Development and Characterization of Liposomal Drug Delivery System Containing Aqueous Extract of Lantana Camara Fresh Leaves

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ABSTRACT :
The present study aimed to formulate and evaluate liposomes loaded with aqueous extract of fresh Lantana camara leaves using the thin film hydration technique. Lantana camara is a medicinal plant known for its antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties. However, the therapeutic effectiveness of herbal extracts is often limited by poor stability and bioavailability. Therefore, a liposomal drug delivery system was developed to improve the encapsulation and controlled release of the plant extract. Preformulation studies were carried out to evaluate the physicochemical properties of the extract. FTIR studies confirmed the compatibility of the extract with the selected excipients, indicating the absence of any significant drug–excipient interaction. Liposomes were prepared using varying concentrations of phosphatidylcholine and cholesterol and evaluated for particle size, zeta potential, entrapment efficiency, morphology, in vitro drug release, and stability. The particle size of the formulations ranged from 185 to 241 nm, while zeta potential values ranged from −23 to −29 mV. SEM analysis confirmed the formation of spherical liposomal vesicles. Among all formulations, F2 exhibited the highest entrapment efficiency of 89.43% and maximum cumulative drug release of 97.43% within 8 hours. The release profile demonstrated an initial burst release followed by sustained drug release. Stability studies conducted over three months indicated that the optimized formulation remained stable under different storage conditions. The results of the study suggest that liposomal encapsulation is a promising strategy for enhancing the delivery and controlled release of Lantana camara extract. The optimized liposomal formulation showed excellent encapsulation efficiency, satisfactory stability, and prolonged drug release characteristics, making it a potential carrier system for herbal therapeutics and future pharmaceutical applications.
Keywords: Lantana camara, Liposomes, Thin Film Hydration Method, Herbal Drug Delivery System, Entrapment Efficiency, Controlled Drug Release, Stability Studies.

I.INTRODUCTION

Liposomes

Liposomes are biocompatible as well as biodegradable bilayer vesicles comprising of a hydrophilic aqueous core and are made of phospholipids. From the last several years the liposomal vesicles have been extensively considered as carrier of preference for the delivery of various potential drug candidates that are lipophilic as well as hydrophilic.[1] Generally, the the liposomes used in clinical practice have the size with diameter in the range of 50 to 300 nm.

Fundamental Structure and Composition

The membranes of liposomal vesicle are linked with the plasma membrane, and are consisting of bilayers made up of phospholipids. Instinctively, vesicles formation occurs upon hydration by means of aqueous media from phospholipids as a consequence of tails of hydrophobic fatty acid, and a head group of hydrophilic phosphatidyl that exist in the typical amphiphilic molecular structure. In the liposomal membrane cholesterol (CH) can be simply incorporated just as plasma membrane in the matching style which stabilizes the membrane and adjust the release of the drug. The drugs hydrophilic in nature are enclosed inside internal aqueous phase, whereas, in the bilayer area of the lipid tail the hydrophobic drugs are located. Liposomal membranes are fluidic in nature like the biomembranes and therefore, the enclosed drugs may be leaked out or released by the liposomes. The release rate is reliant on the components present in the membrane of liposome, like types of phospholipid's fatty

acid acyl chain, degree of unsaturation, charge on lipid and inclusion of CH.

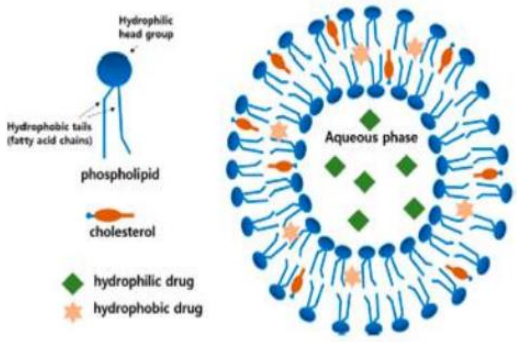

Fig-1: Liposomes

II. EXPERIMENTAL WORK

Materials

Lantana camara was procured from Synpharma Research Labs, Hyd, Phosphotidyl choline, Cholesterol, Chloroformand Methanol were obtained from Synpharma Research Labs, Hyderabad. Other chemicals and the reagents used were of analytical grade.

Methodology

FT-IR spectral study

The stretching vibrations characteristic of the Liposomes was studied by obtaining the FT-IR spectra of liposomes.

Formulation Development

Table-1: Formulation development of Liposomes

Ingredients	F1	F2	F3	F4
Plant extract	25	25	25	25
Phosphatidyl choline	100	200	300	400
Cholesterol	50	100	150	200
Chloroform: methanol	2:1	2:1	2:1	2:1
7.4 Phosphate buffer	10	10	10	10

Precipitation method

Lantana camara extract-loaded liposomes were prepared using the thin film hydration technique. Accurately weighed quantities of phosphatidylcholine and cholesterol according to the formulation batches (F1–F4) were dissolved in a chloroform: methanol mixture (2:1 v/v) in a clean, dry round-bottom flask. The required amount of Lantana camara extract (25 mg) was dissolved in a small quantity of methanol and added to the lipid solution. The organic solvent was then removed using a rotary vacuum evaporator at 40–45°C under reduced pressure with continuous rotation at 60–100 rpm until a thin, uniform lipid film was formed on the inner wall of the flask. To ensure complete removal of residual solvent, the flask was further kept under vacuum for about 30 minutes.

The dried lipid film was hydrated with 10 mL of phosphate buffer (pH 7.4) preheated to approximately 45°C. Hydration was continued with gentle rotation for 30–60 minutes, allowing the lipid film to swell and detach from the flask wall, resulting in the formation of multilamellar liposomal vesicles. Distilled water was then added to obtain the desired volume and to facilitate the formation of a homogeneous liposomal suspension. The resulting dispersion was subjected to sonication for 5–10 minutes using a bath or probe sonicator to reduce the vesicle size and improve uniformity.

Evaluation Of Lantana Camara Extract Loaded Liposomes

Particle Size and Zeta Potential

The particle size of the formulation was determinedbyphotocorrelation spectroscopy with a zeta master (Malvern Instruments, UK) equipped with the Malvern PCS software. Every sample was diluted with distilled water.The surfacecharge (Zeta potential) was determinedbymeasuringtheelectrophoreticmobilityof the liposomes usingaMalvern zetasizer (Malvern Instruments, UK). Samples were prepared by diluting with distilled water.

Morphology

The particle size of the Liposomes was determined by a light scattering particle size analyzer. The morphology of the Liposomes was studied with the help of scanning electron microscopy. The particles were coated with gold sputter on a metal stub and were scanned with an electron beam to obtain magnified image of the surface. The surface characteristics were observed from the image.

Entrapment efficiency

The Entrapment efficiency was studied by dissolving the Lantana camara extract loaded liposomes in 7.4 phosphate buffer and analyzing the solution at255nm by UV spectroscopy and amount of Lantana camara extract was calculated from the calibration curve.

$$\text{Entrapment efficiency (\%)} = \frac{\text{Amount entrapped}}{\text{Total drug loaded}} \times 100$$

In-vitro drug release studies:

In vitro release of Lantana camara extract from the Liposomes was carried out on modified Franz diffusion cell, and the temperature was maintained at 37°C±1°C. The formulation was placed into the donor chamber. Dissolution test was carried out in one media: phosphate buffer (pH 7.4) At specific time intervals (namely 1, 2, 3, 4,5. 6, 7 and 8hours), aliquots of 1 mL were withdrawn and immediately restored with the same volume of fresh dissolution media. The amount of the drug released was determined by measuring the absorbance at 255 nm using a double-beam UV–visible (Vis) spectrophotometer.

Fig-2: Franz diffusion cell apparatus

Stability studies

Selected Formulation was subjected to stability studies as per ICH guidelines.
Following conditions were used for Stability Testing.
1. 25^0C/60% RH analyzed every month for period of three months.
2. 30^0C/75% RH analyzed every month for period of three months.
3. 40^0C/75% RH analyzed every month for period of three months.

III.RESULTS AND DISCUSSION

Drug-excipient compatibility studies (FT-IR)

The compatibility between the drug and the selected excipients was evaluated using FTIR peak matching method. There was no appearance or disappearance of peaks in the extract and excipients mixture, which confirmed the absence of any chemical interaction between the extract and various excipients.

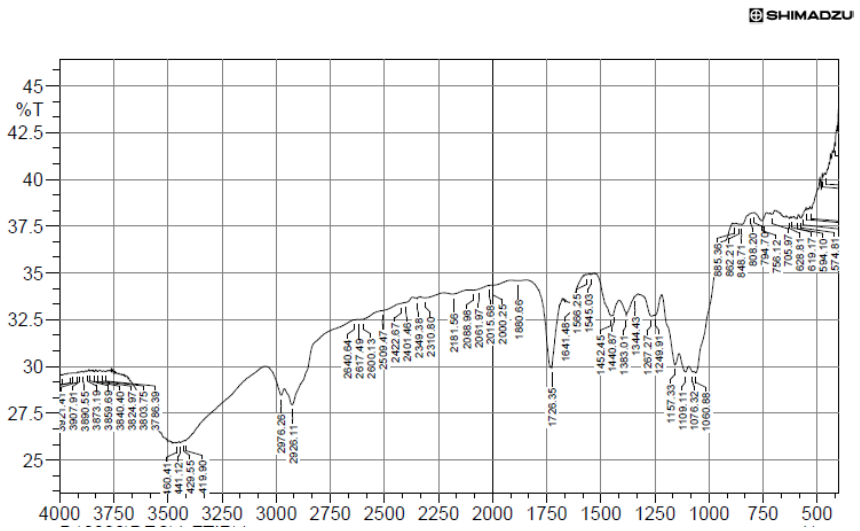

Fig-3: FTIR spectra of Lantana camara extract

Table-2: Characteristic Peaks of Lantana camara extract

S.No.	Characteristic Peaks	Frequency range (cm-1)	Frequency (cm-1)
1	OH stretching	4000-3500	3873.19
2	OH Bending	3000-2500	2976.26
3	C-H stretching	2500-2000	2310.80
4	C=O stretching	1500-1000	1452.45

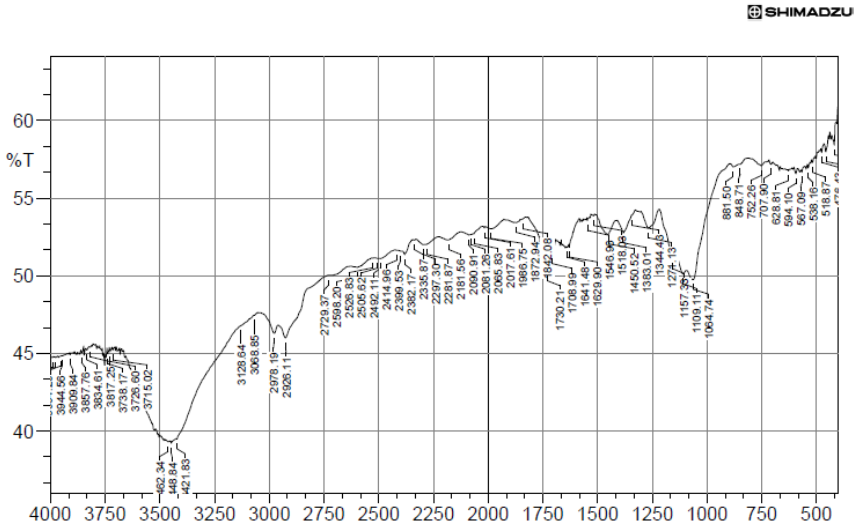

Fig-4: FTIR Spectra of physical mixture of drug and excipients

Table-3: Characteristic Peaks and frequency of physical mixture of drug and excipients

S.No.	Characteristic Peaks	Frequency range (cm-1)	Frequency (cm-1)
1	OH stretching	4000-3500	3857.76
2	OH Bending	3000-2500	2729.19
3	C-H stretching	2500-2000	2335.92
4	C=O stretching	1500-1000	1450.52

Compatibility studies were performed using IR spectrophotometer. The IR spectrum of pure drug and physical mixture of drug and excipients were studied. The characteristic absorption of peaks was obtained as above and as they were in official limits (±100 cm^{-1}) the drug is compatible with excipients.

Evaluation Of Lantana Camara Extract Loaded Liposomes

Determination of Zeta potential:

Zeta potential is a measure of charge present on the vesicle surface. It was determined by using phase analysis light scattering with Malvern zetasizer at field strength of 20V/cm in distilled water and based on electrophoretic mobility of charged particles present in the nanocarrier system. Charged particles were attracted to the electrode with the opposite charge when an electric field is applied.

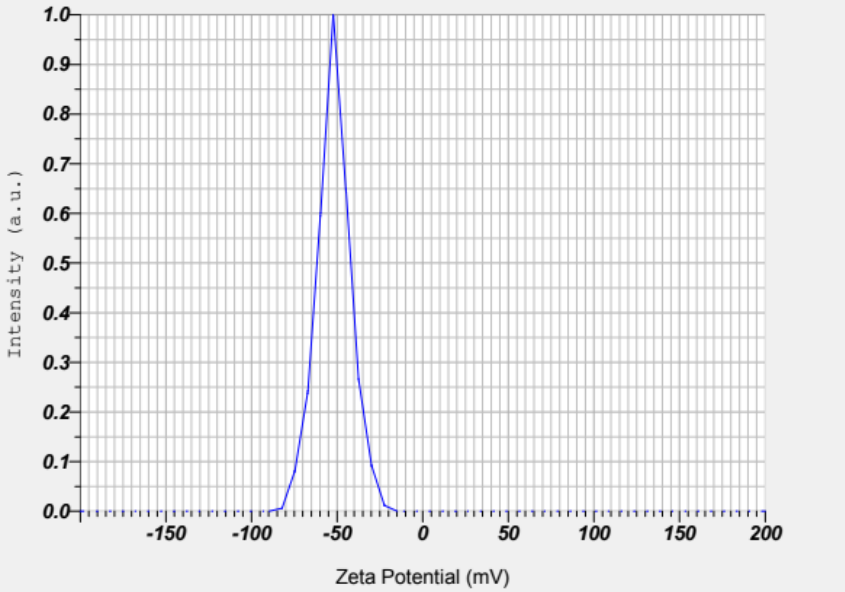

Fig-5: Zeta potential of Optimized formulation

The addition of membrane additives affects zeta potential value depending on the type of membrane additives. Zeta potential of optimized Lantana camara extract Liposomes formulation was measured and found to -23 mv. The obtained result of the zeta potential of the prepared formulation indicates particles in the formulation remains suspended and so were found to be stable.

Particle size

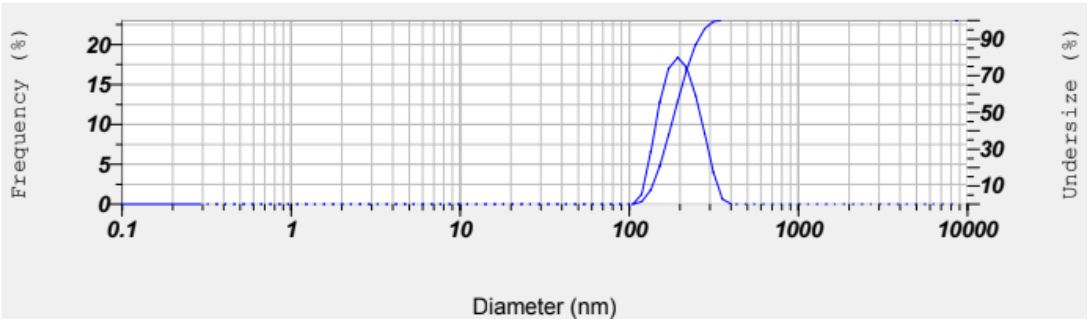

Fig-6: Particle size of optimized formulations

Scanning Electron Microscopy:

The surface characteristic of prepared crystal was studied by SEM (ZEISS Electron Microscope, EVO MA 15). Powder samples was mounted onto aluminium stub using double sided adhesive tape and sputter coated with a thin layer of gold at 10 Torr vacuum before examination. The specimens were scanned with an electron beam of acceleration potential of 20 kV and the images were collected as secondary electron mode.

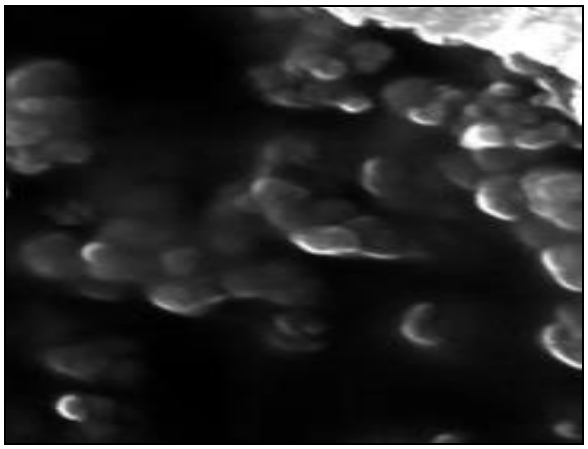

Fig-7: SEM Analysis of Lantana camara Liposomes

Table-4: Evaluation Studies of particle size and Zeta potential of Lantana camara extract Liposomes

F. no	Particle size (nm)	Zeta Potential(mV)
F1	193	-28
F2	185	-23
F3	241	-29
F4	203	-25

Entrapment efficiency

The drug entrapment efficiency of all 4 formulations was evaluated. From the F2 formulation showed maximum drug entrapment efficiency 89.43 % compared to other formulations. The zeta potential or the change on the surface of colloidal particles in Lantana camara extract Liposomes was measured by electrophoretic light scattering mode using zetasizer nano ZS. The particle charge of Lantana camara extract Liposomes were quantified at 25^{o} C. The samples were diluted approximately with the deionized water for the measurements of particle size.

Table-5: Evaluation Studies of Entrapment Efficiency of Liposomes

F. no	Entrapment Efficiency (%)
F1	83.59
F2	89.43
F3	84.10
F4	82.19

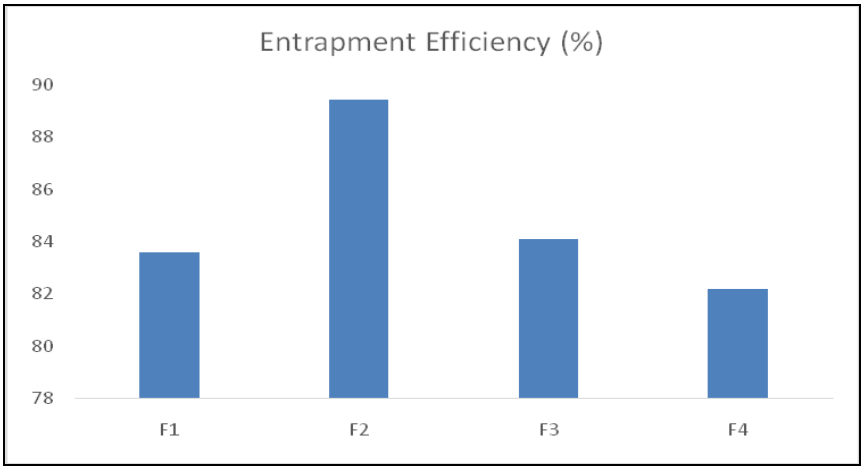

Fig-8: Entrapment efficiency Liposomes

In vitro drug release studies

Table-6: In vitro drug release studies of all formulations

Time (hrs)	F1	F2	F3	F4
0	0	0	0	0
1	25.43	28.42	26.93	24.79
2	34.10	35.46	32.13	33.16
3	41.19	46.48	45.47	43.57
4	54.20	58.10	55.48	53.64
5	64.73	67.13	65.49	68.10
6	75.10	78.90	76.34	74.25
7	82.46	86.46	84.59	83.51
8	94.10	97.43	95.84	96.25

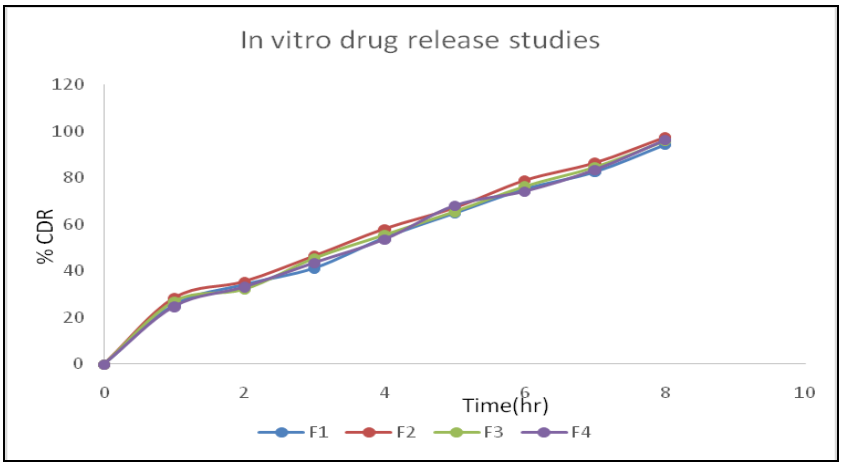

Fig-9: In vitro drug release studies of (F1-F4) Formulations

The drug release studies of all formulations of Lantana camara extract Liposomes were conducted by means of diffusion apparatus for a time period of 8 hrs. From the drug release studies as depicted in Figure, the results showed that 8 formulations showed maximum drug release rate of 97.43 % within 8 hrs.

Stability studies

There was no significant change in physical and chemical properties of the Liposomes of formulation F-2 after 90 days. Parameters quantified at various time intervals were shown.

Table-7: Stability studies of all formulations

F.no	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-2	**25^{0}C/60%RH**	97.43	96.47	95.78	94.36	**Not less than**
F-2	**30^{0}C/75% RH**	97.43	96.53	95.49	94.25	**Not less than**
F-2	**40^{0}C/75% RH**	97.43	96.25	95.3	94.13	**Not less than**

CONCLUSION

The present study aimed to formulate and evaluate liposomes loaded with aqueous extract of fresh *Lantana camara* leaves using the thin-film hydration method. FTIR studies confirmed the compatibility of the extract with the selected excipients. Four liposomal formulations (F1–F4) were prepared and evaluated for particle size, zeta potential, entrapment efficiency, SEM, in vitro drug release, and stability. The formulations showed particle sizes ranging from 185–241 nm and zeta potential values between −23 and −29 mV. Among all formulations, F2 exhibited the highest entrapment efficiency (89.43%) and maximum cumulative drug release

(97.43%) over 8 hours. SEM analysis confirmed spherical vesicles, while stability studies demonstrated that the optimized formulation remained stable for three months.The study successfully developed liposomes containing *Lantana camara* aqueous extract by the thin-film hydration method. Among the formulations, F2 was identified as the optimized formulation due to its smallest particle size (185 nm), highest entrapment efficiency (89.43%), and maximum drug release (97.43%). FTIR and SEM studies confirmed compatibility and successful liposome formation, while stability studies demonstrated good formulation stability.

Overall, the developed liposomal formulation is a promising herbal drug delivery system for improving the therapeutic potential of *Lantana camara*.

REFERENCES

1. Singh A, Singh NB, Hussain I, Singh H, Yadav V, Singh SC (2016) Green synthesis of nano zinc oxide and evaluation of its impact on germination and metabolic activity of Solanum lycopersicum. J Biotechnol 233:84–94
2. Singh A, Singh NB, Hussain I, Singh H, Singh SC (2015) Plant-nanoparticle interaction: an approach to improve agricultural practices and plant productivity. Int J Pharm Sci Invent 4(8):25–40.
3. Hussain I, Singh NB, Singh A, Singh H, Singh SC (2016) Green synthesis of nanoparticles and its potential application. Biotechnol Lett 38(4):545–560
4. Alves, M. J., Grenho, L., Lopes, C., Borges, J., Vaz, F., Vaz, I. P., et al. (2018). Antibacterial Effect and Biocompatibility of a Novel Nanostructured ZnO-Coated Gutta-Percha Cone for Improved Endodontic Treatment. Mater. Sci. Eng. C 92, 840–848. doi:10.1016/j.msec.2018.07.045
5. Gregoriadis G. Liposome technology, Liposome preparation and related techniques, 3rd edition, vol. l 2007. p. 21.
6. Singh S, Gulati M. Advances in liposomal therapeutics, Targeting of drugs to lipid bilayer in liposomes: an approach for the success of liposomes as delivery systems. CBS Publishers and distributors. New Delhi. 2001. p. 4-34.
7. Scherphof G, Morselt H, Regts J. The involvement of the lipid phase transition in the plasma-induced dissolution of multilamellar phosphatidylcholine vesicles. Biochem. Biophys. Acta.1979;556:196–207.
8. Jain NK. Controlled and novel drug Delivery, CBS Publishers and distributors.2002. p. 305,326,330.
9. Bangham AD. Techniques in life Science, lipid membrane biochemistry. Elsevier scientific publications. Ireland. 1982 p.1-25.
10. Drummond DC, Meyer O, Hong K. Pharmacokinetic study of liposome encapsulated drug. Pharmacological reviews 1999; 51(4): 691-743.11.
11. Lasic DD. Stealth liposomes,Microencapsulation Method and Industrial Applications. Vol. 73 1996 p. 297-311.
12. Crommelin DJ, Schreier H. Liposomes, Colloidal Drug Delivery Systems. 1996 p. 73-157.
13. Liu R, Cannon JB, Li Y. Liposomes in Solubilization. Water Insoluble Drug FormulationCRC Press, New York. 2000; 355-358.
14. Patel HM, Ryman BR. Systemic and Oral administration of liposomes. Physical Structure to therapeutic applications 1981; 409-439.
15. Jaggi M, Khar RK. Liposomes as Drug Carriers. Indian Drugs 1989; 27(8):404.
16. Krensky AM, Strom TB. Immunomodulators: Immunosuppressive agents, Tolerogens and Immunostimulants. Goodman and Gilman's The Pharmacological Basis of TherapeuticsLimbird LE (Ed.) 10th Ed, 2001 p. 1466-1480.