/rehydration of SUV[31], and freezethawing of liposomes[29,30] can also be used to prepare LUV.

Small unilamellar vesicles (SUV)

When compared to MLV and LUV, SUV are smaller (less than 0.1 μm) and feature a single bilayer. They are characterised by a low entrapped aqueous volume to lipid ratio and a lengthy circulation half life. SUV can be made via a solvent injection approach (ethanol or ether injection methods)[33] or by sonicating or extruding MLV or LUV under an inert environment such as nitrogen or Argon to reduce their size. A bath sonicator or a probe sonicator can be used for sonication. MLV can also be passed through a tiny orifice under high pressure to achieve SUV. At lower or negligible/no charge, these SUV are vulnerable to aggregation and fusion.[34]

Methods Of Preparation

Solubilization of lipids in organic solvent, drying of lipids from organic solution, dispersion of lipids in aqueous media, purification of resultant liposomes, and analysis of the final product are some of the traditional procedures for making liposomes.[35]

The thin-film hydration method is the simplest and most extensively utilised method for manufacturing liposomes. This approach produces MLV in the size range of 1 to 5µm. If the medication is hydrophilic, it goes into the aqueous buffer, but if it's hydrophobic, it goes into the lipid film. However, this approach has a low encapsulation efficiency for hydrophobic medicines (approximately 5–15 percent). The encapsulation efficiency of the MLV can be improved by hydrating the lipids in the presence of an organic solvent.[36,37] Solvent injection, detergent dialysis, calcium induced fusion, and reverse phase evaporation procedures can all be used to make LUV. Extrusion or sonication of MLV or LUV can be used to make SUV.

All of these preparation processes call for the use of organic solvents or detergents, which can be harmful even in little amounts. Other ways without employing organic solvents or detergents, such as polyol dilution[38], bubble method[39], and heating approach[40], have been devised to avoid this. The literature[21,35] contains detailed protocols for liposome preparation.

Charaterization Of Liposomes

Various methods produce liposomes with different physicochemical properties, resulting in changes in their in vitro (sterilisation and shelf life) and in vivo (disposition) performance.[41-43] For a predictable in vitro and in vivo behaviour of the liposomal therapeutic product, rapid, precise, and repeatable quality control tests are necessary for characterising the liposomes following formulation and storage.[44,45] Some of the parameters listed further down can be used to characterise a liposomal medicinal formulation.

Size and size distribution

When liposomes are designed for inhalation or parenteral delivery, the size distribution is critical because it affects the in vivo fate of liposomes and the encapsulated drug molecules.[46-50] Microscopy (optical microscopy[51], negative stain transmission electron microscopy[42], cryo-transmission electron microscopy[52], freeze fracture electron microscopy, and scanning electron microscopy[45]), diffraction and scattering techniques (laser light scattering and photon correlation spectroscopy)[45], and hydrodynamic techniques

are all used to determine the size of the vesicles (field flow fractionation[53], gel permeation[54] and ultracentrifugation).

Percent drug encapsulation

Percent drug encapsulation is the amount of drug encapsulated/entrapped in a liposome vesicle. The % medication encapsulation of liposomes can be estimated using column chromatography.[55] The medicine is available in two forms: free (unencapsulated) and encapsulated. The free drug is separated from the encapsulated one in order to determine the exact amount of drug encapsulated. The drug-encapsulated fraction of liposomes is then treated with a detergent to achieve lysis, which causes the drug to be released from the vesicles into the surrounding medium. This exposed drug is tested using a suitable method, which yields the percent drug encapsulated, from which encapsulation efficiency can be determined.[56-59]

The percent medication contained in a liposome vesicle can also be calculated using trapped volume per lipid weight. Aqueous volume entrapped per unit quantity of lipid, l/mol or g/mg of total lipid[41,43], is the most used unit of measurement. Various materials, such as radioactive markers, fluorescent markers, and spectroscopically inert fluid[60], can be employed to determine the confined volume. The radioactive approach is commonly used to calculate trapped volume.[41] Dispersing lipid in an aqueous solution containing a non-permeable radioactive solute such as [22Na] or [14C] inulin[61] is used to determine it. To measure the confined volume, water soluble markers such as 6-carboxyfluorescein, 14C or 3H-glucose, or sucrose can be utilized.[45] A unique approach for assessing intravesicular volume via salt entrapment has also been published.[62]

Surface charge

Because the charge on the liposome surface is so important for in vivo disposal, knowing the surface charge on the vesicle surface is crucial. The surface charge of the vesicle can be estimated using two methods: free-flow electrophoresis and zeta potential testing. Estimating the mobility of the liposomal dispersion in a suitable buffer (found using the Helmholtz–Smolochowski equation)[63] can be used to compute the surface charge.

Vesicle shape and lamellarity

The form of the vesicles can be assessed using a variety of electron microscopy techniques. Freezefracture electron microscopy[41] and 31P-Nuclear magnetic resonance analysis[64] can

be used to assess the number of bilayers present in the liposome, i.e. lamellarity. The surface morphology of liposomes can be examined using freeze-fracture and freeze-etch electron microscopy[64], in addition to determining their form and lamellarity.

Phospholipid identification and assay

Liposome chemical components must be examined both before and after production.[45] The phospholipid concentration in the liposomal formulation can be estimated using the Barlett assay[65], Stewart assay[66], and thin layer chromatography.[67] The intensity of blue colour generated at 825 nm against water was measured using a spectrophotometric approach to estimate total phosphorous in a sample.[68] Cholesterol oxidase assays or the ferric perchlorate method[69] as well as gas liquid chromatography techniques[70] can be used to assess cholesterol concentrations.

Stability of Liposomes

The stability of the produced formulation is a crucial factor during the creation of liposomal medicinal products. From the production process to storage and administration, the stability of the liposomes determines the drug's therapeutic effectiveness. The physical stability and chemical integrity of the active molecule are maintained during the formulation and storage of stable dosage forms. The measurement of physical, chemical, and microbiological factors, as well as the assurance of product integrity during the storage duration, are all part of a well-designed stability study. As a result, a stability methodology is required to investigate the drug product's physical and chemical integrity throughout storage.

Physical Stability

When phospholipids are hydrated in water, they form liposomes, which are bilayered vesicles. The vesicles that result from this process are of various sizes. The vesicles tend to combine and grow in size during storage in order to achieve a thermodynamically favourable condition. Drug leakage from the vesicles can occur during storage due to vesicle fusion and breaking, compromising the liposomal drug product's physical stability. As a result, vesicle form, size, and size distribution are crucial characteristics to measure physical stability.[28] A range of techniques, such as light scattering and electron microscopy[71], can be employed to estimate the visual appearance (morphology) and size of the vesicles in order to monitor this.

Chemical stability

Phospholipids are chemically unsaturated fatty acids that are susceptible to oxidation and hydrolysis, thereby compromising the drug's stability. pH, ionic strength, solvent system, and buffering species all play a role in keeping a liposomal composition stable. Even light, oxygen, temperature, and heavy metal ions can cause chemical reactions.

The development of cyclic peroxides and hydroxyperoxidases as a result of free radical creation in the oxidation process is referred to as oxidation degradation. Antioxidants such as alpha-tocopherol or butylated hydroxyl toluene (BHT), manufacturing the product in an inert environment (presence of nitrogen or Argon), or adding EDTA to eliminate trace heavy metals can all help to avoid oxidative destruction of liposomes.[21,28]

The creation of lyso-phosphatidylcholine (lysoPC) is caused by hydrolysis of the ester link at the carbon position of the glycerol moiety of phospholipids, which improves the permeability of the liposomal contents. As a result, controlling the amount of lysoPC in the liposomal medicinal product becomes critical. This can be accomplished by creating liposomes that are phosphatidylcholine-free.[21]

***In Vivo* Behavior Of Liposomes**

Various physico-chemical parameters are changed during the optimization of liposomal formulation in order to obtain the appropriate bio-distribution and cellular uptake of medicines. The parameters that determine liposomes' in vivo (biological) performance are listed below.[72]

Liposome size

Because the fraction cleared by RES is determined by the size of the vesicle, the in vivo fate of liposomes is determined by the size of the vesicle.[73] The rate of liposome uptake by RES increases as the vesicle size grows. When compared to liposomes smaller than 0.1 μm, RES takes up (opsonizes) larger liposomes more quickly.

Liposome extravasation is also determined by the size of the vesicle. Capillaries in tumours are more permeable than capillaries in healthy people. Fluids and microscopic liposomes can travel through the holes in such a leaky vasculature, resulting in an enhanced accumulation of drug-loaded liposomes in tumour tissue. The differential in intravascular hydrostatic and interstitial pressure acts as a driving force for tiny liposome extrvasation.[74]

Surface charge

The nature and quantity of charge on the liposome surface can influence the lipid–cell interaction. The nature and charge of the liposome can be changed by charging the lipid content. The lack of charge in SUV liposomes can cause aggregation, lowering the liposome's stability; however, the interaction of neutrally charged liposomes with cells is almost non-existent.[75,76] The liposome's high electrostatic surface charge may be beneficial in increasing lipid–cell contact. Negatively charged density increases the intracellular absorption of liposomes by target cells and alters the degree of lipid–cell interactions.[77] Positively charged liposomes, on the other hand, are removed faster after systemic injection. Cationic liposomes, unlike negatively charged liposomes, convey their contents to cells via fusing with the cell membrane.[78]

Surface hydration

Liposomes with hydrophilic surface coverings are less likely to opsonize, lowering their absorption by RES cells. This is due to the hydrophilic surface coating, which prevents liposomes from interacting with cell and blood components.[79-81] When compared to liposomes covered with hydrophobic coatings, these sterically stabilised liposomes are more stable in the biological milieu and have longer circulation half lifetimes. Hydrophilic groups such as monogangliosides, hydrogenated phosphotidyl inositol, and polyethylene glycol are responsible for the steric stability of liposomes.[82,83]

Bilayer fluidity

Above and below the phase transition temperature, lipid occurs in several physical states (Tc). Below Tc, they are hard and well ordered, while beyond Tc, they are in a fluid-like liquid – crystalline form. The phase transition temperatures of several phospholipids are listed in Table 2.[3,21] Liposomes having a low Tc (less than 37°C) have a fluid-like consistency and are susceptible to drug content leakage at physiological temperatures. Liposomes having a high Tc (more than 37°C) are stiff and leaky at physiological temperatures.

The liposomal cell contact is also governed by the phase transition temperature. When comparing liposomes with low Tc lipids to those with high Tc lipids, liposomes with low Tc lipids had a higher degree of RES uptake.[80] At temperatures above the phase transition point, cholesterol incorporation in the bilayer can reduce membrane fluidity, giving liposomes stability.

Table 2: Temperatures of phase transitions in various phospholipids.

Name of the phospholipid	Molecular weight	Phase transition temperature (°C)
Dimyristoyl phosphatidylcholine (DMPC)	677.94	23
Dioleoyl PC (DOPC)	786.12	-22
Distearoyl PC (DSPC)	790.15	55
Dipalmitoyl phosphatidylethanolamine (DPPE)	691.97	67
Dipalmitoyl PC (DPPC)	734.05	41
Dipalmitoyl phosphatidylglycerol (DPPG)	744.96	41

Therapeutic Applications Of Liposomes

New drug delivery systems are developed when a traditional dose form fails to produce the intended therapeutic effect. Liposomes are one of these systems that outperform existing formulations in terms of therapeutic performance and safety. The following are some of the most common therapeutic uses for liposomes in medication delivery**.**

Site-avoidance delivery

Anti-cancer medicines' cytotoxicity to normal tissues is due to their limited therapeutic index (TI). In such cases, the TI can be improved by encapsulating the medicine in liposomes to reduce drug distribution to normal cells. The toxicity of free doxorubicin is significant, but when it is packaged into liposomes, the toxicity is lowered without affecting the therapeutic activity.[3, 84]

Site specific targeting

By decreasing the drug's exposure to normal tissues, site specific targeting can deliver a higher fraction of the drug to the desired (diseased) site. Drugs can be encapsulated in liposomes for both active and passive drug targeting, resulting in a safer and more effective treatment.[3] Long circulating immunoliposomes have a higher selectivity for recognising and binding to target cells after systemic treatment.[85,86] When muramyl peptide derivatives were synthesised as liposomes and delivered systemically to patients with recurrent osteosarcoma, monocyte tumoricidal activity was increased.[87]

Intracellular drug delivery

The liposomal drug delivery mechanism can increase the transport of strong medicines to the cytosol (where drug receptors are located).[3] Normally, N-(phosphonacetyl)-L-aspartate

(PALA) is poorly absorbed by cells. In comparison to free medicines, these medications have more action against ovarian carcinoma cell lines when encapsulated in liposomes.[76]

Sustained release drug delivery

Liposomes can be utilised to deliver medications that require a prolonged plasma concentration at therapeutic levels in order to achieve optimal therapeutic efficacy.[3] In vivo, drugs such as cytosine Arabinoside can be encapsulated in liposomes for prolonged release and an optimum drug release rate.[88]

IntraperitoneaI administration

The medicine can be used to treat tumours that form in the intra-peritoneal (i.p.) cavity. However, because the medications are cleared quickly from the i.p. cavity, the drug concentration at the sick location is kept to a minimum. Liposomal encapsulated medications, on the other hand, have a lower clearance rate than free pharmaceuticals and can deliver the maximal proportion of drug to the target site for a longer period of time.[89, 90]

Immunological adjuvants in vaccines

Delivering antigens contained within liposomes can boost immune response. Antigens can be accommodated in the aqueous cavity or incorporated inside the bilayers depending on their lipophilicity.[3] Liposomes were first utilised as immunological adjuvants to boost the immune response to diphtheria toxoid.[91]

CONCLUSION

Using the liposomal drug delivery technology, a number of extremely potent medication candidates with limited therapeutic indication can be targeted to the required sick site. The pharmacokinetics of drugs contained in liposomes can be drastically affected. The ability of the liposomal formulation to deliver the therapeutic molecule to the targeted region over a lengthy period of time while limiting its (drug's) harmful effects is critical to its success. The medications are encased in phospholipid bilayers and are expected to slowly diffuse out of them. During the formulation of liposomal drug delivery systems, various aspects such as drug concentration, drug to lipid ratio, encapsulation efficiency, and in vivo drug release must be taken into account. The manufacturing of deformable liposomes and ethosomes, as well as the inhalation and ocular administration of drug-loaded liposomes, are examples of technological developments. As a result, the liposomal method can be used to improve

pharmacokinetics and therapeutic efficacy whil1qwaQe lowering the toxicity of a variety of very strong medications.

REFERENCES

1. Amidon, G.L., Lennernas, H., Shah, V.P., Crison, J.R. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm Res, 1995; 12: 413-420.
2. Sunil, P., Maru, O., Chan, M. Novel lipid-based formulations enhancing the in vitro dissolution and permeability characteristics of a poorly water-soluble model drug, piroxicam. Int J Pharm, 2005; 301(1-2): 209-216.
3. Sharma, A., Sharma, U.S. Liposomes in drug delivery: progress and limitations. Int J Pharm, 1997; 154: 123-140.
4. Gregoriadis, G. Liposomes, In Gregoriadis, G., (Ed.), Drug Carriers in Biology and Medicine. Academic Press, New York, 1979; Ch. 14. Pp 287- 341.
5. Albertsson, A.C., Donaruma, L.G., Vogl, O. Synthetic polymers as drugs, In Tirrell, D.A., Donaruma, L.G., Turek, A.B., (Eds.), Macromolecules as drugs and drug as carriers for biologically active material. Ann NY Acad Sci, 1985; 446: 105-115.
6. Donaruma, L.G., Warner, R.J. Some biologically active (thiosemicarbazides). In Tirrell, D.A., Donaruma, L.G. and Turek, A.B. (Eds.), Macromolecules as drugs and drug as carriers for biologically active materials. Ann NY Acad Sci, 1985; 446: 116-133.
7. Abra, R.M., Hunt, C.A. Liposome disposition in vivo. III. Dose and vesicle size effects, Biochim Biophys Acta, 1981; 666: 493-503.
8. Tirrell, D.A., Heath, T.D., Colley, C.M., Ryman, B.E. New aspects of liposomes, Biochim Biophys Acta, 1976; 457: 259.
9. Tirrell, D.A., Takigawa, D.Y., Seki, K. pH sensitization of phospholipid vesicles via complexation with synthetic poly (carboxylic acids). In Tirrell, D.A., Donaruma, L.G., Turek, A.B. (Eds.), Macromolecules as drugs and drug as carriers for biologically active materials. Ann NY Acad Sci, 1985; 446: 237- 248.
10. Mozafari, M.R. Liposomes: an overview of manufacturing techniques. Cell Mol Biol Lett, 2005; 10: 711-719.
11. Bangham, A.D., Horne, R.W. Negative Staining of Phospholipids and Their Structural Modification by Surface-Active Agents As Observed in the Electron Microscope. J Mol Biol, 1964; 8: 660-668.

12. Bangham, A.D., Hill, M.W., Miller, N.G.A. Preparation and use of liposomes as models of biological membranes, In Korn, E.D. (Ed.), Methods in Membrane Biology. Vol. 1. Plenum Press, New York, 1974; pp. l-68.
13. Gregoriadis, G., Florence, A.T. Liposomes in drug delivery: Clinical, diagnostic and ophthalmic potential. Drugs, 1993; 45: 15-28.
14. Wu. J., Liu. Q., Lee, R.J. A folate receptor- targeted liposomal formulation for paclitaxel. Int J Pharm, 2006; 316(1-2): 148-153.
15. Pavelic, Z., Skalko-Basnet, N., Filippvic-Grcic, J., Martinac, A., Jalsenjak, I. Development and in vitro evaluation of a liposomal vaginal delivery system for acyclovir. J Control Release, 2005; 106: 34-43.
16. Nagarsenker, M.S., Londhe, V.Y., Nadkarni, G.D. Preparation and evaluation of liposomal formulations of tropicamide for ocular delivery. Int J Pharm, 1999; 190(1): 63-71.
17. Al-Angary, A.A., Al-Meshal, M.A., Bayomi, M.A., Khidr, S.H. Evaluation of liposomal formulations containing the anti-malarial agent arteether. Int J Pharm, 1996; 128(1-2): 163-168.
18. Qiu, L., Jing, N., Jin, Y. Preparation and in vitro evaluation of liposomal chloroquine diphosphate loaded by a transmembrane pH- gradient method. Int J Pharm, 2008; 361(1-2): 56- 63.
19. Al-Meshal, M.A., Khidr, S.H., Bayomi, M.A., Al- Angary, A.A. Oral administration of liposomes containing cyclosporine: a pharmacokinetic study. Int J Pharm. 1998; 168(2): 163-168.
20. Agarwal, R., Katare, O.P., Vyas, S.P. Preparation and in vitro evaluation of liposomal/ niosomal delivery systems for anti-psoriatic drug dithranol. Int J Pharm, 2001; 228(1-2): 43-52.
21. Vyas, S.P., Khar, R.K. In Vyas, S.P., Khar, R.K. (Eds.), Targeted and controlled drug delivery: Novel carrier systems. CBS publishers, 2002; 173- 248.
22. Lasic, D.D. The mechanism of vesicle formation. Biochem J, 1988; 256: 1-11.
23. Lasic, D.D., Joannic, R., Keller, B.C., Frederik, P.M., Auvray, L. Spontaneous vesiculation. Adv Colloid Interfac Sci, 2001; 89-90: 337-349.
24. Olson, F., Hunt, T., Szoka, F., Vail, W.J., Papahadjopoulos, D. Preparation of liposomes of defined size distribution by extrusion through polycarbonate membranes. Biochim Biophys Acta, 1979; 557: 9-23.

25. Barenholz, Y., Gibbes, D., Litman, B.J., Gall, J., Thompson, T.E., Carlson, R.D. A simple method for the preparation of homogeneous phospholipid vesicles. Biochemistry, 1977; 16: 2806-2810.
26. Ohsawa, T., Miura, H., Harada, K. A novel method for preparing liposome with a high capacity to encapsulate proteinous drugs: freeze-drying method. Chem Pharm Bull, 1984; 32: 2442-2445.
27. Kirby, C.J., Gregoriadis, G. A simple procedure for preparing liposomes capable of high encapsulation efficiency under mild conditions, In Liposome Technology, Vol. 1. CRC Press, Boca Raton, FL, 1984; pp 19-27.
28. Vemuri, S., Rhodes, C.T. Preparation and characterization of liposomes as therapeutic delivery systems: a review. Pharmaceutica Acta Helvetiae, 1995; 70: 95-111.
29. Pick, U.I. Liposomes with a large trapping capacity prepared by freezing and thawing of sonicated phospholipid mixtures. Arch Biochem Biophys, 1981; 212: 186-194.
30. Kasahara, M., Hinkle, P.C. Reconstitution and purification of the o-glucose transporter from human erythrocytes. J Biol Chem, 1977; 252: 7384-7390.
31. Shew, R.L., Deamer, D. A novel method for encapsulation of macromolecules in liposomes. Biochim Biophys Acta, 1985; 816: 1-8.
32. Reeves, J.P., Dowben, R.M. Formation and properties of thin-walled phospholipid vesicles. J Cell Physiol, 1969; 734: 49-60.
33. Deamer, D.W., Bangham, A.D. Large volume liposomes by an ether vaporization method. Biochim Biophys Acta, 1976; 443: 629-634.
34. Hamilton, R.L., Goerke, J., Guo, L. Unilamellar liposomes made with the French pressure cell: A simple preparative and semi-quantitative technique. J Lipid Res, 1980; 21: 981-992.
35. New, R.R.C. Preparation of liposomes, In: New, R.R.C. (Ed.), Lipsomes: a practical approach. IRL Press, Oxford, 1990; pp. 33-104.
36. Papahadjopoulos, D., Watkins, J.C. Phospholipid model membranes. II. Permeability properties of hydrated liquid crystals. Biochim Biophys Acta, 1967; 135: 639-652.
37. Gruner, S.M., Lenk, R.P., Janoff, A.S., Ostro, M.J. Novel multilayered lipid vesicles: comparison of physical characteristics of multi-lamellar liposomes and stable pluri-lamellar vesicles. Biochemistry, 1985; 24: 2833-2842.
38. Kikuchi, H. Yamauchi, H., Hirota, S. A polyol dilution method for mass production of liposomes. J Liposome Res, 1994; 4: 71-91.

39. Talsma, H., Van Steenbergen, M.J., Borchert, J.C.H., Crommelin, D.J.A. A novel technique for the one-step preparation of liposomes and nonionic surfactant vesicles without the use of organic solvents. Liposome formation in a continuous gas stream: The bubble method. J Pharm Sci, 1994; 83: 276-280.
40. Mozafari, M.R., Reed, C.J., Rostron, C., Kocum, C., Piskin, E. Construction of stable anionic liposome-plasmid particles using the heating method: A preliminary investigation. Cell Mol Biol Lett, 2002; 7: 923- 927.
41. Ostro, M.J. In: Liposomes: from Biophysics to therapeutics. Marcel Dekker, New York, 1987, pp 383.
42. New, R.R.C. In: New, R.R.C. (Ed.), Lipsomes: a practical approach. OIRL Press, Oxford, London, 1989; 1.
43. Weiner, N., Martin, F., Riaz, M. Liposomes as drug delivery systems. Drug Dev Ind Pharm, 1989; 18: 1523-1554.
44. Talsma, H., Crommelin, D.J.A. Liposomes as drug delivery systems, part II: Characterization. Pharmaceutical Technology, 1992b; 16: 52-58.
45. Barenholz, Y., Cromellin, D.J.A. In: Encyclopedia of pharmaceutical technology. Swabrick, J. (Ed.), Marcel Dekker, New York, 1994; 1-39.
46. Vemuri, S., Yu, T., De Groot, J., Roosdrop, N. In- vitro interaction of sized and unsized liposome vesicles with high density lipoproteins. Drug Dev Ind Pharm, 1990; 16: 1579-1584.
47. Ellens, H., Mayhew, E., Rustum, Y.M. Reversible depression of the reticulo-endothelial system by liposomes. Biochim Biophys Acta, 1982; 714: 479- 485.
48. Kao, Y.J., Juliano, R.L. Interaction of liposomes with the reticulo-endothelial system. Biochim Biophys Acta, 1981; 677: 453- 461.
49. Juliano, R.L., Stamp, D. Effect of particle size and charge on the clearance rate of liposomes and liposome encapsulated drugs. Biochem Biophys Res Commun, 1975; 63: 651.
50. Guiot, P., Baudhuin, P., Gotfredsen, C. Morphological characterization of liposome suspensions by stereological analysis of freeze- fracture replicas from spray-frozen samples. J Microsc, 1980; 120: 159-174.
51. Katare, O.P., Vyas, S.P. Proliposomes of Indomethacin for oral administration. J Microencap, 1991; 8: 1-7.

52. Schmidtgen, M.C., Drechsler, M., Lasch, J., Schubert, R. Energy-filtered cryotransmission electron microscopy of liposomes prepared from human stratum corneum lipids. J Microsc, 1998; 191: 177-186.
53. Moon, M.H., Giddings, J.C. Size distribution of liposomes by flow field-flow fractionation. J Pharm Biomed Anal, 1993; 11: 911-920.
54. Andrieux, K., Lesieur, S., Ollivon, M., Grabielle- Madelmont, C. Methodology for vesicle permeability study by high-performance gel exclusion chromatography. J Chromatogr Biomed Sci Appl, 1998; 706(1): 141-147.
55. Maddan, T.D., Harrigan, P.R., Tai, L.C.L., Bally, M.B., Mayer, L.D., Redelmeier, T.E., et al. The accumulation of drugs within large unilamellar vesicles exhibiting a proton gradient: a survey. Chem Phys Lipids, 1990; 53: 37.
56. Vemuri, S., Rhodes, CT. Development and characterization of a liposome preparation by a pH gradient method. J Pharm Pharmacol, 1994a; 46: 778-783.
57. Vemuri, S., Rhodes, CT. Separation of liposomes by a gel filtration chromatographic technique: a preliminary evaluation. Pharmaceutica Acta Helvetiae, 1994b; 69: 107-113.
58. Vemuri, S., Rhodes, CT. Encapsulation of a water soluble drug in a liposome preparation: removal of free drug by washing. Drug Dev Ind Pharm, 1995; 21(11): 1329-1338.
59. Vemuri, S., Rhodes, CT. Development and validation of a drug release rate method for a water soluble drug in a liposome preparation. Drug Dev Ind Pharm, 1995; 21(11): 1353-1364.
60. Yoss, N.L., Propescu, O., Pop, V.I., Porutiu, D., Kummerow, F.A., Benga, G. Comparison of liposome entrapment parameters by optical and atomic absorption spectrophotometry. Biosci Rep, 1985; 5: 1-5.
61. Hope, M.J., Bally, M.B., Webb, G., Cullis, P. Production of large unilamellar vesicles by rapid extrusion procedure: characterization of size distribution, trapped volume, and ability to maintain a membrane potential. Biochim Biophys Acta, 1985; 812: 55-65.
62. Gruber, H.J., Wilmsen, H.U., Schurga, A., Pilger, A., Schindler, H. Measurement of intravesicular volumes by salt entrapment, Biochim Biophys Acta, 1995; 1240: 266-276.
63. Adamson, A.W. In: Physical chemistry of surface, II Ed., Interscience, New York, 1967; 23.
64. Mandal, T.K., Downing, D.T. Freeze-fracture electron microscopic and osmotic water permeability studies of epidermal lipid liposomes derived from stratum corneum lipids of porcine epidermis. Derm Venereol, 1993; 73: 12-17.

65. Barlett, G.R. Phosphorus assay in column chromatography. J Biol Chem, 1959; 234: 466- 468.
66. Stewart, J.C.M. Colorimetric determination of phospholipids with ammonium ferrothiocyanate. Anal Biochem, 1959; 104: 10-14.
67. Terao, J., Asano, I., Matsushito, S. Preparation of hydroperoxy and hydroxy derivatives of rat liver phosphatidylcholine and phosphatidylethanolamine. Lipids, 1985; 20(5): 312-317.
68. McClare, C.W.F. An accurate and convenient organic phosphorus assay. Anal Biochem, 1971; 38: 527-530.
69. Wybenga, D.R., Pileggi, V.J., Dirstine, P.H., Di Giorgio, J. Direct manual determination of serum total cholesterol with single stable reagent. J Clin Chem. 1970; 16: 980-984.
70. Brooks, C.J.W., MacLachlan, J., Cole, W.J., Lawrie, T.D.V. In: Proceedings of symposium on anlaysis of steroids, Szeged, Hungary, 1984; 349.
71. Szoka, F., Papahadjopoulos, D. Comparative properties and methods of preparation of lipid vesicles (liposomes). Ann Rev Biophys Bioeng, 1980; 9: 467-508.
72. Straubinger, R., Sharma, A., Murray, M., Mayhew, E. Novel taxol formulations: taxol containing liposomes. J Natl Cancer Inst Monograph, 1993; 15: 69-78.
73. Harashima, H., Sakata, K., Funato, K., Kiwada, H. Enhanced hepatic uptake of liposomes through complement activation depending on the size of liposomes. Pharm Res, 1994; 11: 402-406.
74. Yuan, F., Leunig, M., Huang, S.K., Berk, D.A., Papahadjopoulos, D., Jain, R.K. Microvascular permeability and interstitial penetration of sterically stabilized (stealth) liposomes in a human xenograft. Cancer Res, 1994; 54: 3352- 3356.
75. Sharma, A., Straubinger, R.M. Novel taxol formulations: preparation and characterization of taxol-containing liposomes. Pharm Res, 1994; 11: 889-896.
76. Sharma, A., Straubinger, N.L., Straubinger, R.M. Modulation of human ovarian tumor cell sensitivity to N-(phosphonacetyl)-L-aspartate (PALA) by liposome drug carriers. Pharm Res, 1993a; 10: 1434-1441.
77. Gabizon, A., Price, D.C., Huberty, J., Bresalier, R.S., Papahadjopoulos, D. Effect of liposome composition and other factors on the targeting of liposomes to experimental tumors: biodistribution and imaging studies. Cancer Res, 1990; 50: 6371-6378.
78. Felgner, J.H., Kumar, R., Sridhar, C.N., Wheeler, C.J., Tsai, Y.J., Border, R. et al. Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations. J Biol Chem, 1994; 269: 2550-2561.

79. Allen, T.M., Ryan, J.L., Papahadjopoulos, D. Gangliosides reduce leakage of aqueous-space markers from liposomes in the presence of human plasma. Biochim Biophys Acta, 1985; 818: 205-210.
80. Gabizon, A., Papahadjopoulos, D. Liposome formulations with prolonged circulation time in blood and enhanced uptake by tumors. Proc Natl Acad Sci, USA, 1988; 85: 6949-6953.
81. Papahadjopoulos, D., Allen, T.M., Gabizon, A., Mayhew, E., Matthay, K., Huang, et al. Sterically stabilized liposomes: improvements in pharmacokinetics and antitumor therapeutic efficacy. Proc Natl Acad Sci, USA, 1991; 88: 11460-11464.
82. Klibanov, A.L., Maruyama, K., Beckerleg, A.M., Torchilin, V.P., Huang, L. Activity of amphipathic poly(ethylene glycol) 5000 to prolong the circulation time of liposomes depends on the liposome size and is unfavorable for immunoliposome binding to target. Biochim Biophys Acta, 1991; 1062: 142-148.
83. Lasic, D.D., Martin, F.J., Gabizon, A., Huang, S.K., Papahadjopoulos,D. Sterically stabilized liposomes: a hypothesis on the molecular origin of the extended circulation times. Biochim Biophys Acta, 1991; 1070: 187-192.
84. Szoka, F.C. Liposomal drug delivery: current status and future prospects. In: Wilschut, J., Hoekstra, D. (Eds.), Membrane Fusion, Marcel Dekker, New York, 1991; 845-890.
85. Gregoriadis, G. Engineering liposomes for drug delivery: progress and problems. Trends Biotechnol, 1995; 13: 527-537.
86. Lasic, D.D., Papahadjopoulos, D. Liposomes revisited. Science, 1995; 267: 1275-1276.
87. Killion, J.J., Fidler, I.J. Systemic targeting of liposome encapsulated immuno-modulators to macrophages for treatment of cancer metastasis. Immuno methods, 1994; 4: 273-279.
88. Allen, T.M., Mehra, T., Hansen, C., Chin, Y.C. Stealth liposomes: an improved sustained release system for 1- beta-D- arabinofuranosylcytosine. Cancer Res, 1992; 52: 2431- 2439.
89. Dedrick, R.L., Myers, C.E., Bungay, P.M., DeVita, V.T. Jr. Pharmacokinetic rationale for peritoneal drug administration in the treatment of ovarian cancer. Cancer Treat Rep, 1978; 62: 1-11.
90. Markman, M., Hakes, T., Reichman, B., Hoskins, W., Rubin, S., Jones, W., et al. Intra-peritoneal therapy in the management of ovarian cancer. Yale J Biol Med, 1989; 62: 393-403.
91. Allison, A.C., Gregoriadis, G. Liposomes as immunological adjuvants. Nature, 1974; 252: 252-255.