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DESIGN, DEVELOPMENT, AND CHARACTERIZATION OF LIPOSOMAL FORMULATIONS FOR IMPROVED BIOAVAILABILITY OF EPROSARTAN

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Abstract:
The purpose of this study was to design, development, and characterization of liposomal formulations for improved bioavailability of Eprosartan. liposomal formulations, was prepared by dry film hydration method. Box-behnken design was introduced to optimize the formulation of polymeric nanoparticles Results: Fourier Transform Infra-Red (FTIR) studies indicate that the excipients added were compatible with the drug. Cumulative drug release in 7.4 pH phosphate buffer was found to be more than95.85%. formation of Liposomes from preliposomes having small Multilamellar vesicles and spherical in shape. Entrapment efficiency of optimized batch was found to be 75.80±0.35 %.Optimized batch having particle size 345.80nm±0.13 nm and polydispersity index of 0.281 which conclude that particles were in mono-disperse state. Scanning Electron microscopy confirmed that Liposomes are in spherical in shape. It concluded that most of the drug was entrapped in liposomes..
Key words: Bioavailability, solvent diffusion, Box-behnken design, Scanning electron microscopy & Particle size.

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INTRODUCTION:

Hypertension is an important risk factor for the future development of cardiovascular disease. It is defined as the condition in which blood pressure has risen to the extent that its reduction leads to clinical benefits. The measurement of BP includes systolic and diastolic parts, and both are important in assessing a patient's heart problem risk. Although BP of 140/90 mmHg is regarded the top limit for normal BP, there is no well-defined threshold for hypertensive and normal BP patients. Stroke and myocardial infarction are the most common and serious cardiovascular problems. A 5 mmHg increase in normal diastolic BP is linked to a 35–40% increase in the risk of stroke. Antihypertensive medication combinations in modest dosages are frequently tolerated better than single medicines in high doses. Because the hypertensive population is so big, the expense of individual preparations must be considered [1-6].

MATERIALS AND METHODS

Materials

Eprosartan was obtained from Alembic Pharma. All other reagents are of either laboratory/analytical grade as per the requirement.

Methods

Drug Excipients Compatibility Study

Drug Excipients compatibility study was done as part of Preformulation experiments after completion of screening. All components were considered for further process only if it was compatible with drug. Fourier Transform Infrared Spectrophotometer (FTIR) (Bruker) was used to obtained the Infrared spectra of the drug substance to check the compatibility of drug with screened components. The method involves preparation of pellets in that drug added in KBr 10% mixture and triturate in mortar pestle and compressed into the KBr pellets. The pellets were treated with excipients by putting drop of each liquid component on pellets and scanned over a wave number range of 400-4000 cm-1 using FTIR. The binary mixture of (Drug+Lipid) (Drug+Cholesterol) (Drug+Mannitol) and Physical Mixture of (Drug+Cholesterol+Phospholipon90H +Mannitol) were also analyzed by same procedure[7-9].

Formulation of Eprosartan. liposomes

Dry film hydration method was used to prepare the Liposomes by using Rota evaporator (ROTA). Weighed required amount of drug (20mg) and lipid: cholesterol was dissolved in 20 ml of organic solvent mixture containing methanol: chloroform (4:2) (V/V). Above mixture was transferred in to a 250mL of round bottom flask and the flask is attached to Rota evaporator till solvent was evaporated under the pressure. Once solvent was completely evaporated, the films formed were dried for overnight. Hydration of the film was done by using hydration media. The prepared liposomal suspension was kept 24 hr to get complete hydration.

Optimization of Liposomes by Box Behnken Design[122]

The product and process variables which were screened from Taguchi design of Liposomes were optimized by the Box Behnken design as response surface methodology. Independent variables Amount of lipid, Amount of Cholesterol and Sonication time were selected beside dependent variables were particle size and entrapment efficiency recorded in form of 3D plot, contour plots and ANOVA analysis were carried out the variance in responses and the best fitted model is predicted according to regression analysis. Different Variables and Levels in BBD were tabulated in Table 14. Total 15 batches were prepared as shown in Table 15. Experimental design was validated to determine the actual and predicted response by formulating from the design space[10-12].

Table: 1 Variables and their levels for liposome optimization

Independent variables	Low level	High level
A= Amount of Lipid (mg) (Phospholipon 90H)	16	75
B = Amount of Cholesterol (mg)	8	35
C = Sonication time (min)	5	15
Dependent variables	R1 = Particle Size R2= Entrapment Efficiency	

Method of preparation of Preliposomes

Preliposomes were prepared by slurry method. Drug, Lipid (Phospholipon 90 H) and Cholesterol were weighed and dissolved in methanol: chloroform mixture in the ratio of (4:2) (V/V). Transferred this mixture into the round bottom flask add Mannitol into the above flask and dispersed in the above mixture. Attached this flask with rotary evaporator at specific speed, temperature and pressure till complete evaporation of the organic solvent. Once Solvent get evaporated kept this flask in vacuum oven to get complete free flowing preliposomal powder. Prepared preliposomes scraped out from the round bottom flask and stored in desiccator and evaluated[13-16].

Table: 2 Variables and their levels for Preliposomes Optimization

Independent variables	**Low level**	**High level**
A= Amount of Lipid (mg) (Phospholipon 90H)	30	90
B = Amount of Cholesterol (mg)	10	30
C = Amount of Carrier (mg)	300	400
Dependent variables	R1 = Particle Size R2= Entrapment Efficiency	

Evaluation of Optimized formulation

Percentage yield

The total percentage yield of the prepared formulation was determined by using following equation.

% yield = (Practical yield / Theoretical yield) * 100

Formation of vesicular structures from preliposome powder

Prepared Preliposomal powder was taken and hydrated with the help of distilled water in watch glass. Take this hydrated liposomal suspension in the glass slide cover with the help of coverslip and observed in inverted microscope at 450X magnification. Formation of liposomes from the preliposomes was seen in micrographs were taken in inverted microscope

Flow Property

The flow properties of prepared preliposomal powders were determined by Bulk density, Tapped density Angle of repose, Carr's Compressibility index and Hausners'ratio.

Angle of repose

Angle of repose measured by fixed funnel method in that 2cm gap between bottom of funnel and horizontal plane should be maintained. Weighed 5 gm of powder transferred in to the funnel keep the orifice of the funnel blocked with the help of thumb after that removed the thumb. Measured the diameter and calculate the Angle of repose.

Formula for Angle of Repose: $\tan\theta = h/r$

Where, h = height of pile r = radius of the pile

Bulk density

Bulk density is defined as ratio of weight of powder to the bulk volume. Weighed accurately 5 gm of powder passed from the sieve no 40 and transferred into the 100 ml of graduated cylinder with help of funnel and volume measured and the bulk density is calculated as per given formula[17].

Bulk density =Wt. of powder / Bulk volume

Tapped density

Tapped density is defined as the ratio of weight of powder to the tapped volume. Tapped density measured by tapped densitometer. Weighed accurately 5 gm of Preliposomal powder and add into the 100 ml of measuring cylinder. Fixed this cylinder on the densitometer apparatus and timer knob is set for 100 tapping. Note down the tapped volume and the tapped density calculated by given formula.

Tapped density = Weight of powder
Tapped volume

Carr's index (% Compressibility)

Carr's Index defined as the ratio of difference in between tapped density and bulk density to that of tapped density multiplied by 100. Calculated by given formula[18].

Carr's index = Tapped density – Bulk density X 100/ Tapped density

Hausner's ratio

Hausner's ratio is defined as the ratio of tapped density to the bulk density.

Calculated by given formula.

Hausner's ratio =Tapped density/ Bulk density

Surface morphology

Scanning Electron Microscope is used for the surface morphological characterization of preliposomes. The Preliposomal powder was hydrated with distilled water observed in SEM and micrograph was taken.

Determination of Particle size and size distribution of Preliposomes99,123

Particle size and size distribution were measured by Malvern Zetasizer 2000MU instrument. Approximately 500 gm of Preliposomal powder was hydrated with 2 ml of distilled water and mix it properly for 30 min. This hydrated suspension was centrifuged for 5 min at 400 rpm to remove the carrier. The supernatant from liposomal suspension was used for particle size & PDI determination.

Drug content

Preliposome powder equivalent to 20 mg of Eprosartan was taken and lysed in 5ml of methanol mix the above mixture by bath sonicator for 15 min to solubilized the lipids and finally make up the volume up to 10 ml with 0.1N HCl. Take the 1 ml of sample and analyzed by using UV-visible spectrophotometer at 296nm[19-20].

Entrapment efficiency

Entrapment efficiency was determined by using Refrigerated centrifugation. Weighed accurately 100mg preliposomal powder, add 10 ml of distilled water and subjected to the centrifugation at 10,000 rpm at 4 ℃ for 20 min. The clear supernatant was taken to get unentrapped drug. This supernatant was diluted with methanol and subjected to analyze by UV spectrophotometer at 296 nm. The absorbance was converted into drug concentration using standard curve. The encapsulation efficiency was calculated. Entrapment efficiency=(Total amount of drug added-Unentrapped drug) X 100/ Total amount of drug taken

In-Vitro Dissolution Study Comparison with Marketed Formulation

In-vitro dissolution study of preliposomes powder encapsulated in capsule and Marketed formulation of Eprosartan (Brand name: Telminorm 20 mg Tablet) was performed according to method described in Chang chu et al, china pharmacopoeia (2010, Paddle method) by some modification. The dissolution media used was 0.1 N HCl and 7.4 pH phosphate buffer, 900ml and temperature at 37±0.5℃, with paddle speed 50 rpm throughout the study. An aliquot of 2ml of sample withdrawn from a predetermined time interval and placed fresh dissolution media into the bath. Dilute the sample and analyzed by UV-visible spectrophotometer at λmax 296nm.Cumulative Percentage drug release was calculated.

RESULTS & DISCUSSION:

Optimization of Liposomes by Box Behnken Design

Response surface methodology one of the valuable statistical technique for optimizing multiple variant by lowest no of experiments. It represents the relationship between dependent and independent variables, there interaction effects. Box–Behnken design (BBD) mainly used design for the optimization of the three variables.

Table: 3 Design Matrix as per BBD and responses for liposomes

Batch	Independent Variables			Dependant Variables	
	X1	X2	X3	Y1(nm)	Y2(%)
L1	16	8	10	221.5±0.05	53.16±0.090
L2	75	8	10	260.68±0.100	70.47±0.017
L3	16	35	10	232.8±0.201	43.17±0.056
L4	75	35	10	228.7±0.022	50.83±0.02
L5	16	21.5	5	258.3±0.071	60.74±0.14
L6	75	21.5	5	244.2±0.011	53.13±0.074
L7	16	21.5	15	219.35±0.15	52.96±0.060
L8	75	21.5	15	246.3±0.07	68.65±0.01
L9	45.5	8	5	270.9±0.110	65.54±0.071
L10	45.5	35	5	263.1±0.030	56.14±0.053
L11	45.5	8	15	265.3±0.020	65.78±0.43

Optimization of Preliposomes by Box Behnken Design

Response surface methodology one of the valuable statistical technique for optimizing multiple variant by lowest no of experiments. It represents the relationship between dependent and independent variables, there interaction effects. Box–Behnken design (BBD) mainly used design for the optimization of the three variables in preliposomes.

Table: 4 Design Matrix as per BBD and responses for preliposomes

Batch code	Independent Variables			Dependant Variables	
	X1	X2	X3	Y1(nm)	Y2(%)
PL1	30	10	350	321.5±0.21	53.14±0.03
PL2	90	10	350	359.6±0.015	56.47±0.058
PL3	30	30	350	334.8±0.01	58.13±0.081
PL4	90	30	350	327.5±0.065	60.74±0.054
PL5	30	20	300	354.8±0.34	35.73±0.031
PL6	90	20	300	346.7±0.08	55.78±0.034
PL7	30	20	400	321.2±0.02	60.14±0.057
PL8	90	20	400	345.5±0.048	52.83±0.011
PL9	60	10	300	370.8±0.09	50.13±0.060
PL10	60	30	300	364.7±0.023	58.16±0.044
PL11	60	10	400	367.4±0.06	60.7±0.07
PL12	60	30	400	349.2±0.017	75.96±0.062
PL13	60	20	350	366.5±0.47	81.54±0.041
PL14	60	20	350	368.4±0.04	80.65±0.020
PL15	60	20	350	367.3±0.042	79.17±0.078

Evaluation of Final Formulation

%Percentage Yield

Percentage yield of optimized batch of preliposomes was found to be 91.2% by using Rota evaporator slurry method.

%Yield= (4.56*100)/5 = 91.2 %

Formation of vesicular structures from Preliposome powder

Preliposomes after complete immediately converted into the Liposomes whenever contact with body fluid. Results showed the formation of Liposomes from preliposomes having small Multilamellar vesicles and spherical in shape.

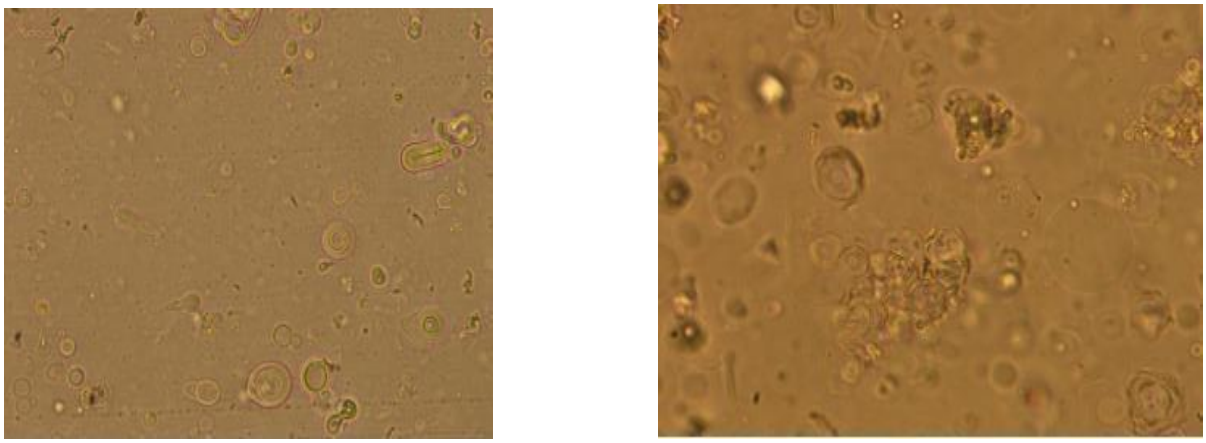

Figure 1: Inverted microscopy images of Preliposomes powder immediately after hydration with distilled water

Flow Property

Flow property was important parameter for Preliposome powder. The flow property of optimized batch was found to be in acceptable range. From the above tabulates data it was found that Preliposome powder has excellent flow regarding standards for flow property.

Table:5 Flow Property of optimized batch

Bulk Density gm/ml	Tapped Density gm/ml	Angle of repose °	Carr's Index %	Hausner's ratio	Flow
0.3082	0.5870	22.07°	14.11	1.10	Excellent

Surface morphology

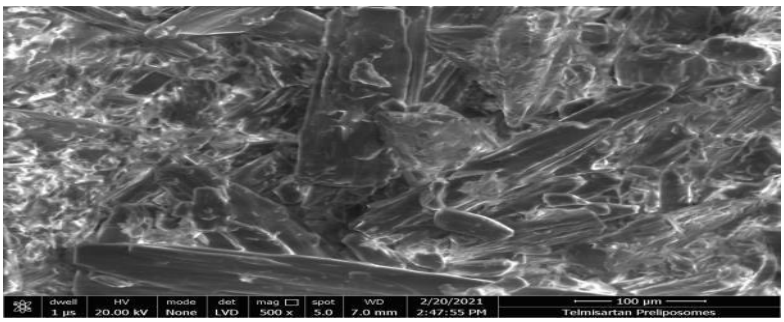

Figure 2 A: SEM images of Preliposomes

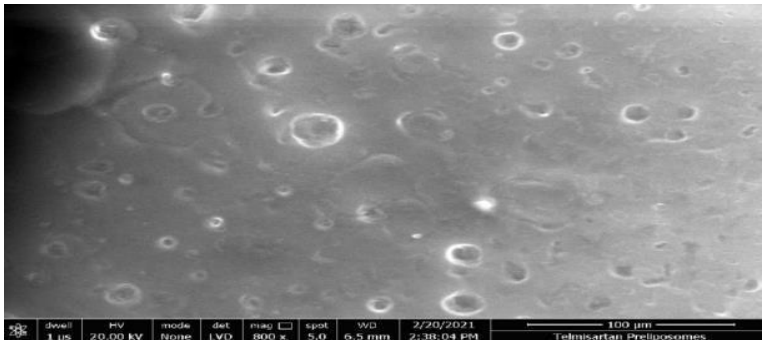

Figure 3 B: SEM images of hydrated Liposomes dispersion

Scanning Electron microscopy confirmed that Liposomes are in spherical in shape. It concluded that most of the drug was entrapped in liposomes.

Determination of Particle size

Particle size analysed by Malvern zeta size analyser. Optimized batch having particle size 345.80nm±0.13 nm and polydispersity index of 0.281 which conclude that particles were in mono-disperse state .

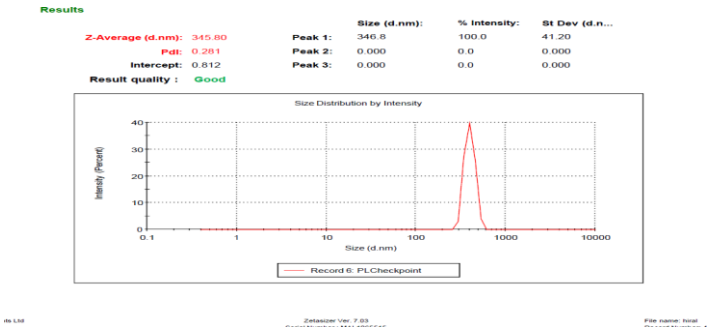

Figure 4: Particle size of optimized batch

Drug content

Total drug content was determined by UV-Visible spectrophotometer at 296 nm and was found to be 90.54% w/w of the Eprosartan.

Entrapment efficiency

Entrapment efficiency of optimized batch was found to be 75.80±0.35 %.

In Vitro Dissolution Study Comparison with Marketed Formulation

The dissolution profile of Eprosartan release from Preliposomes vs Conventional Tablet was evaluated in 0.1 N HCl and 7.4 pH phosphate buffer. The data showed that drug release was faster in phosphate buffer of pH 7.4 than other dissolution media. As shown in figure 23 after 1 hr of drug release the conventional tablet showed a 94.88 %. The release of preliposomes formulation showed a sustained release of Eprosartan during the 10 hrs. Cumulative drug release in 7.4 pH phosphate buffer was found to be more than 95.85%.

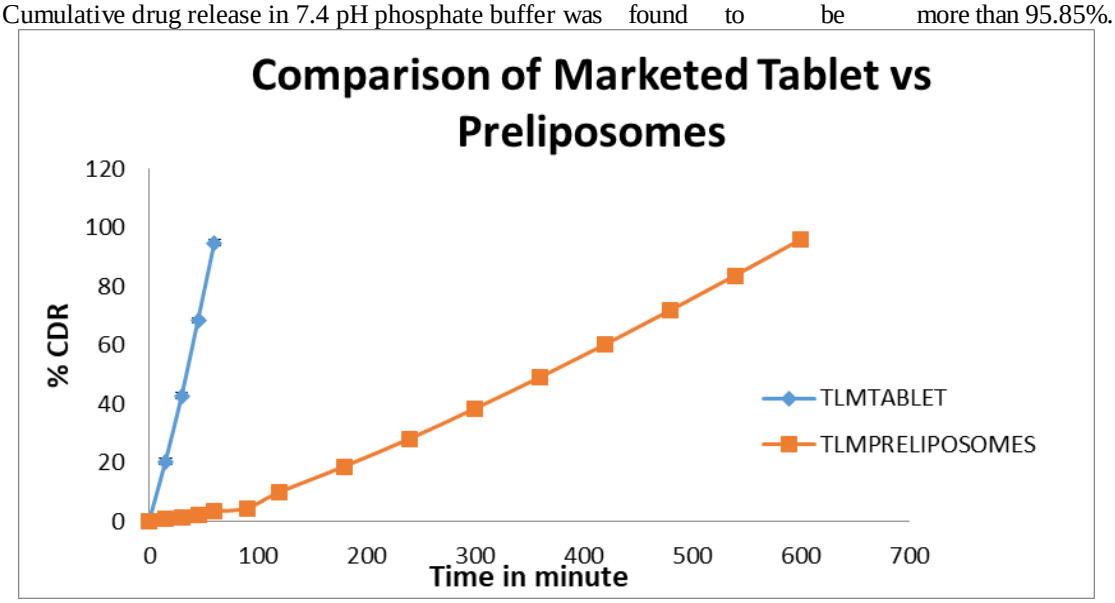

Figure 5: *In vitro* Release of Optimized Batch

Drug Excipients Compatibility Study

IR spectra and bands in the Eprosartan Pure drug and in the mixtures with different excipients like Cholesterol, Phospholipon 90 H and Mannitol was found shown in figure 12A to 12E. No significant interaction with these all excipients was found thus it is compatible with each other. So, it is concluded that all Screened excipients are compatible with each other and found stable

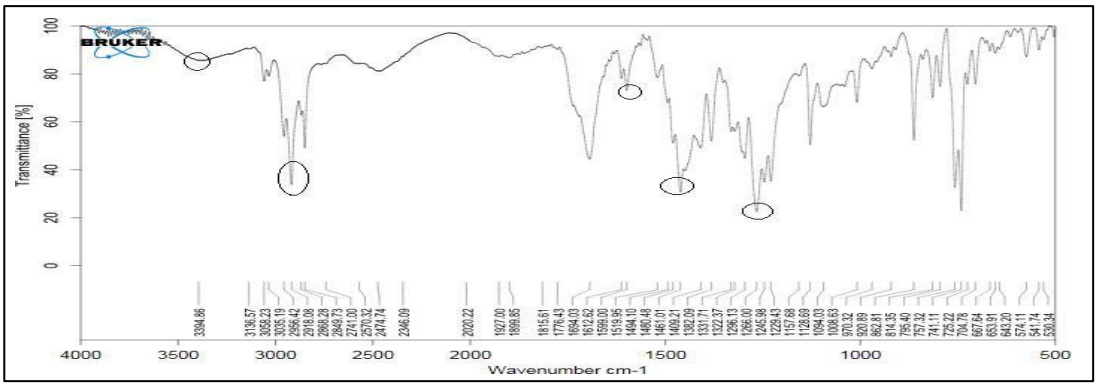

Figure 1: E FTIR of Physical Mixture (Drug + Cholesterol + Phospholipon 90H)

CONCLUSION:

Vesicular formulation development for the bioavaibility enhancement of Eprosartan BCS class-II drug. Various Lipids and Carriers were selected on basis of screening study. Screening of processing parameters was done by using taguchi design. It concluded that Amount of lipid, Amount of Cholesterol and Sonication time were most effective parameters. Box Behnken design was utilized for development of liposomes and

preliposomes formulation. Amount of lipid, Amount of Cholesterol and Sonication time showed significant impact in %EE and particle size. After Comparative study of both formulations, it concludes that preliposomes were most stable as compare to liposomes and preliposomes formulation incorporated in capsule formulation and further evaluated for different parameters including bioavailability study. The checkpoint batch was selected from the design space. It shows similarity of values for experimental and predicted. The checkpoint formulation batches were studied for % Yield, Microscopy study, flow property, SEM study, particle size, entrapment efficiency, drug content, in-vitro release study comparison with marketed tablet formulation.

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Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES:

1. Reddy SB, Reddy PV. Formulation, evaluation, and pharmacokinetics of isradipine proliposomes for oral delivery. Journal of Liposome Research. 2012, 22: 285-294.
2. Abdullah H, Alomrani A, Gamal A, et al.Itraconazole-hydroxypropyl-- cyclodextrin loaded deformable liposomes: In vitro skin penetration studies and antifungal efficacy using Candida albicans as model. Colloids and Surfaces B: Biointerfaces. 2014, 121: 74–81.
3. Gangishetty H, Eedara B, Bandar S. Development of ketoprofen loaded proliposomal powders for improved gastric absorption and gastric tolerance: in vitro and in situ evaluation. Pharmaceutical Development and Technology 2014.
4. Deshpande PB, Gurram A, Deshpande A, et al. A novel nanoproliposomes of lercanidipine: Development, in vitro and preclinical studies to support its effectiveness in hypertension therapy. Elsevier Life Sciences 2016,162: 125-137.
5. Zhao L, Xiong H, Peng H, et al. PEG-coated lyophilized proliposomes: preparation, characterizations and in vitro release evaluation of vitamin E. Eur Food Res Technol. 2011, 232: 647–654.
6. Sun C, Wang J, Liu J,at el. Liquid Proliposomes of Nimodipine Drug Delivery System: Preparation, Characterization, and Pharmacokinetics, AAPS PharmSciTech. 2013,13.
7. Anonymous Eprosartan. In: British Pharmacopoeia British Pharmacopoeia Commission Office, MHRA, London, 2015,(2): 979-980.
8. Budvari S, O' Neil MJ, Heckelman PE, Koch CB, Roman KJ, Kenny Cm and D'Arecca. Eprosartan In: The Merck Index - An encyclopedia of chemicals, drugs and biological.14th Edition, Merck Research Laboratory, Division of Merck and Co., Inc., Whitehouse Station, New Jersey, 1996,1569.
9. Sweetman SC. Eprosartan. In: Martindale - The Complete Drug Reference. 36th Edition, Pharmaceutical Press, London, 2009,1409Freitas C, Muller R H. Stability determination of solid lipid nanoparticles (SLNTM) in aqueous dispersion after addition of electrolyte. J. Microencapsul. 1999; 16: 59–71.
10. Park JM, Ahn BN, Yoon EJ, Et al. The pharmacokinetics of methotrexate after intravenous administration of methotrexate loaded proliposomes to rats, Scholars Portal Journals proliposomes. 1994,15,391-407.
11. Katare OP, Vyas SP, Dixit VK. Proliposomes of indomethacin for oral Administration, Journal of Microencapsulation. 1991, 8: 1-7.
12. Jung BH, Chung BC, Chung SJ, et al. Prolonged delivery of nicotine in rats via nasal administration of proliposomes. Scholars Portal Journals proliposomes. 2000, 66: 73-79.
13. Manjula D, Shabaraya AR, Shyale S. Formulation, Characterization and in vitro release of Proliposomes for topical delivery of Aceclofenac. Journal of Pharmacy Research. 2014, 8(5):674-679.
14. Xiong F, Xiong C, Ge LC, et al. Preparation, characterization, and biodistribution of breviscapine proliposomes in heart. Journal of Drug Targeting, Journal of Drug Targeting. 2009; 17(5): 408-414.
15. FU Q, FU H, LUO H, et al. Preparation of Cefquinome Sulfate Proliposome and its Pharmacokinetics in Rabbit. Iranian journal of pharmaceutical research. 2013; 12(4): 611–621.
16. Chunqing B, Hailong P, Hua X, et al. Carboxymethyl chitosan-coated proliposomes containing coix seed oil: Characterization, stability and in vitro release evaluation. Elsevier food chemistry 129. 2011,1695-1702.
17. Deepali D, Deshmukha, William R, et al. Improved delivery of cromolyn from oral

proliposomal beads. International Journal of Pharmaceutics. 2008, 358: 128- 136.
18. Kurakula M, Srinivas C, Kasturi N, et al. Formulation and Evaluation of Prednisolone Proliposomal Gel for Effective Topical Pharmacotherapy. International Journal of Pharmaceutical Sciences and Drug Research. 2012 4: 35- 43.
19. Vanic Z, Planinsek O, Skalko-Basnet N, et al. Tablets of pre-liposomes govern in situ formation of liposomes: Concept and potential of the novel drug delivery system. European Journal of Pharmaceutics and Biopharmaceutics 2014.
20. Pauli G, Tang W, and Li SD. Development and Characterization of the Solvent- Assisted Active Loading Technology (SALT) for Liposomal Loading of Poorly Water-Soluble Compounds. Pharmaceutics. 2019 (11): 465.