



Liposomal Drug Delivery Systems – A Review

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ABSTRACT

Liposomes are spherical shaped lipoidal vesicles which are under extensive investigation as drug carriers for improving the bioavailability and delivery of therapeutic agents. Due to innovative developments in liposome technology, numerous liposome based drug delivery systems are currently in clinical trial, and recently some of them have been approved for clinical use. Formulation of drugs in liposomes has provided an opportunity to enhance the therapeutic indices of various agents mainly through alteration in their bio distribution. This review discusses the classification, formulation, characterization and potential applications of liposomes in drug delivery.

Keywords: Liposomes, phospholipids, formulation, characterization and applications.

INTRODUCTION

Liposomes are spherical shaped small vesicles that can be produced from cholesterol, non toxic surfactants, sphingolipids, glycolipids, long chain fatty acids and even membrane proteins. Phospholipids spontaneously form a closed structure when dissolved in water with internal aqueous environment bounded by phospholipids bilayer membranes, this vesicular system is called as liposome.¹ Liposomes are the drug carrier loaded with different variety of molecules such as small drug molecules, proteins, nucleotides and even plasmids.

Liposomes were first described in 1961 by British hematologist Dr. Alec D Bangham. Liposomes were discovered when Bangham and R. W. Horne were testing the institute's new electron microscope by adding negative stain to dry phospholipids. The resemblance to the plasma lemma and the microscope pictures served as the first real evidence for the cell membrane being a bilayer lipid structure. Liposome can be formulated and processed to differ in size, composition, charge and lamellarity.² Liposomal formulations of various therapeutic drugs have been commercialized.

CLASSIFICATION OF LIPOSOMES^{3,4,5}

The liposomes may be classified based on

1. Structure
2. Method of preparation
3. Composition
4. Conventional liposome
5. Specialty liposome

1. Classification Based on Structure

Vesicle Types with their Size and Number of Lipid Layers

Vesicle type	Abbreviation	Diameter Size	No. of Lipid Layers
Unilamellar	UV	All size ranges	One
Small Unilamellar	SUV	20-100nm	One
Medium Unilamellar	MUV	More than 100nm	One
Large Unilamellar	LUV	More than 100nm	One
Giant Unilamellar	GUV	More than 1.0 µm	One
Oligo lamellar	OLV	0.1-1.0 µm	Approx 0.5
Multi lamellar	MLV	More than 0.5 µm	5-25
Multi vesicular	MV	More than 1.0 µm	Multi compartmental structure

2. Based on Method of Preparation

Different Preparation Methods and the Vesicles Formed by these Methods

Preparation Method	Vesicle Type
Single or oligolamellar vesicle made by reverse phase evaporation	REV
Multilamellar vesicle made by reverse phase evaporation method	MLV-REV
Stable pluri lamellar vesicle	SPLV
Frozen and thawed multi lamellar vesicle	FATMLV
Vesicle prepared by extrusion technique	VET
Dehydration- Rehydration method	DRV



3. Based on Composition

Different Liposome with their Compositions

Type	Abbreviation	Composition
Conventional	CL	Neutral or negatively charge phospholipids and cholesterol
Fusogenic	RSVE	Reconstituted sendai virus envelops
pH sensitive	-	Phospholipids such as PER or DOPE with either CHEMS or OA
Cationic	-	Cationic lipid with DOPE
Long circulatory	LCL	Neutral high temp, cholesterol and 5-10% PEG, DSP
Immuno	IL	CL or LCL with attached monoclonal antibody or recognition sequences

4. Based Upon Conventional Liposome

- Natural lecithin mixtures
- Synthetic identical, chain phospholipids
- Liposome with Glycolipids

5. Based Upon Speciality Liposome

- Bipolar fatty acid.
- Antibody directed
- Methyl/ Methylene x- linked
- Lipoprotein coated
- Carbohydrate coated
- Multiple encapsulated

STRUCTURAL COMPONENTS OF LIPOSOMES

The main components of liposomes are

- Phospholipids.
- Cholesterol.

Phospholipids

Phospholipids are the major structural components of biological membranes. The most common phospholipid used in liposomal preparation is phosphatidylcholine (PC).

Phosphatidylcholine is an amphipatic molecule containing^{6,7}

- A hydrophilic polar head group, phosphocholine
- A glycerol bridge
- A pair of hydrophobic acyl hydrocarbon chains

Molecules of phosphatidylcholine are not soluble in water. In aqueous media they align themselves closely in planar bilayer sheets in order to minimize the unfavourable action between the bulk aqueous phase and the long hydrocarbon fatty chain. Then the sheets fold on themselves to form closed sealed vesicles.

There are several phospholipids that can be used for the liposome preparation such as Dilauryl phosphotidyl

choline (DLPC), Dimyristoyl phosphotidyl choline (DMPC), Dipalmitoyl phosphotidyl choline (DPPC), Distearoyl phosphotidyl choline (DSPC), Dioleoyl phosphotidyl choline (DOPC), Dilauryl phosphotidyl ethanolamine (DLPE), Dimyristoyl phosphotidyl ethanolamine (DMPE), Distearoyl phosphotidyl ethanolamine (DSPE), Dioleoyl phosphotidyl ethanolamine (DOPE), Dilauryl phosphotidyl glycerol (DLPG), Distearoyl phosphotidyl serine (DSPS).

Cholesterol

The role of cholesterol in formulation of liposomes was given below:⁸

- Incorporation of sterols in liposome bilayer produces major changes in the preparation of these membranes.
- Cholesterol itself does not form a bilayer structure.
- However, cholesterol acts as a fluidity buffer. It makes the membrane less ordered and slightly more permeable below the phase transition and makes the membrane more ordered and stable above the phase transition. It can be incorporated into phospholipid membranes in very high concentration up to 1:1 or even 2:1 molar ratios of cholesterol to phospholipids.

MECHANISM OF VESICLE FORMATION

Lipid vesicles are formed when thin lipid films are hydrated and swelled. The hydrated lipid sheets detach during agitation and form large MLV which prevents the interaction of water with the hydrocarbon core of the bilayer at the edges. Once the particles formed, it is size reduced by sonication or by extrusion.⁸

METHOD OF PREPARATION

I. Passive loading technique⁹⁻¹¹

1. Mechanical dispersion

Lipid Hydration Method

This is the common and most widely used method for the preparation of MLV. Round bottomed flask can be used for the preparation. The method involves formation of a thin film by drying the lipid solution and then hydrating the film by adding aqueous buffer and vortexing the dispersion. The hydration step is done at a temperature above the gel liquid crystalline transition temperature of the lipid or above the transition temperature of the highest melting component in the lipid mixture. Depending upon their solubilities the compounds to be encapsulated are added either to aqueous buffer or to organic solvent containing lipids. The disadvantages of the method include low internal volume, less encapsulation efficiency and varying size. The less encapsulation efficiency can be overcome by hydrating the lipids in presence of immiscible organic solvents like petroleum ether, diethyl ether. Then it is emulsified by sonication. MLVs are formed by removing organic layer by passing nitrogen.

Micro emulsification

This method is used for preparing small lipid vesicles in commercial quantities. This can be achieved by microemulsifying lipid compositions using high shearing stress generated from high pressure homogenizer. Microemulsion for biological applications can be produced by adjusting the speed of rotations from 20 to 200.

Sonication

In this method MLVs are sonicated either with a bath type sonicator or probe sonicator. The main drawbacks of this method are very low internal volume/encapsulation efficiency, degradation of phospholipids, exclusion of large molecules, metal contamination from probe tip and presence of MLV along with SUV.

French Pressure Cell Method

The method involves the extrusion of MLV through a small orifice at 20,000 psi at 4°C. The method has several advantages over sonication method. The method is simple, rapid, and reproducible and involves gentle handling of unstable materials. The resulting liposomes are larger than sonicated SUVs. The disadvantages include difficulty in achieving temperature and less working volume (about 50 mL maximum).

Membrane extrusion

In this method, suspension of heterogeneous size liposomes is passed through a polymer filter having a web-like construction providing a tortuous-path capillary pore, network of interconnected, and a membrane thickness of at least about 100 microns. The processed liposomes have a narrow size distribution and selected average size less than about 0.4 microns.

Dried reconstituted vesicles

In this method the preformed liposomes are added to an aqueous solution containing drug or mixed with a lyophilized protein, followed by dehydration of mixture.

Freeze-Thaw Method

In this method the SUVs are rapidly frozen, followed by slow thawing. The sonication disperses aggregated materials to LUV. The fusion of SUV during the processes of freezing and thawing leads to the formation of ULV. This type of fusion is strongly inhibited by increasing the ionic strength of the medium and by increasing the phospholipid concentration. The entrapment efficiencies of 20 to 30% were obtained by this method.

2. Solvent dispersion**Ethanol Injection Method**

A lipid solution of ethanol is rapidly injected to an excess of buffer, which leads to the immediate formation of MLVs. The major drawback of the method is that the particles may be with heterogeneous size distribution (30-

110 nm). Another drawback is removal of all ethanol is difficult, which may lead to form azeotrope with water.

Ether Infusion Method

A solution of lipids dissolved in diethyl ether or ether-methanol mixture is slowly injected to an aqueous solution of the drug, to be encapsulated at a temperature of 55-65°C under reduced pressure. The liposomes reformed by subsequent removal of ether under vacuum. The main drawbacks of the method are exposure of drugs and lipids to organic solvents and high temperature which may cause degradation. Further the size may vary from 70 -190 nm.

Double emulsification

In this method, a primary emulsion is prepared by dissolving the drug in an aqueous phase (w_1) which is then emulsified in an organic solvent of a polymer to make a primary w_1/o emulsion. This primary emulsion is further mixed in an emulsifier-containing aqueous solution (w_2) to make a $w_1/o/ w_2$ double emulsion. The removal of the solvent leaves microspheres in the aqueous continuous phase, which are collected by filtering/centrifuging.

Reverse-phase evaporation

The lipid mixture is taken in a round bottom flask followed by removal of solvent under reduced pressure by a rotary evaporator. The system is purged with nitrogen and the lipids are re-dissolved in the organic phase. The reverse phase vesicles will form in this phase. The usual solvents used are diethyl ether and isopropyl ether. Aqueous phase which contains drug to be encapsulated is added after the lipids are re-dispersed in this phase. The system is kept under continuous nitrogen and the two phase system is sonicated until the mixture becomes clear one-phase dispersion. The mixture is then placed on the rotary evaporator and the removal of organic solvent is done until a gel is formed followed by removal of non-encapsulated material. The resulting liposomes are called reverse-phase evaporation vesicles.

3. Detergent removal

Lipids are solubilised by the detergents at their critical micellar concentrations. The micelles become progressively richer in phospholipid as the detergent is removed by dialysis and finally combine to form LUVs. The advantages of detergent dialysis method are outstanding reproducibility and production of liposome populations of homogenous size. The main drawback of the method is the retention of detergent contaminants.

II. Active loading technique¹²⁻¹⁴**Proliposome**

Lipid and drug are coated onto a soluble carrier to form free-flowing granular material in pro-liposome which forms an isotonic liposomal suspension on hydration. The pro-liposome approach may provide an opportunity for

cost-effective large scale manufacture of liposomes containing particularly lipophilic drugs.

Lyophilization

The removal of water from products in the frozen state at extremely reduced pressure is called lyophilization (freeze drying). The process is generally used to dry products that are thermolabile which may be destroyed by heat-drying. This technique has a great potential to solve long term stability problems with respect to liposomal stability. Leakage of entrapped materials may take place during the process of freeze- drying and on reconstitution.

SIZING OF LIPOSOMES

Size characteristics of liposome have a major effect on the application they can be used. Physical integrity and stability of lipid bilayers structure influence the therapeutic applications of liposome. Therefore particle size of the liposome must be considered for the liposome production procedure and it must be predictable and reproducible with particle size distribution within a certain size range. Sequential extrusion, gel chromatography and sonication are the common methods of sizing of liposomes,¹⁵

But ultimately these methods have the following disadvantages:

1. Exclusion of oxygen is difficult which result in per oxidation reaction.
2. Titanium probes shed metal particle resulting in contamination.
3. They can generate aerosols, which exclude them from use with certain agents.

These above problems are mainly related with the probe sonication but these problems can be removed by using the bath sonication.

CHARACTERIZATION OF LIPOSOMES¹⁶⁻¹⁸

Liposome should be characterized for visual appearance, turbidity, size distribution, lamellarity, concentration, composition, presence of degradation products, and stability. The behaviour of liposomes in both physical and biological system is governed by these factors; therefore liposomes are characterized for physical attributes and chemical compositions.

A. Biological characterization

- Sterility - Aerobic/anaerobic culture
- Pyrogenicity - Temperature (Rabbit) response
- Animal toxicity - Monitoring survival of animals (rats)

B. Chemical characterization

- Phospholipids concentration - HPLC/Barrlet assay
- Cholesterol concentration - HPLC / cholesterol oxide assay

- Drug concentration - Assay method
- Phospholipids peroxidation - UV observance
- Phospholipids hydrolysis - HPLC/ TLC
- Cholesterol auto-oxidation - HPLC/ TLC
- Anti-oxidant degradation - HPLC/TLC
- pH - pH meter
- Osmolarity - Osmometer

C. Physical Characterization

- Vesicle shape, and surface morphology – SEM / TEM
- Vesicle size and size distribution - Dynamic light scattering, TEM.
- Surface charge - Free flow electrophoresis
- Electrical surface Potential and pH - Zeta potential and pH sensitive probes
- Lamellarity - NMR
- Phase behaviour - DSC, freeze fracture electron microscopy
- Percent capture - Mini column centrifugation, gel exclusion
- Drug release - Diffuse cell / dialysis

1. Visual Appearance

Based on the particle size and composition the appearance of the liposomal suspension may be varying from translucent to milky. The samples are homogeneous if the turbidity has a bluish shade; the presence of a nonliposomal dispersion is by flat, grey colour and is most likely a disperse inverse hexagonal phase or dispersed micro crystallites. An optical microscope can detect liposome of size greater than 0.3 μm as well as contamination with larger particles.

2. Determination of Liposomal Size

Size Distribution

It is usually measured by dynamic light scattering. Liposomes with relatively homogeneous size distribution are reliable for this method. Gel exclusion chromatography is a simple method, in which a truly hydrodynamic radius can be detected. Sephacryl-S100 can separate liposome in size range of 30-300nm. Sepharose -4B and -2B columns can separate SUV from micelles.

3. Determination of lamellarity

The lamellarity of liposomes can be measured by electron microscopy or spectroscopic techniques. The NMR spectrum of liposome is recorded most frequently with and without the addition of a paramagnetic agent that shifts or bleaches the signal of the observed nuclei on the outer surface of liposome.



4. Liposome Stability

Liposome should be physically, chemically, and biologically stable. Physical stability indicates the ratio of lipid to therapeutic agent and steadiness of the size. The chemical stability may be affected by two degradation pathways, oxidative and hydrolytic. Oxidation of phospholipids in liposomes mainly takes place in unsaturated fatty acyl chain-carrying phospholipids. These chains are oxidised in the absence of particular oxidants. Reduction of oxidation can be achieved by storage at low temperatures and protection from light and oxygen.

5. Entrapped Volume

The entrapped volume of liposome (in µL/ mg phospholipids) can often be deduced from measurements of the total quantity of solute entrapped inside liposome assuring that the concentration of solute in the aqueous medium inside liposomes is the same after separation from untrapped material. For example, in two phase method of preparation, water can be lost from the internal compartment during the drying down step to remove organic solvent.

$$\% \text{ Entrapment Efficiency} = \frac{\text{Entrapped drug}}{\text{Total added drug}} \times 100$$

6. Surface Charge

Liposomes are usually prepared using charge imparting / constituting lipids and hence it is imparting to study the charge on the vesicle surface. The two methods used in general to assess the charge are free flow electrophoresis and zeta potential measurement.

STABILIZATION OF LIPOSOME

Usually liposomes may create problem in stability during the storage period. In general certain parameters should be considered to achieve successful formulation of stable liposomal drug product:¹⁹

- Processing with fresh, purified lipids and solvents.
- Avoidance of high temperature and excessive shearing stress.
- Maintenance of low oxygen potential
- Use of antioxidant or metal chelators.
- Formulating at neutral pH.
- Use of lyo-protectant when freeze drying.

ENTRAPMENT OF DRUGS INTO LIPOSOME BILAYERS

Liposomes, because of their biphasic character, can act as carrier for both lipophilic and hydrophilic drugs. Depending upon their solubility and partitioning characteristics, the drug molecules are located differently in the liposomal environment and exhibit different entrapment and release properties.²⁰

Lipophilic drugs	Hydrophilic drugs	Amphiphilic drugs	Biphasic insoluble drugs
High Entrapment Low Leakage	Low Entrapment Bilayer Composition Dependent Leakage	High Entrapment Rapid Leakage	Poor Entrapment
Chemical Stability	Hydrolytic Degradation		

APPLICATIONS OF LIPOSOMES

Liposomes have great pharmaceutical applications in oral and transdermal drug delivery systems. Reduction in the toxic effect and enhancement of the effectiveness of drugs are achieved by this drug delivery system. The targeting of liposome to the site of action takes place by the attachment of amino acid fragment that target specific receptors cell. Several modes of drug delivery application have been proposed for the liposomal drug delivery system, few of them are as follows:

1. Enhancement of solubilisation (Amphotericin-B, Paclitaxel)
2. Protection of sensitive drug molecules (Cytosine arabinosa, DNA, RNA, Ribozymes)
3. Enhancement of intracellular uptake (Anticancer, anti-viral and antimicrobial drugs)
4. Alteration in pharmacokinetics and bio-distribution (prolonged or SR drugs with short circulatory half life)

Several recent applications of liposomal drug delivery system are as follows

A. Liposome for Respiratory Drug Delivery System²¹

Liposome is widely used in several types of respiratory disorders. Liposomal aerosols can be formulated to achieve sustained release, prevent local irritation, reduced toxicity and improved stability. Whilst preparing liposomes for lung delivery, composition, size, charge, drug/lipid ratio and drug delivery method should be considered. The liquid or dry form is taken for the inhalation during nebulisation. Drug powder liposome is produced by milling or by spray drying.

B. Liposome in Eye Disorders²²

Liposome has been used widely to treat disorders of eye. The disease of eye includes dryness, keratitis, corneal transplant rejection, endophthalmitis and proliferative vitreo retinopathy. Retinal diseases are important cause of blindness. Liposome is used as vector for genetic transfection and monoclonal antibody directed vehicle. Applying of focal laser to heat induced release of liposomal drugs and dyes are the recent techniques of the treatment of selective tumour and neo-vascular vessels occlusion, angiography, retinal and choroidal blood vessel stasis.

C. Liposome as Vaccine Adjuvant^{23,24}

Liposome has been established firmly as immuno-adjuvant that is potentiating both cell mediated and non-cell mediated immunity. Liposomal immuno-adjuvant acts



by slow release of encapsulated antigen on intramuscular injection and also by passive accumulation within regional lymph node. The accumulation of liposome to lymphoid is done by the targeting of liposome with the help of phosphotidyl serine. Liposomal vaccine can be prepared by inoculating microbes, soluble antigen and cytokines of deoxyribonucleic acid with liposome.

D. Liposomes for Brain Targeting²⁵⁻²⁷

The biocompatible and biodegradable character of liposomes makes its used in brain drug delivery system. Liposomes with a small diameter (100 nm) and large diameter undergo free diffusion through the BBB. However small unilamellar vesicles (SUVs) coupled to brain drug transport vectors may be transported by receptor mediated or absorptive mediated transcytosis through the BBB. Cationic liposomes undergo absorptive mediated endocytosis into cells whereas the same undergoing absorptive mediated transcytosis through the BBB has not yet been determined. Liposomes coated with the mannose reach brain and assist transport of loaded drug through BBB. The neuropeptides, leu-enkephalin and mefenkephalin normally do not cross BBB when given systemically. The anti depressant amitriptyline normally penetrate the BBB, due to versatility of this method.

E. Liposome as Anti-Infective Agents²⁸⁻³⁰

The diseases like leishmaniasis, candidiasis, aspergilosis, histoplasmosis, erythrocytosis, gerardiasis, malaria and tuberculosis can be treated by incorporating and targeting the drug using liposomal carrier.

F. Liposome in cancer therapy^{31,32}

All cancer drugs on long term usage produce stern toxic effects. The liposomal approach causes targeting of drug to tumour with lesser toxic effects. The small and stable liposome is passively targeted to different tumour because they can circulate for longer time. Nowadays many anti-cancer herbal drugs also formulated into liposomes to provide better targeting with enhanced bioavailability.

CONCLUSION

Liposomes are one of the classical specific drug delivery system, which can be used for controlled and targeted action. These systems can be administered through oral, parenteral as well as topical route. This wide range of selection of route of administration makes its flexible in designing the drug delivery system. Also these systems provide as an effective carrier for cosmetic formulations also. The major problem in the formulation of liposome is its stability problem. These problems can be overcome by employing modification in the preparation method and also by using some specialized carriers. Nowadays liposomes are used as carrier for wide variety of drugs. In spite of its few disadvantages liposomes serve as versatile carrier for wide range of drugs.

REFERENCES

1. Ravichandiran V, Masilamani K, Senthilnathan B. Liposome- A Versatile Drug Delivery System. *Der Pharmacia Sinica*, 2(1), 2011, 19-30.
2. Torchilin VP. Liposomes as pharmaceutical carriers. *Nature reviews/Drug discovery*, 4, 2005, 145-160.
3. Sultana ASY, Aqil M. Liposomal Drug Delivery Systems, 4, 2007, 297-305.
4. Riaz M. Liposome preparation method. *Pakistan Journal of Pharmaceutical Sciences*, 1, 1996, 65-77.
5. Gregoriadis G, Florence AT. Liposomes in drug delivery, Clinical, diagnostic and ophthalmic potential, *Drugs*, 45, 1993, 15-28.
6. A.D. Bangham, M.M. Standish, J.C. Watkins, Diffusion of univalent ions across the lamellae of swollen phospholipids, *J. Mol. Biol.* 13, 1965, 238-252.
7. McIntosh TJ, The effect of cholesterol on the structure of phosphatidylcholine bilayers, *Biochim. Biophys. Acta*, 513, 1978, 43-58.
8. P.R. Cullis, Lateral diffusion rates of phosphatidylcholine in vesicle membranes, effects of cholesterol and hydrocarbon phase transitions, *FEBS Lett.* 70, 1976, 223-228.
9. Martin, F.J., Pharmaceutical manufacturing of liposomes. In, Tyle, P. (Ed.), *Specialized Drug Delivery System, Manufacturing and Production Technology*, Marcell Dekker, New York, 1990, 267-316.
10. Gregoriadis G. Engineering liposomes for drug delivery, progress and problems. *Trends Biotechnol.* 13, 1995, 527-537.
11. Amarnath Sharma, Uma S.Sharma, Elsevier- Liposomes in drug delivery, progress and limitations, *International Journal of Pharmaceutics*, 1997, 123-140.
12. Winden EC. Freezy-drying of liposomes, theory and practice. *Meth. Enzymol.* 367, 2003, 99-110.
13. Lo, Y. L., Tsai, J. C. & Kuo, J. H. Liposomes and disaccharides as carriers in spray-dried powder formulations of superoxide dismutase. *J. Control. Release* 94, 2009, 259-272
14. Alving CR. Liposomes as carriers of antigens and adjuvants. *J. Immunol. Methods*, 140, 1991, 1-13.
15. Koshkina, N. V., Golunski, E., Roberts, L. E., Gilbert, B. E. & Knight, V. Cyclosporin A aerosol improves the anticancer effect of paclitaxel aerosol in mice. *J. Aerosol Med.* 17, 2004, 7-14.
16. Ehrlich, P. The relations existing between chemical constitution, distribution and pharmacological action. *Collected Studies on Immunity*, Vol. 2, Wiley, New York, 1906, 404-443.
17. Sawant RR, Torchilin VP. Challenges in development of targeted liposomal therapeutics, *AAPSJ*, 14 (2), 2012, 303-315.
18. Torchilin, V. P. Liposomes as targetable drug carriers. *CRC Crit. Rev. Ther. Drug Carrier Syst*, 1, 1985, 65-115.



19. Taira, M. C., Chiaramoni, N. S., Pecuch, K. M. & Alonso-Romanowski, S. Stability of liposomal formulations in physiological conditions for oral drug delivery. *Drug Deliv.* 11, 2004, 123–128.
20. G. Gregoriadis, Drug entrapment in liposomes, *FEBS Lett.* 36, 1973, 292–296.
21. Blume G, Cevc G. Liposomes for the sustained drug release in vivo, *Biochim. Biophys. Acta*, 1029, 1990, 91–97.
22. Abra RM, Bosworth ME, Hunt CA. Liposome disposition in vivo, effects of pre-dosing with liposomes, *Res. Commun. Chem. Pathol. Pharmacol*, 29, 1980, 349–360.
23. A.G. Allison, G. Gregoriadis, Liposomes as immunological adjuvants, *Nature*, 252, 1974, 252.
24. D.S. Watson, A.N. Endsley, L. Huang, Design considerations for liposomal vaccines, influence of formulation parameters on antibody and cell-mediated immune responses to liposome associated antigens, *Vaccine*, 30, 2012, 2256–2272.
25. Monica Gulati, Manish Grover, Saranjit Singh, Mandip Singh, Lipophilic drug derivatives in liposomes, *International Journal of Pharmaceutics*, 165, 1998, 129–168.
26. Lokling KE, Fosshem SL, Klaveness J, Skurtveit R. Biodistribution of pH-responsive liposomes for MRI and a novel approach to improve the pH-responsiveness. *J. Control. Release*, 98, 2004, 87–95.
27. M.B. Yatvin, W. Kreutz, B.A. Horwitz, M. Shinitzky, pH sensitive liposomes, possible clinical implications, *Science*, 210, 1980, 1253–1255.
28. Sundar, S. et al. Single-dose liposomal amphotericin B in the treatment of visceral leishmaniasis in India, a multicentre study. *Clin. Infect. Dis.* 37, 2003, 800–804.
29. Jolck RI, Feldborg LN, Andersen S, Moghimi SM, Andresen TL. Engineering liposomes and nanoparticles for biological targeting, *Adv. Biochem. Eng. Biotechnol*, 125, 2011, 251–280.
30. Alving CR, Steck EA, Chapman WL, Waits VB, Hendricks LD, Therapy of leishmaniasis, superior efficacies of liposome encapsulated drugs, *Proc. Natl. Acad. Sci. U.S.A.*, 75, 1978, 2959–2963.
31. Klibanov AL, Maruyama K, Torchilin VP, Huang L. Amphipatic polyethylene glycols effectively prolong the circulation time of liposomes. *FEBS Lett.* 268, 1990, 235–238.
32. V.P. Torchilin, Recent approaches to intracellular delivery of drugs and DNA and organelle targeting, *Annu. Rev. Biomed. Eng.* 8, 2006, 343–375.

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