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DESIGN AND DEVELOPMENT OF TELMISARTAN-LOADED LIPOSOMES USING THIN LAYER HYDRATION METHOD

JHA LL[1] AND MANANI HM[2, 3*]

1: Department of Pharmaceutics, School of Pharmacy, Parul University, P.O. Limda, Ta. Waghodia – 391760, Gujarat, India

2: Ph.D Scholar, School of Pharmacy, Parul University, P.O. Limda, Ta. Waghodia – 391760, Gujarat, India

3: Assistant Professor, Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, India

***Corresponding Author: Hiral Manani: E Mail:** hiralmanani812@gmail.com

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ABSTRACT

In recent times more focus is to formulate an innovative drug delivery system for poorly water-soluble drugs to increase the bioavailability of the drug as compared to conventional dosage forms. The present work aimed to develop, optimize and characterize a Liposomal drug delivery system of telmisartan for the treatment of hypertension. Liposomes were developed by using the thin film Hydration technique using the Rota evaporator. Preliminary screening of lipids was done to select the lipid for formulation. Optimization of Liposomal formulation was done by Box Behnken design (BBD) by using design expert 12 version software. Independent factors selected were the amount of lipid, amount of cholesterol & sonication time and dependent variables were entrapment efficiency and particle size. From the result of the preliminary study Phospholipon, 90 H was selected. Batch S7 showed a particle size of 315.7±0.10 and Maximum entrapment efficiency of 80.14±0.02 %. The optimized formulation showed the entrapment efficiency & particle size of the optimized batch (L2) were 260.60 ± 0.1 and 70.4 ± 0.017 respectively. *In-vitro* drug release of 12 hrs of the optimized batch was found to be 98.57 ± 0.02. Transmission Electron microscopy (TEM) showed that the drug was satisfactorily entrapped and

liposomes were spherical. As per ICH guidelines stability studies were conducted and found to be stable. It was concluded that telmisartan Liposomes have all the required qualities of a vesicular drug delivery system. Throughout, 15 batches L2 was found to be most appropriate with low particle size and higher entrapment efficiency. Also found stable in the stability study.

Keywords: Telmisartan, Liposomes, Thin Film hydration method, Box Behnken design

INTRODUCTION

This study was undertaken to formulate liposomes of poorly water-soluble drugs to improve oral bioavailability **[1]**. The oral route is the convenient route of administration and also improves patient compliance **[2, 3].** In research and development, most of the compounds were fail due to their low bioavailability and low absorption. So many reasons for poor bioavailability, one of the reasons was the poor solubility of the drug and first-pass metabolism different approaches have been carried out to increase the oral bioavailability of the drug such as solid dispersion, micronization, and hydrotrophy **[4, 5]**. Telmisartan is a BCS-II drug used for the treatment of hypertension and its direct-acting (Angiotensin receptor blockers) ARBs. Telmisartan has only 50% oral bioavailability **[6]**.

Recently, Novel approaches are there to proliferate the oral bioavailability of poorly water-soluble drugs. Novel drug delivery systems increase therapeutic efficiency and reduce problems like low solubility and poor oral bioavailability. A novel vesicular drug delivery system provides low dosing frequency improves bioavailability & reduced side effects **[7]**.

Liposomes are vesicular structures composed of phospholipid and cholesterol bilayer liposome shells encapsulated in a protein hormone, enzymes & anticancer agents. The vesicular structure also prevents encapsulated drugs from the acidic environment vesicular system improved oral bioavailability by avoiding first-pass metabolism due to the lymphatic delivery of the drug. Liposomes have bilayer structure properties that it was used as the carrier for hydrophilic and lipophilic molecules. Lipophilic compound entrapped within the lipid bilayer, and small size improves the cellular uptake than other nanoparticulate system & improve the oral bioavailability of the drug **[8, 9]**.

Here optimization of liposomes was done by using response surface methodology, in that box Behnken design was selected it determined the optimum concentration of experimental factors to get good quality of the liposomes.

Three factors selected as independent variables can be fixed throughout an experiment and three different levels are selected for the factors. The output variables (response) were measured and can be altered based on the level of the factors. BBD has fewer experimental batches and is frequently used whenever the number of factors was three **[10]**.

MATERIALS AND METHODS

The study consists of Telmisartan (Alembic Pharma Limited, Vadodara), Phospholipon 90H, (lipoid Ludwigshafen, Germany), and Cholesterol (SD Fine Chem Ltd, Mumbai), Moreover, All solvents and reagents such as chloroform, methanol and phosphate buffer used were analytical grades from Merck.

Preparation of liposomes;

The liposomes were prepared using the thin layer hydration method. Thin-film hydration method was used for the liposome preparation by using a Rota evaporator. Phospholipon 90H, cholesterol, and telmisartan were dissolved in a mixture of methanol & chloroform in the ratio (4:2) in a 250ml round bottom flask. The flask was attached to the rota evaporator which form a thin film at 50℃ and 60 rpm. The film was vacuum dried overnight and then hydrated with phosphate buffer pH 7.4 at 60℃ at 200 rpm **[11-13]**.

Optimization of liposome by using Response Surface Methodology;

Response surface methodology was implicated for the optimization of liposome formulation here to estimate the effect of various formulation & processing parameters on different product attributes. BBD is the most useful approach for developing one variable at a time. Design of expert software 12 version was used to generate the number of batches based on the 3 levels and 3 factors selected. Box Behnken design was selected in which Amount of lipid, Amount of cholesterol, and sonication time were selected as independent variables as shown in **Table 1**. While the particle size and entrapment efficiency were selected as quality attributes for the optimization of liposomes. BBD consisting of 15 experiments was created by using DOE Trial version 12 software as shown in **Table 2**.

Table 1: Variables and their levels in Box Behnken Design

Independent Variables	Levels		
	Low (-1)	Medium (0)	High (+1)
X1= Amount of Lipid (mg)	16	45.5	75
X2= Amount of Cholesterol (mg)	8	21.5	35
X3= Sonication Time (min)	5	10	15

Table 2: Box-Behnken Experimental Design with Measured Responses

Runs	Independent Variables			Dependent Variables	
	X1	X2	X3	Y1 (nm)	Y2(%)
1	-1	-1	0	221.5±0.060	53.16±0.090
2	+1	+1	0	260.68±0.102	70.47±0.017
3	-1	-1	0	232.8±0.203	43.17±0.056
4	+1	+1	0	228.7±0.024	50.83±0.02
5	-1	0	-1	258.3±0.076	60.74±0.14
6	+1	0	-1	244.2±0.015	53.13±0.074
7	-1	0	+1	219.35±0.16	52.96±0.060
8	+1	0	+1	246.3±0.08	68.65±0.01
9	-1	-1	-1	270.9±0.110	65.54±0.071
10	+1	+1	-1	263.1±0.200	56.14±0.053
11	-1	-1	+1	265.3±0.040	65.78±0.43
12	+1	+1	+1	247.5±0.06	67.13±0.09
13	-1	0	0	269.8±0.140	64.14±0.087
14	+1	0	0	267.3±0.27	65.73±0.04
15	-1	0	0	268.3±0.42	63.87±0.16

Characterization of liposomes;

Vesicle surface morphology;

Liposome shape and the surface morphological study was carried out by (TEM) Transmission Electron Microscopy from Sicart Analytical laboratory, V.V. Nagar Anand **[14]**.

Particle size determination;

Particle sizes were determined by the Malvern zeta sizer instrument. That sample was taken in a transparent cuvette at a specific temperature. Particle size was determined by the zeta sizer and the graph was shown in software with size and polydispersity index **[15-16]**.

Entrapment efficiency;

To determine the entrapment efficiency refrigerated centrifuge instrument was used. Take the liposomal suspension in the Eppendorf tube and centrifuge in the Remicool centrifuge at 4000 rpm for 20 min at 4°C temperature. The supernatant contained the liposomes in the form of dispersion and unentrapped drugs on the wall of the centrifuge tube. Supernatant again centrifuged at 4°C temp 12,000 rpm for 30 min. Sedimented pellets were dispersed in the inorganic solvent and analyzed by U.V. Spectrophotometer for the number of drugs entrapped **[17, 18]**. % EE was calculated by this formula:

%EE= 1-Unentrupped drug/Total drug X 100.

Stability study;

A stability study was carried out to obtain a stable formulation to assure its safety and efficiency throughout the shelf life of the product. The sample was placed in the plastic tube at room temp (37±5°C) and in a refrigerator at 2-8°C for 3 months the sample was evaluated for particle size and

entrapment efficiency at a specific time interval **[19]**.

RESULTS AND DISCUSSION

Owing to its efficiency as well as convenience of use, the thin layer hydration technique was selected for the production of liposomes. To develop the lipid bilayer in liposomes, phospholipon 90H and cholesterol have been used as phospholipids. Additionally, the structure of liposomes is significantly influenced by cholesterol. In order to create a physically stable equation, the proportion of lipids was optimized, as evidenced by the outcomes of the characterization in the form of particle size and zeta potential values. A current study demonstrated that adding cholesterol to liposomes increases their physical stability **[20-25]**.

Box-Behnken Data Analysis;

BBD Response surface design with different three independent variables at three levels has studied the effect on dependent variables. A total of 15 batches of liposomes were evaluated for particle size and percentage drug efficiency. Box-Behnken experimental design has the benefit of fewer experiments (15 batches) than would a full factorial design (27 batches). Decoded values of all 15 batches along with their results are shown in **Table 3**. Batch codes 2, 9, and 15 had the highest percentage of drug entrapment (PDE). The PDE and Particle size (dependent variable) obtained at various levels of the 3 independent variables (X1, X2, and X3) were subjected to multiple regression to yield a second-order polynomial equation (full model):

PDE = +66.28+4.13A-4.71B+5.83AC-8.33A2…….(1)

The value of the correlation coefficient r2 of Equation 1 was found to be 0.9755, indicating a good fit. The PDE values measured for the different batches showed wide variation (ie, values ranged from a minimum of 43.17 to a maximum of 70.47). The results distinctly show that the PDE value is strongly affected by the variables selected for the study. That is also reflected by the wide range of values for coefficients of the terms of an equation. Some terms and interaction effects are omitted from Equation 1 to obtain a reduced second-order polynomial equation and the significant terms are <0.005. It is concluded that the omitted terms do not significantly affect the PDE.

The particle size of the liposome batches, measured by using the zeta sizer instrument, was found to be in the range of 219.5 nm to 272.5 nm. The reduced polynomial equation was also developed for Particle size.

PS= +268.94+5.99A-5.78B-7.26C-10.82AB+10.26AC-26.34A² .....(2)

The value of the correlation coefficient r2 of equation 2 was found to be 0.9439 shows a good fit. From the independent variables selected and their interaction, A, B, AB, AC and A2 were found to be significant <0.05 indicating a major effect on particle size.

The 3 center points in the Box Behnken design made it possible to identify the pure error of the experiment and the model's lack of fit to be checked. In this study lack of fit was checked for both responses PDE and PS by using statistical analysis. The p-value of Lack of fit was obtained at 0.11 and 0.08 respectively. Hence, the current model gives a satisfactory fit to the data (P > 0.05).

The correlation between the dependent and independent variables was elucidated by using contour plots. The effect of A and B with their interaction on PDE art fixed levels of C are shown in **Figure 1**.

The plots were to be linear up to 55% PDE, but above this value, the plots were found to be nonlinear shows a nonlinear relationship between A & B. It shows that a low level of cholesterol and a medium level of amount of lipids favors the higher PDE of liposomes. This observation showed cholesterol decreased the percentage of drug entrapment **[26]**. The Lipids in medium amount, which is hydrophobic, result in an increased percentage of drug entrapment because of hydrophobic interaction with the drug.

Checkpoint Analysis;

Table 3 shows the checkpoint batches that were organized and assessed for PDE and PS. The analysis shows that the expected PDE and PS are equivalent. from that, we can conclude that the acquired mathematical formula is valid for forecasting PDE and PS.

Optimum Formula;

After examining the effect of independent variables on the chosen responses, the levels of these variables that produce the best results are confirmed. The finest formulation provides high PDE and Low PS. The new fresh formulation was prepared with the independent variables at their optimal range, and the resulting liposomes have been tested for PDE and PS. The measured values of PDE and PS were 66.35 % and 256.43nm, respectively, which agreed well with the theoretical values.

Vesicle surface morphology;

Surface morphology images as shown in **Figure 3** present vesicles. This image confirms that one LUV formed and having spherical.

Particle Size Determination;

Particle size determination was performed to confirm required liposome size has been obtained by using the optimized formula. As shown in **Figure 3**, particle size 256.43±0.25 nm the small size LUVs result in advanced drug delivery. The PDI value is 0.405 indicates the heterogeneity and more physical stability of particle sizes in a Formulation.

Stability study;

Phospholipon 90 H and cholesterol were used to create liposomes by using the thin-film hydration technique. It is significant to note that the properties of liposomes were sustained at 37°C and 2°C–8°C temperature with respect to particle size and entrapment efficiency. According to the stability studies, the optimized batch of telmisartan-containing liposomes remained relatively stable for three months in terms of particle size and entrapment effectiveness at both room temperature and the refrigerator. There is no change in Particle size and entrapment effectiveness. After 90 days, there is no crystallization of the drug. It shows the steady nature of the prepared formulations.

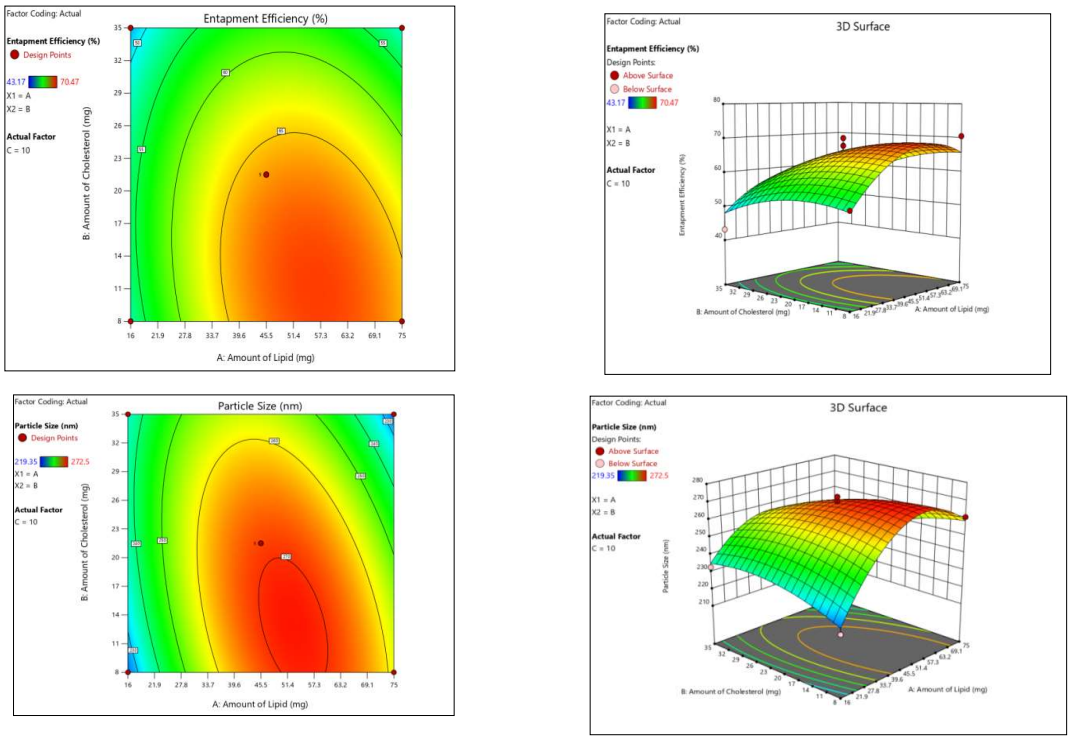

Figure 1: Counter plot and the 3D surface of particle size and PDE

Table 3: Validation of Checkpoint Batch

Parameters	Results given by DOE® 9.0.1 software	Checkpoint batch
Particle size (PS)	258.73 nm	256±0.25 nm
% drug entrapment (PDE)	65.83%	66.35±0.23

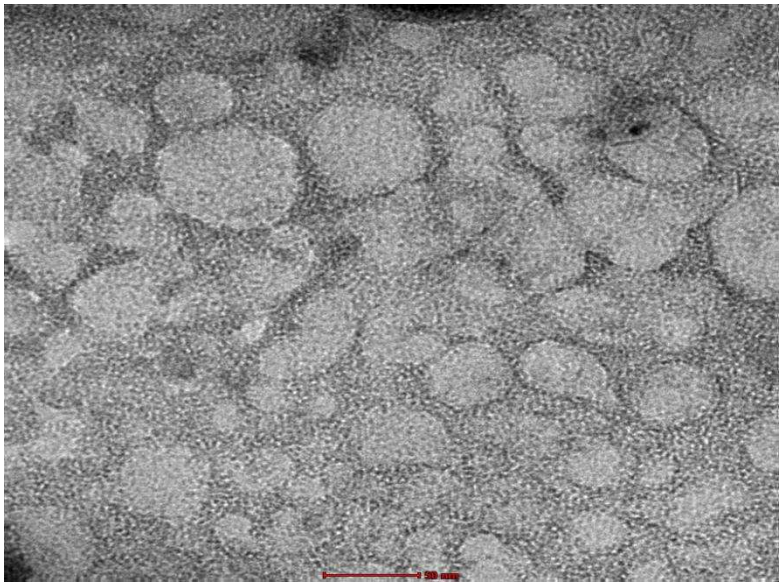

Figure 2: TEM image of Telmisartan-loaded liposomes

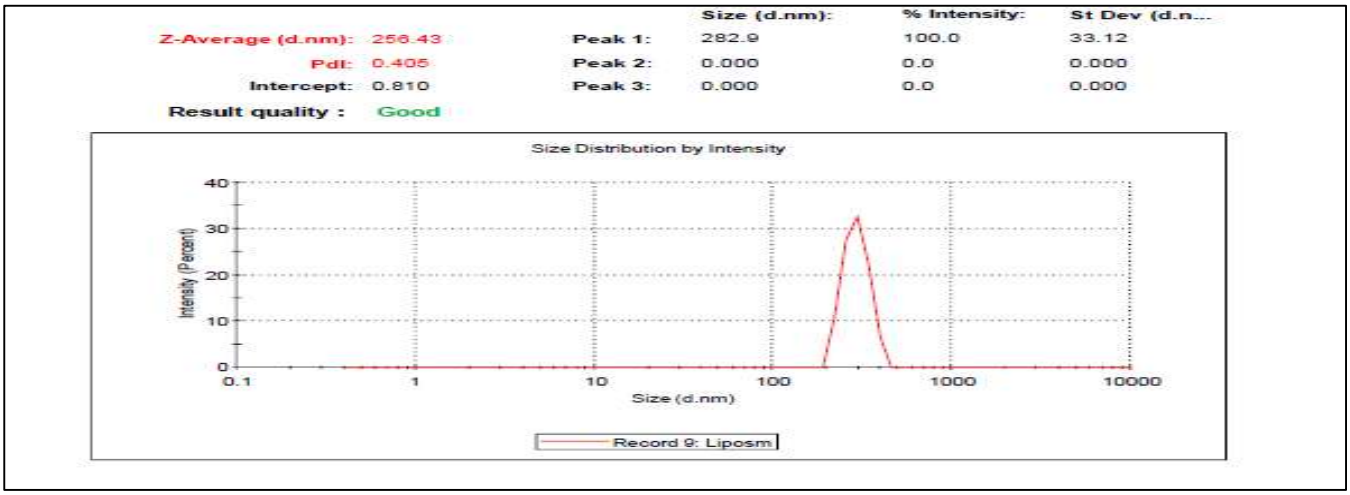

Figure 3: Particle Size Analysis of the Checkpoint Batch

CONCLUSION

The methodology of optimizing a liposome formulation is a complex process that involves a variety of factors and their interactions. Liposomes were made through the rota evaporator method and the TEM images show adequate morphology and LUVs. The current trials conclusively prove the use of a Box-Behnken design in the optimization of liposome development **[27,**

28]. In order to create the best liposome formulations with the ideal characteristics which are helpful to forecast the values of a few independent variables using the derived polynomial equations and contour plots.

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REFERENCES

[1] Singh A, Jha KK, Mittal A, Kumar A. A Review on Telmisartan. J sci innov res. 2013; 2:160-175.

[2] Akbarzadeh A, Sadabady RR, Davaran S, Woo-Joo S, Zraghami N, *et al.* Liposome: classification, preparation, and applications. Nanoscale Res Lett. 2013; 8:1-9.

[3] Dwivedi C, Verma S. Review on Preparation and Characterization of Liposomes with Application. J sci innov Res. 2013; 2:486-508.

[4] Sharma VK, Mishra DN, Sharma AK, Srivastava B. Liposomes: Present Perspective and Future Challenges. Int J Curr Pharm Rev Res. 2010; 1:1-26.

[5] Storm G, Daan JA, Crommelin. Liposomes: quo Vadis. PSTT. 1998; 1:19-31.

[6] Kim H, Kim Y, Lee J. Liposomal formulations for enhanced lymphatic drug delivery. Asian J Pharm Sci. 2013; 8:96-103.

[7] Sharma M, Sharma R, Jain DK. Nanotechnology-Based Approaches for Enhancing Oral Bioavailability of Poorly Water-Soluble Antihypertensive Drugs. Scientifica. 2016; 2016:1-11.

[8] Shashi K, Satinder K, Bharat PA. complete review on liposomes. Int Res J Pharm. 2012; 3:10-16.

[9] Murthy BRS, Vishakha K, Prakash P, Basha DJ, Govardhan T, Prathap MS. Formulation and in vitro evaluation of liposomes loaded with mupirocin. Int j res pharm nano sci. 2015; 4:162-174.

[10] Amin SG, Shah DA, Dave RH. Formulation and Evaluation of Liposomes of Fenofibrate Prepared by Thin Film Hydration Technique. Int J Pharm Sci Res. 2018; 9:3621-3637.

[11] Yang T, Cui F, Choi M, Lin H, Chung SJ, Shim CK, Kim DD. Liposome Formulation of Paclitaxel

with Enhanced Solubility and Stability. Drug Deliv. 2007; 14:301–308.

[12] Dua JS, Rana AC, Bhandari AK. Liposome: Methods of Preparation and Applications. Int J Pharm Sci Res. 2012; 3:14-20.

[13] Moghimipour E, Salami A, Monjez M. Formulation and Evaluation of Liposomes for Transdermal Delivery of Celecoxib. J Nat Pharm Prod. 2015;10:176-153.

[14] Yadav AV, Murthy MS, Shete AS, Sakhre S. Stability Aspects of Liposomes. Indian J Pharm Educ Res. 2011; 45:402-413.

[15] Abdullah H, Alomrani A, Gamal A. Itraconazole-hydroxypropyl--cyclodextrin loaded deformable liposomes: In vitro skin penetration studies and antifungal efficacy using Candida albicans as a model. Colloids Surf B Biointerfaces. 2014; 121:74–81.

[16] Chinnala KM, Panigrahy R. Formulation and Evaluation of Acyclovir Liposomes. Int J Pharma Bio Sci. 2017; 4:5-15.

[17] Lankalapalli S, Tenneti VS, Adama R. Preparation and evaluation of liposome formulations for poorly soluble drug itraconazole by complexation. Der Pharm Lett. 2015; 7:1-17.

[18] Yan C, Qingqing W, Zhang Z, Yuan L, Liu X, *et al.* Preparation of Curcumin-Loaded Liposomes and Evaluation of Their Skin Permeation and Pharmacodynamics. Molecules. 2012; 17:5972-5987.

[19] Muppidi K, Andrew SP, Jeffrey W, Guru B. Development and Stability Studies of Novel Liposomal Vancomycin Formulations. ISRN Pharma. 2012;636743

[20] Baillie AJ, Florence AT, Hume LR, Muirhead GT, Rogerson A. The preparation and properties of niosomes non-ionic surfactant vesicles. J Pharm Pharmacol. 1985; 37:863Y868.

[21] Rathod S, Deshpande SG. Design and Evaluation of Liposomal Formulation of Pilocarpine Nitrate. Indian J Pharm Sci. 2010; 72:155-160.

[22] Roy AS, Das A, Samanta A. Design, Formulation and Evaluation of Liposome Containing Isoniazid. Int J Appl Pharm. 2018; 10: 52-56.

[23] Nguyen TL, Nguyen TH, Nguyen DH. Development and In Vitro Evaluation of Liposomes Using Soy Lecithin to Encapsulate Paclitaxel. Int J Biomater. 2017; 2:1-7.

[24] Babu MK, Rajesh K, Sujatha M, Suanand V, Reddy GJ. Design and development of Telmisartan transferosomes. RJRPB. 2018; 6:111-115.

[25] Paun J. Design, development, and evaluation of nanosuspensions for enhancement of oral bioavailability of poorly soluble drugs. Sodhganga. 2018.

[26] Shaker S, Gardouh AR, Ghorab MM. Factors affecting liposomes particle size prepared by ethanol injection method. Res Pharm Sci. 2017; 12:346-352.

[27] Pasha K, Banu S. Formulation and Evaluation of Glimepiride Liposomal Drug Delivery System. Int J Pharma Bio Sci. 2017; 4:39-44.

[28] Fugate A, Nagoba S, Hyam SR. Formulation Development and evaluation of Liposomal Drug Delivery System Containing Etoposide. J Complement Med Res. 2021; 12:7-20.