IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Short Communication
May 2020 Vol.:18, Issue:2

Liposomes: A Novel Drug Delivery System

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

R. R. Thenge, M. P. Chandak*, V. S. Adhao

Dr. Rajendra Gode College of Pharmacy, Malkapur-443101, Maharashtra, India.

Submission: 23 April 2020
Accepted: 01 May 2020
Published: 30 May 2020

www.ijppr.humanjournals.com

Keywords: Liposomes, Method of preparation of liposome, Evaluation of liposome, Novel drug delivery system

ABSTRACT

The discovery of liposome or lipid vesicle emerged from self-forming enclosed lipid bi-layer upon hydration; liposome drug delivery systems have played a significant role in the formulation of potent drugs to improve therapeutics. Liposomes are spherical vesicles composed of one or more lipid bilayers, involving an aqueous compartment. The term liposome means lipid body. It has been derived based on the name of subcellular particles, ribosomes. Liposomes were first made by A.D. Bangham in the early 1960s. Their size ranges from 25to 500nm. Today, they are a very useful tool in various scientific disciplines, including chemistry, colloid science, biochemistry, biology & pharmaceutical science. Along with many new drug delivery systems, liposomes distinguish and advanced technology to transport active molecules to the site of action, and at present, several dosage forms are in clinical use. This paper summarizes exclusively focuses on classification, methods of preparations, stability, and applications concerning liposomal drug formulations.

INTRODUCTION

Paul Ehrlich in 1906 initiated the era of development for targeted delivery.[1]When phospholipids are dispersed in water, they spontaneously form a closed structure with an internal aqueous environment bounded by phospholipid bilayer membranes, this vesicular system is called a liposome.[2]The name liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body. Liposomes are microparticulate or colloidal carriers, usually 0.05- 5.0 µm in diameter which forms spontaneously when certain lipids are hydrated in aqueous media.[3]Liposomes are the small vesicle of spherical shape that can be produced from cholesterols, non-toxic surfactants, sphingolipids, glycolipids, long-chain fatty acids, and even membrane proteins. Liposomes are the drug carrier loaded with a great variety of molecules such as small drug molecules, proteins, nucleotides, and even plasmids.[2]Liposomes were first produced in England in 1961 by Alec D. Bangham.[4] The sphere-like shell encapsulated a liquid interior that contains substances such as peptides and protein, hormones, enzymes, antibiotics, anti-fungal, and anticancer agents.[5]Techniques like membrane extrusion, sonication, homogenization, and/or freeze-thawing are being employed to control the size and size distribution. Liposomes can be formulated and processed to differ in size, composition, charge, and lamellarity.[6] Although liposomes have been extensively studied as promising carriers for therapeutically active compounds, some of the major drawbacks for liposomes used in pharmaceutics are the rapid degradation due to the reticuloendothelial system(RES)and inability to achieve sustained drug delivery over a prolonged period.[7]Liposomal drug delivery is gaining interest due to its contribution to varied areas like drug delivery, cosmetics, and the structure of the biological membrane.[8]There are several mechanisms by which liposomes act within and outside the body which are as follows: 1) Liposome attaches to the cellular membrane and appears to fuse with them, releasing their content into the cell. 2) Sometimes they are taken up by the cell and their phospholipids are incorporated into the cell membrane by which the drug trapped inside is released. 3)In the case of phagocyte cell, the liposomes are taken up, the phospholipid walls are acted upon by organelles called lysosomes and the active pharmaceutical ingredients are released. [2]

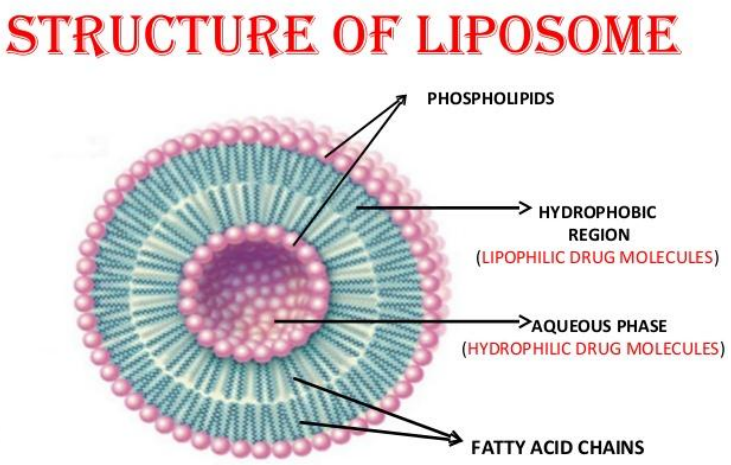

Figure No. 1: Structure of Liposomes

Advantages of liposome: [1,6,8,9,10,11,12,13]

- Stability increased if liposome prepared via encapsulation.
- Liposomes increased the efficacy and therapeutic index of a drug (actinomycin-D).
- Liposomes reduce the toxicity of the encapsulated agent (amphotericin B, Taxol).
- Liposomes help reduce the exposure of sensitive tissues to toxic drugs.
- Site avoidance effect.
- Liposomes are flexible, non-toxic, biocompatible, completely biodegradable, and non-immunogenic for systemic and non-systemic administrations.
- Flexibility to couple with site-specific ligands to achieve active targeting.
- Suitable for delivery of hydrophobic, amphipathic, and hydrophilic drugs.

Disadvantages of liposome:[1,4,7,9,10,11,12,13,14]

- Short half-life.
- Low solubility.

- Leakage and fusion of encapsulated drug/ molecules.
- The production cost is high.
- Sometimes phospholipids undergo oxidation and hydrolysis-like reaction.
- Time-consuming procedure.
- Less stability.
- Allergic reactions may occur to liposomal constituents.

Methods of liposome preparation:

The main goal of an ideal method of liposome formulation is to obtain efficient drug entrapment, narrow particle size distribution, and long-term stability of liposome products.

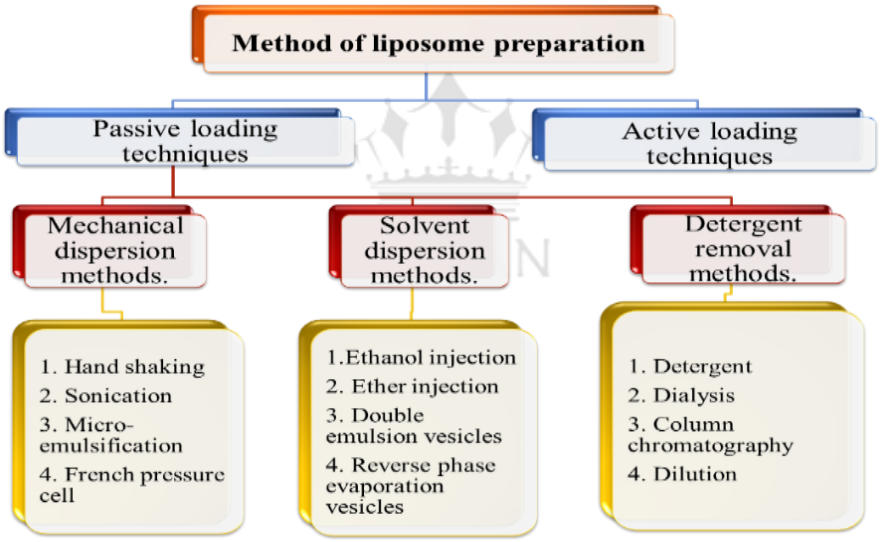

Figure No. 2: Methods of liposome preparation

1. Mechanical dispersion methods:

Liposomes are produced when thin lipid films or lipid cakes are hydrated and stacks of liquid crystalline bilayers become fluid and swell. The hydrated lipid sheets cut off from lipid film during agitation and self-close to form large, multilamellar vesicles (LMV), which prevents interaction of water with the hydrocarbon core of the bilayer at the edges. once these particles have formed, particle size reduction requires energy input in the form of sonic energy (sonication)or mechanical energy (extrusion).

Lipid film hydration by handshaking:

Initially a mixture of phospholipid and cholesterol was dispersed in the organic solvent. Afterward, the organic solvent was removed by evaporation generally a Rotary Evaporator is used at reduced pressure. Finally, the dry lipidic film formed on the flask wall was hydrated by the addition of an aqueous buffer solution under agitation at a temperature above the lipid transition temperature. This method is most popularly used and easy to handle, dispersed-phospholipids in aqueous buffer produce a population of multilamellar liposomes (MLVs) that differ both in size and shape (1–5nm diameter).

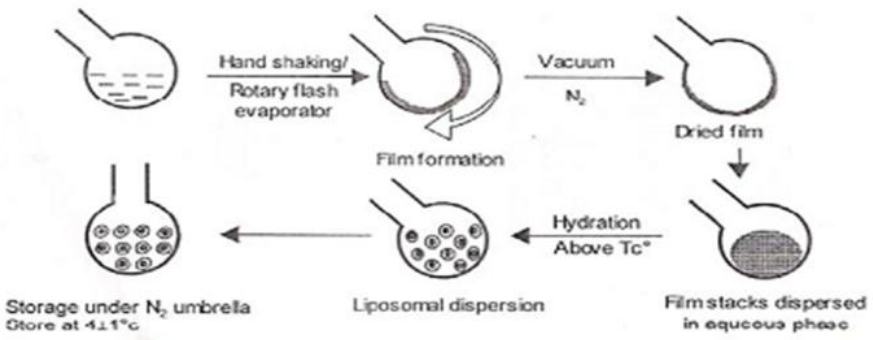

Figure No. 3: Multilaminar vesicles (MLVs) formed by handshaking technique [15,16]

Sonication method: [15,17,18,19,20,21,22,23,24,25,26]

The sonication method is based on size transformation and involves the subsequent sonication of MLVs prepared by the thin-film hydration method, using sonic energy usually under an inert atmosphere including nitrogen or argon. The sonication method enables homogenous dispersion of small vesicles using bath type or probe-type sonicator with a potential for greater tissue penetration. The probe tip sonicator delivers high energy to the lipid suspension. The possibility of overheating of the lipid suspension causes degradation. Sonication tips tend to release titanium particles into the lipid suspension which must be removed by centrifugation before use. The bath sonicators are the most widely used instrumentation for the preparation of SUV. They are used for a large volume of dilute lipids. The oxidation of unsaturated bonds in the fatty acid chains of phospholipids and hydrolysis to lysophospholipids and free fatty acids, as well as denaturation of thermolabile substances and very low encapsulation efficiency of the internal volume, are the main drawbacks of the method.

French Pressure Cell Method:[27]

The method involves the extrusion of MLV at 20,000 psi at 4°C through a small orifice. The method has several advantages over the sonication method. The method is simple, rapid, reproducible, and involves the gentle handling of unstable materials (Hamilton and Guo, 1984). The resulting liposomes are somewhat larger than sonicated SUVs. The drawbacks of the method are that the temperature is difficult to achieve and the working volumes are relatively small (about 50 mL maximum).

2. Solvent dispersion method:

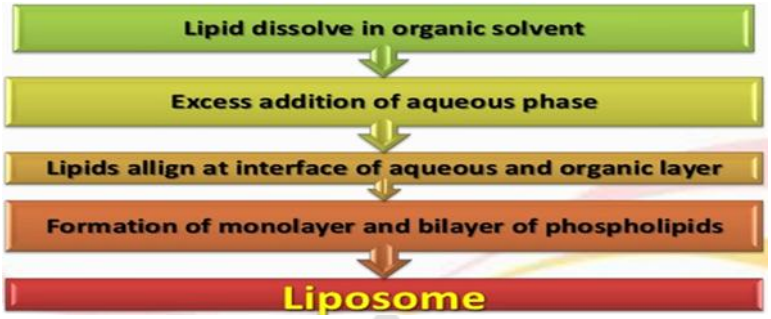

Figure No. 4: Solvent dispersion method

Ethanol Injection Method: [26,28,29]

In the ethanol injection method the ethanolic lipid solution is rapidly injected to a vast excess of preheated distilled water or TRIS-HCl buffer5. The incorporation of the drug in liposomal vesicles depends on its hydrophilic/hydrophobic character. Nimesulide as a lipid-soluble component incorporates better in liposomes than 5-fluorouracil which migrates to the external aqueous phase. The main advantage of the ethanol injection method is including of non-harmful solvent as ethanol, as well as easy scale-up of the method. The possibility of the formation of an azeotrope with water reduces its applicability.

Ether Injection Method:[26,29,30]

In the ether injection method a solution of lipids is dissolved in ether or diethyl ether/methanol mixture which is slowly injected to an aqueous solution of the material to be capsulated. The subsequent removal of the organic solvent under reduced pressure leads to the formation of liposomes. The main disadvantage of the method is the heterogeneous

population and the exposure of compounds to be encapsulated to organic solvents or high temperatures.

Reverse phase evaporation vehicle:[15,16]

The mixture of two phases is subjected to bath sonication. The droplets thus formed are dried down to a semisolid gel in a rotary evaporator under reduced pressure. The monolayers of phospholipids at this stage surround each water compartment, which is closely opposed to each other. This is followed by mechanical shaking with the help of a vortex shaker causing the collapse of certain water droplets during this process the lipid monolayer, which enclosed the collapsed vesicle, becomes the part of the adjacent vesicle to form outer leaflet of the bilayer of LUV. The dispersion medium for these newly formed liposomes is provided by the aqueous content of collapsed droplets.

3. Detergent removal method:[15,31]

The method involves intimate contact of phospholipids with the aqueous phase through detergent, which links with phospholipid molecules and act as divider of the hydrophobic portions of the molecules from water. The configuration created as a result of this union is recognized as micelles. The conc. of detergent at which micelles are formed is called as CMC.

Column chromatography: [16,17,18]

1) Phospholipids in the form of either sonicated vesicles or as a dry film, at a molar ratio of 2:1 with deoxycholate form unilamellar vesicles of 100 nm on the removal of deoxycholate by column chromatography.

2) This could be achieved by passing the dispersion over a Sephadex G-25 column pre-saturated with constitutive lipids and pre-equilibrated with a hydrating buffer.

Evaluation of liposomes:[1,8,32,33]

1. Particle size analysis:

One drop of the Liposomal formulation was homogeneously spread onto a glass slide and left to dry overnight. After platinum coating the sample with a Polaron E5100 sputter coater (Polaron, England), the samples were observed under a Philips 505 scanning electron

microscope at an accelerating voltage of 20 kV. Photographs were taken at 70, 100, 200, and 300 magnifications wherever necessary.

2. Drug entrapment studies:

To aliquots of liposome sample (0.5 ml), 5 ml of 10% sodium lauryl sulfate (SLS) was added and the volume was made up to 50 ml. The sample was warmed on a water bath at 70°C for 30 min. Similarly, a blank liposome (without drug) suspension (0.5 ml), 5 ml of 10% SLS were taken in a 50 ml volumetric flask and the volume was made up with distilled water. The blank was warmed on a water bath at 70°C for 30 min. The absorbance of the test solution was taken in a UV spectrophotometer at 263 nm against the blank solution.

3. Percentage of entrapment efficiency:

It was determined by using the ratio of the entrapped drug (mg) to the total drug (mg), which may be expressed by the following formula.

Entrapment efficiency = amount of drug entrapped /total amount of drug X100

4. *In-vitro* drug release study from liposomes:

Concentrated liposomal suspension, 0.5 ml was taken in a test tube of an opening diameter of 20 mm. The open end was covered with a semi-permeable dialysis membrane and tied with a thread. The test tube was inverted and placed over the surface of 100 ml water present in a 250 ml beaker in such a way that the membrane just touched the water surface. The test tube was secured by a clamp fixed with a stand. The water in the beaker was stirred with a magnetic stirrer so that no vortex could form in the beaker. The temperature was maintained at 37 °C. The drug released from the liposomes permeates across the membrane and enters into the receptor chamber medium. Samples of 2 ml were taken out from the receptor chamber medium, suitably diluted, and the absorbances were taken by UV-spectrophotometer at 263 nm against a blank of fresh medium. At the same time, 2 ml of fresh medium was added to the beaker to keep the volume of the medium constant in the beaker.

5. Stability test:

The behavior of the liposome to retain the drug was studied by storing the liposome at 4 to 8 ° C (refrigerator RF) for a period of 1 mo. The liposomal preparations were kept in sealed vials.

Application of liposomes:[1,2,4,7,9,11, 15,34]

- In gene delivery.
- As drug delivery carriers.
- Enzyme replacement therapy.
- Liposomes in anti-viral / anti-microbial therapy.
- In multidrug resistance.
- In Tumour therapy.
- In Immunology.
- In Cosmetology.
- Liposomes as vaccine carriers.
- Liposomes as artificial blood Surrogates.
- Liposomes as radiopharmaceuticals and radio diagnostic carriers.

Table No. 1: Drug formulated in the form of liposomes

Sr. No.	Drug	Liposome method	Use	Ref No.
1.	Doxorubicin	Lipid-film hydration method and extrusion	Cancer activity	35
2.	Tamoxifen	Thin-film hydration method	Cancer activity	36
3.	Vinblastine sulfate	Thin-film hydration method and sonication	Cancer activity	37
4.	Amphotericin B	Thin-film hydration method	Antifungal activity	38
5.	Clotrimazole	Rotary evaporation method	Antifungal activity	39
6.	Fluconazole	Thin-film hydration method	Antifungal activity	40
7.	5-fluorouracil	Lipid-film hydration method, extrusion, ethanol injection and reverse-phase evaporation	Cancer activity	28
8.	Amikacin	Reverse phase evaporation method	Antibiotic drugs	41
9.	Mafenide acetate	Solvent evaporation and microencapsulation	Antibiotic drugs	42
10.	Sirolimus	Thin-film hydration method	Immunosuppressive activity	43
11.	Tacrolimus (Fk-506)	Thin-film hydration method	Immunosuppressive activity	44
12.	Ketorolac tromethamine	Thin-film hydration method	Analgesic activity	45
13.	Nimesulide	Ethanol injection method and rotary evaporation method	Analgesic activity	29
14.	Acetazolamide	Reverse phase evaporation and thin-film hydration method	Ophthalmic activity	23
15.	Brimonidine tartrate	Thin-film hydration method and sonication	Ophthalmic activity	18
16.	Tranexamic acid	Chloroform film and sonication	Antifibrinolytic activity	46
17.	Cyproterone acetate	Thin-film hydration method	Hormone activity	47
18.	Sodium glycocholate	Reverse phase evaporation	Potential drugs as oral insulin	48
19.	Metformin hydrochloride	Thin-film hydration method MLV	Potential drugs as oral insulin	49

REFERENCES

1. Kant Shashi et al "A complete Rev on Liposomes" IRJP,2012;3(7):10-16.
2. Abdus Samad, Y. Sultana and M. Aqil "Liposomal Drug Delivery Systems: An Update Rev Current Drug Delivery, 2007; 4: 297-305.
3. Sharma Shailesh, Sharma Neelam, Kumar Sandeep, Gupta GD "Liposomes: A Rev" J. of Pharm Res 2009;2(7):1163-1167.
4. Maluta S. Mufamadi et al "A Rev won Composite Liposomal Technologies for Specialized Drug Delivery" J. of Drug Delivery,2011:1-19.
5. Mansoori M et al "A Rev on Liposome" IJARPB, 2012; 2 (4):453-464.
6. Saraswathi Marripati et al "A Rev on Liposome" Int J of Res in Pharmaceutical and Nano Sciences, 2014;3(3):159 - 169.
7. Upendra Bulbake et al "Liposomal Formulations in Clinical Use: An Updated Rev" Pharm 2017; 9(12):1-33.
8. Sandeep Kalepu et al "Liposomal drug delivery system - A Comprehensive Rev" Int. J. Drug Dev. & Res., 2013;5(4):62-75.
9. Devender Sharma et al "An Updated Rev on Liposomes as drug delivery system" PharmaTutor, 2018;6(2):49-62.
10. MU Uhumwangho and RS Okor "Current trends in the production and biomedical applications of liposomes: A Rev" J of Medicine and Biomedical Res, 2005; 4(1): 9-21.
11. Abolfazl Akbarzadeh et al "Liposome: Classification, Preparation, and Applications" Nanoscale Res, 2013;8:1-9.
12. A.V. Yadav et al "Stability Aspects of Liposomes" Ind J Pharm Edu Res, 2011;45(4):402-413.
13. Mehran Alavi et al "Application of Various Types of Liposomes in Drug Delivery Systems" Adv Pharm Bull, 2017;7(1):3-9.
14. Amol R. Bodke et al "A Rev on Liposomes" Int J of Pharm and Chemical Res, 2017;3(2):120-127.
15. N.K. Jain Introduction to Novel Drug Delivery Systems Vallabh Prakashan 2010:154-174.
16. Olga Popovska et al "An Overview: Methods for Preparation and Characterization of Liposomes as Drug Delivery Systems" Int J of Pharm and Phytopharmacological Res 2013; 3 (3): 182-189.
17. Hwang TL, Lee WR, et al "Cisplatin encapsulated in phosphatidylethanolamine liposomes enhances the in vitro cytotoxicity and in vivo intratumor drug accumulation against melanomas" J Dermatol Sci, 2007, 46: 11-20.
18. Prabhu P, Kumar N, et al "Preparation and evaluation of liposomes of brimonidine tartrate as an ocular drug delivery system" Int J Res Pharm Sci, 2010, 1(4): 502-508.
19. Lopes LB, Scarpa MV, et al "Interaction of sodium diclofenac with freeze-dried soya phosphatidylcholine and unilamellar liposomes" Braz J Pharm Sci, 2006, 42 (4): 497-504.
20. Da Costa CAM, Moraes AM "Encapsulation of 5-fluorouracil in liposomes for topical administration" Maringa, 2003, 25 (1): 53-61.
21. Jadhav MP, Nagarsenker MS, et al "Formulation and evaluation of long-circulating liposomal amphotericin B: a scinti-kinetic study using 99mTc in BALB/C mice" Indian J Pharm Sci, 2011, 73 (1): 57-64.
22. Makhmalzadeh BS, Azh Z, et al "Preparation and evaluation of mafenide acetate liposomal formulation as eschar delivery system" Int J Drug Dev & Res, 2011, 3 (4): 129-140.
23. Hathout RM, Mansour S, et al "Liposomes as an ocular delivery system for acetazolamide: in vitro and in vivo studies" AAPS PharmSciTech, 2007, 8 (1): E1-E12.
24. Rathod S, Deshpande SG "Design and evaluation of liposomal formulation of pilocarpine nitrate" Indian J Pharm Sci, 2010, 72 (2): 155-160.
25. Channarong S, Chaicumpa W, et al "Development and evaluation of chitosan-coated liposomes for oral DNA vaccine: the improvement of Peyer's patch targeting using a polyplex-loaded liposome" AAPS PharmSciTech, 2011, 12 (1): 192-200.
26. Sipai ABM, Vandana Y, et al "Liposomes: an overview" JPSI, 2012, 1 (1): 13-21.
27. Riaz M.; "review: liposomes preparation methods," Pak. J. Pharm. Sci.; 1996, 19, 65-77.
28. Da Costa CAM, Moraes AM "Encapsulation of 5-fluorouracil in liposomes for topical administration" Maringa, 2003, 25 (1): 53-61.

29. Kumar A, Badde S, et al "Development and characterization of liposomal drug delivery system for nimesulide" Int J Pharm Pharm Sci, 2010, 2 (4): 87-89.
30. Nidhal KM, Athmar DH "Preparation and evaluation of salbutamol liposomal suspension using chloroform film method" Mustansiriya Medical Journal, 2012, 11 (2): 39-44.
31. Aditi Gujrati et al. Rev liposomal drug delivery system and its applications. World J of Pharm Res.2019;8(11):1375-1391.
32. Akshay Singha Roy et al "Design, formulation and evaluation of liposome containing Isoniazid" Int J App Pharm, 2018;10(2):52-56.
33. Sunitha Sampathi et al "Preparation and evaluation of liposome entrapped hydrogel complex systems of itraconazole for enhanced transdermal permeation" J of Pharm and Scientific Innovation, 2014;3(1):25-29.
34. Claudia Zylberberg & Sandro Matosevic "Pharm liposomal drug delivery: A Rev of new delivery systems and a look at the regulatory landscape" Drug Delivery, 2016; 23(9): 3319–3329.
35. Achim M, Precup C, et al "Thermosensitive liposomes containing doxorubicin. Preparation and in vitro evaluation" Farmacia,2009;57(6):703-710.
36. Bhatia A "Tamoxifen in topical liposomes: development, characterization and in vitro evaluation" J Pharm Pharm Sci, 2004;7(2):252-259.
37. Patel H, Patel H, et al "Preparation and characterization of calcium phosphate coated vinblastine sulfate liposomes" IJPRBS, 2013;2(2):263-280.
38. Devi M, Kumar MS, et al "Amphotericin-B loaded vesicular systems for the treatment of topical fungal infection" IJRAPR, 2011;4:37-46.
39. Kumar R, Rana AC, et al "Formulation and evaluation of elastic liposomes of clotrimazole" Int J Drug Dev & Res, 2012;4(3):348-355.
40. Chanda H, Das P, et al "Development and evaluation of liposomes of fluconazole" JPBMS, 2011;5(27):1-9.
41. Mirzaee M, Owlia P, et al "Comparison of the bactericidal activity of amikacin in free and liposomal formulation against gram-negative and gram-positive bacteria" JJNPP, 2009:4(1): 1-7.
42. Makhmalzadeh BS, Azh Z, et al "Preparation and evaluation of mafenide acetate liposomal formulation as eschar delivery system" Int J Drug Dev & Res, 2011;3(4):129-140.
43. Ghanbarzadeh S, Valizadeh H, et al "Application of response surface methodology in development of sirolimus liposomes prepared by thin-film hydration technique" Bio Impacts, 2013;3(2):75-81.
44. Patel SS, Patel MS, et al "Development and evaluation of liposomes for topical delivery of tacrolimus (Fk-506)" J Sci Res, 2010;2(3):585-596.
45. Nava G, Pinon E, et al "Formulation and in vitro, ex vivo and in vivo evaluation of elastic liposomes for transdermal delivery of ketorolac tromethamine" Pharm, 2011;3:954-970.
46. Manosroi A, Podjanasoonthon K, et al "Development of novel topical tranexamic acid liposome formulations" Int J Pharm, 2002;235:61-70.
47. Mohammadi-Samani S, Montaseri H, et al "Preparation and evaluation of cyproterone acetate liposome for topical drug delivery" Indian J Pharm Sci, 2009; 5(4):199-204.
48. Niu M, Lu Y, et al "Liposomes containing glycocholate as potential oral insulin delivery systems: preparation, in vitro characterization, and improved protection against enzymatic degradation" Int J Nanomedicine, 2011;6:1115-1166.
49. Divakar P, Kumar DP, et al "Formulation and in vitro evaluation of liposomes containing metformin hydrochloride" IJRPBS, 2013; 4(2):479-485.