JChrDD | Vol 2 | Issue 1 | 2011: 7-13
ISSN: 2249-6785
Journal of Chronotherapy and Drug Delivery

Received: Jan 05, 2011
Revised: Feb 6, 2011
Accepted: April 06, 2011

Special Focus: Colloidal Drug Delivery (Article 1)

LIPOSOME – A NOVEL COLLOIDAL DRUG DELIVERY SYSTEM

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Abstract

The main benefit of targeting drug delivery system is the reduction in frequency of dosing and more effectively the active drug agent is utilized. Nowadays, liposomes are used as a new targeting and controlled drug delivery system. They have attracted more attention because it can be used as a carrier for drugs, diagnostics agents, vaccines products, genetic material such as genes and other bioactive agents. Liposomes are vesicles of phospholipids and consists aqueous medium surrounded by single or more concentric bilayers of phospholipids. When modification is made in the physical and chemical property of liposomes, they can be used in various fields including textiles, cosmetics, etc. Such alteration includes change in size, composition of lipid, charge on surface, surface coating and rigidity of liposomes. In liposomes, both the hydrophobic as well as hydrophilic drugs and agents can be entrapped and targeted to specific required site in the body by use of an appropriate carrier. In addition, the moisturizing effect on skin can be achieved by liposomes due to their ability to enhance the drugs penetration through the skin so that there may be a slow release of drug and may result in moisturizing effect. This article aims to provide an overview of introduction, composition, advantages, applications, preparation methods and problem in liposomes preparation.

Key Words: Carrier systems, lipid vesicles, drug delivery

Introduction

In 1961, liposomes were first discovered in England by Alec D. Bangham, during his study on phospholipids and blood clotting. In his studies, he founded that when phospholipids combined with water, it immediately forms a spherical particle because the one end of phospholipids molecule is hydrophobic while the opposite end hydrophilic. The word consist of two Greek words 'Lipos' and 'Soma' means fat and body respectively. A liposome is composed constituents that are similar to components of cell membrane.[1] According to the solubility of the drug, it may either present in aqueous region or intercession between the bilayers of lipid. Liposomes deliver thier content to specific sites by fusing lipid bilayer with cell membrane or other lipoid layer. Liposomes can be categorized as MLV (multilamellar vesicles), SUV (Small Unilamellar Vesicles) and LUV (Large Unilamellar Vesicles). Liposomes can be differentiated from lipid monolayer structure as the one have a clear separation between hydrophilic and hydrophobic compartments. The molecules which are used to form liposomes may be of amphipathic nature. Depending on manufacturing techniques and control of parameters, liposomes may vary in size. The size range for SUV is 0.02-0.05 µm and for LUV is greater than 0.06 µm and for MLV is 0.1-0.5 µm. Nowadays, liposome technology is growing at fastest rate and used in various fields including gene delivery and cosmetics. The ideal characteristics of

liposomal formulations include; high drug entrapment efficiency, narrow size distributions, stabilities to a long-term period and be able to provide protection against degradation to the agents that are encapsulated and also shows ideal release. Numerous techniques have been used for preparation of liposomes which may give rise to the different size of vesicles that ranges from 20 nm to several microns (in diameter) and consist of single or more bilayers.[1] Liposomes can entrap chemical agents of different sizes. In choosing the preparation method for liposomes the main focusing point is to select a procedure that yields vesicles of correct size, rigidity and vesicles which show no leakage of entrapped content. [2] In the diseases which affect the phagocyte cells of immune system the liposomal drug delivery systems are very effective because phagocyte cells recognize liposome as foreign invaders then the liposomes are engulfed by the phagocyte cells, accumulate in cells and act on the phospholipid layer and entrapped material are released. Liposomal delivery systems used as a target to the infected tissues (due any disease) and mechanism of action in the human body are in under studies. They have also been used in gene therapy, so as to deliver normal gene as well to replaced one that is responsible for any pathogenesis. Liposomes have wide applications in field of cosmetics because of their moisturizing qualities. The present article aims to provide a concise review of advantages and applications of liposomes.

Advantages and limitations of liposomes

The liposomal drug delivery system offers various advantages over other drug delivery systems, which are enlisted below:[1,2]

1. They are biodegradable and biologically compatible to the body.
2. On systemic and non-systemic administrations they are non-toxic and non- immunogenic.
3. They are suitable for liphophilic as well as hydrophilic drugs delivery.
4. They have the ability to encapsulate drug and protect them from the external environment.
5. They can act as sustained release depots.
6. They can be available in various forms such as suspension, semisolid (ointments, gels, lotions) or as lyophilize dry powder (proliposomes).
7. Wide ranges of administration route are available for Leptosomes such as oral, intravenous, subcutaneous, pulmonary, ocular, nasal, topical and other routes.
8. They could encapsulate both small molecules as well as macromolecules.
9. There is reduction in toxicity of entrapped material and increase in stability.
10. They increase efficacy and therapeutic index of drug.
11. They help to reduce exposure of sensitive tissues to toxic drugs.
12. They improves pharmacokinetic effects (reduce elimination, increased circulation life time).
13. They have ability to couple with a carrier or any other site-specific ligands to achieve targeting.

There are certain limitations or disadvantages associated with liposomal drug delivery systems, which include the following:

1. Their production cost is very high.
2. There may be chances of leakage of encapsulated drug or molecules.
3. Sometimes there may be chances that phospholipids undergo oxidation and hydrolysis like reaction.
4. They may have low solubility in aqueous medium.
5. They may have stability problem.
6. There may chances of microbial attack due to presence of aqueous phase.

Composition of liposomes

Liposomes that made up of natural lipids which are biodegradable in nature have weak immunogenicity[2,3] and non-antigenic properties. These liposomes do not cause any pyrogenic reactions and exhibit little intrinsic toxicity.[3] As cell membranes composed of phospholipids, in similar way the liposomes are formed by the polar (head) and nonpolar (tail) groups of phospholipids. The water molecules are attracted towards the polar group of phospholipid and repelled by nonpolar groups of the phospholipids. Most of the times naturally derived phospholipids are used in the liposomes preparations. Surfactant like dioleoyphosphatidylethanolamine (DOPE) is also used in liposomes preparation. The main components which constitute the liposomes are cholesterol and phospholipids.

Cholesterol

The liposomal bilayers are not formed by cholesterol but it gets incorporated into tail of phospholipids. Due to the amphipathic nature of cholesterol, it gets incorporated in the lipid layer in such a way that the hydroxyl group of cholesterol is towards the aqueous region and the long hydrophobic chain in aligned position to the acyl chain at the center of lipid layers.

Phospholipids

They constitute the major part of liposomes. Phospholipids may be of natural or synthetic origin. Phospatidylcholine is the most commonly used natural phospholipid. Lecithin (amphipathic molecule) is the other name of phospatidylcholine. The major source of lecithin is hen's egg and vegetable oil (soya bean oil).

Commercial manufacturing of liposomes

The main point in preparation of lipid vesicles is dispersion of phospholipids such as lecithin in aqueous phase under high energy supply as shown in Fig. 1.

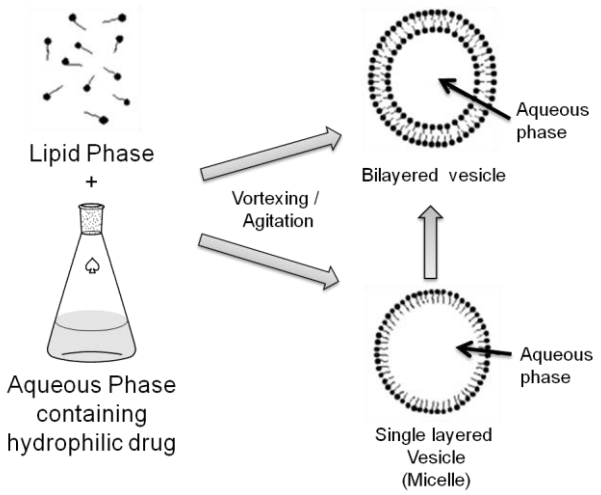

Fig. 1. Mechanism of the formation of bilayer from the lipid precursors

Commercially, liposomal drugs are produced by two major methods, hydration method and emulsion method. The officially approved organic solvent like chloroform, methanol and tertiary butanol are used to dissolve lipid. A proper stirring is to be maintained. The organic solvents are removed by using evaporation or lyophilization, etc. Then hydration in presence of continuous stirring of dry lipid paste or film with aqueous medium is done. This result in entrapment of aqueous medium into lipid vesicles and large MLV are formed. Extrusion or sonications are methods to reduce the size of large MLV. When high amount of energy is supplied, smaller vesicles are formed due to bilayers fragmentation.[4,5] According to solubility drug either dispersed in aqueous phase or lipid. In second method lipid is dissolved in an organic solvent (such as chloroform) then add an aqueous medium with vigorous stirring or agitation. Using reduced pressure organic solvent is removed. Filtration or extrusion is used to reduce the size of liposomal dispersion. Note that there is no spontaneous process to produce liposomes and nanoliposomes.

Choice of preparation method

The correct choice of preparation method depends on the following: [4,5]

1. Concentration and toxicity of substance to be entrapped;
2. Physicochemical properties of the material to be entrapped;
3. Properties of the medium in which lipid vesicles dispersion to be done;
4. Mechanism of delivery of the vesicles;
5. Shelf-life and optimum size of liposomes;
6. Batch-to-batch reproducibility.

Characterization of liposomes

Characterizations of liposomes are necessary to ensure or to predict *in vitro* and *in vivo* performance of liposomes.[4] The characterization includes the following:

1. *Physical characterization*: The parameters are mentioned in Table 1.
2. *Chemical characterization*: Chemical analysis methods are essential to check the stability of liposomes. The parameters mentioned in Table 1.

Table 1. Characterization of liposomes

Parameters	Method / Technique
Physical Characterization	
Liposomes vesicle shape and size	Electron microscopic techniques
Vesicle lamellarity (number of bilayers present)	Freeze fracture electron microscopy
Surface morphology of liposomes	Freeze fracture electron microscopy, X-ray diffraction technique
Appearance	Optical microscopy, Scanning electon microscopy, Light scattering technique, Correlation spectroscopy, Gel permeation technique
Percent fusion	Fluroscent microscopic technique
Amount of entrapped material	Ion exchange chromatography, Size exclusion chromatography
In-vitro release rate of entrapped material	Dissolution apparatus
Size distribution	Size exclusion chromatography
Charge on liposomes	Electrophoresis, Measurement of zeta-potential

Chemical Characterization	
Peroxidation of phospholipids	UV absorbance, GLC technique
Concentration of cholesterol	Cholesterol Oxidase assay
Concentration of phospholipids	Phosphorus content of lipid using Barlett assay
Hydrolysis of phospholipids	HPLC, HPTLC
Concentration of drug	Methods given for drug in monographs
Concentration of lysolecithin (product of lecithin hydrolysis)	Densitometry

There are various formulation factors that influence release of drug from liposomes,[5] which are described in Table 2.

Table 2. Factors influencing drug release from liposomes

Factor	Effect
Phospholipid amount	Higher the amount of phospholipid show higher the rate of absorption.
Charge of vesicle	Negatively charged vesicles absorbed twice as fast in comparison to neutral vesicles.
Size of liposomes	Smaller the size show higher the rate of absorption.
Amount of cholesterol	When Cholesterol and phospholipids are present with saturated hydrocarbon chains, it increases the drug residence time within the lung. The presence of cholesterol and saturated phospholipids decrease liposome membrane permeability to encapsulated drug and to protect liposomes from *in-vivo* destabilization.

Liposome toxicity

The first stage in the Liposome preparation is to dissolve the lipids. In most of liposomes preparation methods for dissolving the lipids there is use of organic solvents which are volatile in nature such as chloroform, ether, etc. These solvents may be present in the final preparation of Liposome and influence the chemical properties of the entrapped material as a result of which it may contribute to toxicity and reduce vesicles stability.[6] Volatile organic solvents may be hazardous to human health as well as to the environment.[7] The mechanism by which the Liposome's solvent shows toxicity towards body tissues include the following:

Mechanism at the molecular level: When organic solvents get dissolved within the aqueous phase it causes entrapped enzyme inhibition, denaturation of protein and modifications in membrane such as membrane expansion, structure alteration and changes in permeability. The effects caused by these alterations are the molecular toxicity.[7,8]

Mechanism at the phase level: Cell nutrients are extracted out from a cell which ultimately leads to cell coating and emulsions formation. To minimize the content of solvents in liposomes various methods can be used such as gel filtration and dialysis. A report suggested that even after the treatment of liposomes (prepared by reverse-phase evaporation technique) with gel filtration method, a little amount of ether still remain in it and as a result there is more leakage of entrapped material as compared to the liposomes prepared in ether absence.[9]

Pharmaceutical applications of liposomes

Liposomes can be used as artificial cells. Apart from the pharmaceutical applications, liposomes are used in textiles to deliver dyes, pesticides to plants, etc. Various pharmaceutical applications of liposomes are given in Table 3 and are described below.

Table 3. Pharmaceutical applications of liposomes

Pharmaceutical applications	Examples
Enhance solubilisation of entrapped drug	Minoxidil, Amphotericin B
Accumulation of drug in stratum corneum	Lidocaine, Prostaglandins
Drug molecule protection	Interleukins, Cytosine arabinoside
Achieving sustained-release	Antineoplastic drugs (eg:5-Flurouracil), Antitubercular drugs (Isonizid and rifampicin), Hormones, Corticosteroids (eg: hydrocortisone)
Targeting and avoiding side effects	Amphotericin B –nephrotoxicity reduction, Doxorubicin – reduce cardiotoxicity
Enhance penetration through skin	Ketoconazole, Topical vehicles
Targeting	Immunomodulators (eg: Paclitaxel-loaded polyethyleneglycolated immunoliposome), Antimalarials, Vaccines
Enhancement of half life of drug	Antifungal drugs (eg: Ketoconazole)

Use of liposomes in targeting

The term targeted delivery refers to the systemic administration of a drug-carrier and targeted delivery can be achieved by using liposomes. Liposomes have ability to couple with a carrier to achieve active targeting. There are various methods to achieve active targeting of drug such as noncovalent bonding of cell specific antibodies to the liposomes,[10] coating the surface of with the immunoglobulin M (IgM),[11] covalent linked of monoclonal antibodies.[12–20] The various benefits of targeting with the help of liposomes can be as followed:

1. They are helpful in controlling the delivery of active agent at predetermined rate.
2. To maintain effective drug level for prolonged duration.
3. To reduce irritation in GIT.
4. There is an increase in patient compliance.
5. To reduce unwanted effect.
6. To reduce the frequency of dosing.
7. To deliver drug in the vicinity of site of action.
8. More effective utilization of active agents.

Liposomes in cancer therapy

Liposomes have ability to target cancer cells. The endothelial cells of blood vessel bound in such a way to form tight junctions. These tight junctions inhibit the leakage of large particle (present in the blood) from the blood vessel. Liposomes having size less than 400 nm may target the cancer cells in the body because such a small size allows penetrating through the blood vessel. Nowadays, several anticancer Liposomal drugs are available in the market and some under clinical trials.[21] 5-fluorouracil liposomes were prepared by film hydration technique and the factors effecting drug release were investigated. If an increase in cholesterol level and drug/aqueous phase mass ratio, it may result in decreased release rate of 5-fluorouracil. *In vitro* release data showed that liposomes acted as reservoir systems for the sustained delivery of encapsulated antineoplastic drug.[22] To target mylenoma cells in breast of human an sterically stabilized paclitaxel-loaded liposome were prepared and it was reported that paclitaxel-loaded Polyethyleneglycolated immunoliposome could prove to be model for upcoming time cancer therapy of breast cancers.[23]

Liposomes in tuberculosis therapy

A co-encapsulated Liposomal drug delivery system for rifampicin and isoniazid were formulated by employing lipid layer hydration method. The *in vitro* release data of isoniazid and rifampicin from liposomes show that there may be sustained release of drug. It was observed that co-encapsulated Liposomal rifampicin and isoniazid have more half life in plasma, lower rate of elimination and show better stability characteristics.[24]

Liposomes in dermatology and cosmetology

On topical application the liposomal drug shows more penetration through skin but limited systemic absorption of drug, so there are very low chances of adverse effects. Various studies suggested that, when drug encapsulated in liposomes, then it is capable of minimizing some well-known problems in drug delivery to the skin. The penetration of drug into the stratum corneum,[25-32] may be enhanced by liposomes carrier system and it maintains drug release in the epidermis.[33] For the liposome skin interactions and penetration through skin there are various proposed mechanisms. An experiment was conducted on guinea pigs by applying liposome-encapsulated lidocaine and derma base (o/w) cream of lidocaine. The result shows that in the epidermis liposomal lidocaine had higher concentrations as compared to cream formulation.[34] The liposomes encapsulated Ketoconazole shows sustained release, increase in half life and fewer side effects as compared to the Ketoconazole cream.[35] Thus it may be beneficial to use liposomes encapsulated drug in topical diseases. Other studies suggest that phosphatidylcholine used to form MLV liposomes of hydrocortisone and such MLV liposomes on topical application shows higher concentration of drug in epidermis which leads to decrease systemic absorption and fewer systemic adverse effects.[36-37]

Future Prospective

Liposomal drug delivery system is of great importance to overcome the problem of low solubility, absorption and to improve the target selectivity. In present scenario, there have been huge number of drugs which cannot be suitably formulated for sufficient delivery or the drug does not reach at the target site of action. Liposome technology provides great advantage while overcoming these problems. Liposomes represent a suitable category of the dosage forms with much scope for formulating and improving delivery of drugs which are very difficult to deliver on the site of action, for example genes, peptides, RNA, protein and growth hormones etc. Delivery through liposomal drugs is much optimized way of drug delivery with characteristics of leading to the

improved potency of liposomal drugs. The features are dynamic control of drug release rate and also the improved cell specific targeting.

Liposomes are nanoscale drug delivery systems. Present liposomal drugs improve the biodistribution as compared to free drugs. To avoid identification of liposomes by reticuloendothelial cells, modification on liposomes can be possible such as pegylated liposomes. Targeting to tumors cells can be achieved by using long circulating liposomes such as pegylated, immobilized liposome and also by monoclonal antibody linked liposomes. Future liposome therapeutic benefits depend on correct design and delivery mechanisms.

Declaration of Interest

It is hereby declared that this paper does not have any conflict of interest.

References

1. Lichtenberg D, Barenholz Y Liposomes: preparation, characterisation and preservation methods. Biochem Anal. 1988; 33: 337-462.
2. Rooijen N, Nieuwmegen R. Liposomes in immunology: multilamellar phosphatidylcholine liposome as a simple biodegradable and harmless adjuvant without any immunogenic activity of its own. Immunol Commun. 1980; 9: 243-256.
3. Campbell PI. Toxicity of some charged lipids used in liposome preparation. Cytobios. 1983; 37: 21-26.
4. Vyas SP, Khar RK. Targeted and controlled drug delivery. 1st ed. Delhi: CBS Publishers; 2002.
5. Lasic A. The mechanism of liposome formation. A review Biochem J. 1988; 256: 1-11.
6. Cortesi R, Esposito E, Gambarin S, Telloli P, Menegatti E, Nastruzzi C. Preparation of liposomes by reverse-phase evaporation using alternative organic solvents. J Microencapsul. 1999; 16: 251-256.
7. Dwivedi AM. Residual solvent analysis in pharmaceuticals. Pharm Technol. 2002; 14: 26-28.
8. Ivanov IT. Rapid method for comparing the cytotoxicity of organic solvents and their ability to destabilize proteins of the erythrocyte membrane. Pharmazie. 2001; 56: 808-809.
9. Allen TM. Removal of detergent and solvent traces from liposomes. In: Gregoriadis G, editor. Liposome Technology Preparation of Liposomes. Florida: CRC Press; 1984. p. 109-122.
10. Gregoriadis G, Neerunjun ED. Homing of liposomes to target cells. Biochem Biophys Res Commun. 1975; 65: 537-544.
11. Weissmann G, Bloomgerden D, Kaplan R, Cohen C, Hoffstein S, Collins T, Gotlieb A, Nagle D. A general method for introduction of enzymes, by means of immunoglobulin coated liposomes into lysosomes of deficient cells. Proc Natl Acad Sci. 1975; 72: 88-94.
12. Torchilin VP, Khaw BA, Smirnow VN, Haber E. Preservation of antimyosin antibody activity after covalent coupling to liposomes. Biochem Biophys Res Commun. 1979; 89: 1114-1119.
13. Huang A, Huang L, Kennel SJ. Monoclonal antibody covalently coupled with fatty acid. J Biol Chem. 1980; 255: 8015–8018.
14. Harsch M, Walther P, Weder HG, Hengartner H. Targeting of monoclonal antibody coated liposomes to sheep red blood cells. Biochem Biophys Res Commun. 1981; 103: 1069-1074.
15. Hashimoto Y, Sugawara M, Endoh H. Coating of liposomes with subunits of monoclonal IgM antibody and targeting of the liposomes. J Immunol Meth. 1983; 62: 155-162.
16. Tooaen PAHM, Crommelin DJA. Immunoglobulins as targeting agent for liposomes encapsulated drugs. Pharm Weekbl Sci. 1983; 5: 269-275.
17. Sugano M, Egilmez NK, Yokota SJ, Chen FA, Harding J, Huang SK, Bankert RB. Antibody targeting of doxorubicin-loaded liposomes suppresses the growth and metastatic spread of established human lung tumor xenografts in severe combined immunodeficient mice. Cancer Res. 2000; 60: 6942-6949.
18. Koning GA, Kamps JA, Scherphof GL. Efficient intracellular delivery of 5-fluorodeoxyuridine into colon cancer cells by targeted immunoliposomes. Cancer Detect Prev. 2002; 26: 299-307.
19. Moribe K, Maruyama K. Pharmaceutical design of the liposomal antimicrobial agents for infectious disease. Curr Pharm Des. 2002; 8: 441-454.
20. Maesaki S. Drug delivery system of anti-fungal and parasitic agents. Curr Pharm Des. 2002; 8: 433-440.
21. Hofheinz RD, Gnad-Vogt SU, Beyer U. Liposomal encapsulated anti-cancer drugs. Anticancer Drugs. 2005; 16: 691-707.
22. Glava M, Goracinova K, Calis S, Simonoska M, Hincal AA. 5-Fluorouracil in topical liposome

gels for anticancer treatment formulation and characterisation. Acta Pharm. 2003; 53: 241-250.
23. Yang T. Preparation and evaluation of paclitaxel-loaded PEGylated immunoliposome. J Control Rel. 2007; 120: 169-177.
24. Karki R, Subramanya G. Formulation and evaluation of coencapsulated rifampicin and isoniazid liposomes using different lipids. Acta Pharm Sciencia. 2009; 51: 177- 188.
25. El Maghraby GMM, Williams AC, Barry BW. Oestradiol skin delivery from ultradeformable liposomes: refinement of surfactant concentration. Int J Pharm. 2000; 196: 63-74.
26. Ganesan MG, Weiner ND, Flynn GL, Ho NFH. Influence of liposomal drug entrapment on percutaneous absorption. Int J Pharm. 1984; 20: 139-154.
27. Niemiec SM, Latta JM, Ramachandran C, Weiner ND, Roessler BJ. Perifollicular transgenic expression of human Interleukin-1 receptor antagonist protein following topical application of novel liposome-plasmid DNA formulations *in vivo*. J Pharm Sci. 1997; 86: 701-708.
28. Fleisher D, Niemiec SM, Oh CK, Hu Z, Ramachandran C, Weiner N. Topical delivery of growth hormone releasing peptide using liposomal systems: an *in vitro* study using hairless mouse skin. Life Sci. 1995; 57: 1293-1297.
29. Egbaria K, Ramachandran C, Kittayanond D, Weiner N. Topical delivery of liposomally encapsulated interferon evaluated by in-vitro diffusion studies. Antimicrob Agents Chemother. 1990; 34: 107-110.
30. Krowczynski L, Stozek T. Liposomes as drug-carriers in the transdermal therapy. Pharmazie. 1984; 39: 627-629.
31. Patel HM. Liposomes as a controlled-release system. Biochem Soc Trans. 1985; 13: 513-516.
32. Price CI, Horton JW, Baxter CR. Liposome delivery of amino glycosides in burn wounds. Surg Gynecol Obstet. 1992; 174: 414-418.
33. Price CI, Horton JW, Baxter CR. Topical liposomal delivery of antibiotics in soft tissue infection. J Surg Res. 1990; 49: 174-178.
34. Foldvari M, Gesztes A, Mezei M. Dermal drug delivery by liposome encapsulation: clinical and electron microscopic studies. J Microencapsul. 1990; 7: 479-489.
35. Patel RP, Patel HH, Baria AH. Formulation and evaluation of carbopol gel containing liposomes of ketoconazole. Int J Drug Deli Tech. 2009; 1: 42-45.
36. Wohlarb W, Lasch J. Penetration kinetics of liposomal hydrocortisone in human skin. Dermatologica. 1987; 174: 18-22.
37. Harsanyi BB, Hilchie JC, Mezei M. Liposomes as drug carriers for oral ulcers. J Dent Res. 1986; 65: 1133-1141.

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