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PREPARATION AND EVALUATION OF NON-IONIC SURFACTANT VESICLE USING STATISTICAL OPTIMIZATION: OLMESARTAN USED AS MODEL DRUG

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Keywords:

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ABSTRACT: The purpose of the present work was to prepare and evaluate olmesartan-loaded non-ionic surfactant vesicles (niosomes) to get sustained release by increasing the solubility and bioavailability. Ether injection and thin-film hydration methods were used for the preparation of all formulations as per the factorial design to study the effect of the three independent variables X1 (amounts of span 60), X2 (amount of cholesterol) and X3 (amount of chitosan/ PEG-6000) on two dependent variable Y1 (% DEE), and Y2 (% CDR), respectively. Prepared niosomes were characterized by % DEE, % CDR, FTIR, SEM, and zeta potential, *etc*. Statistical analysis was performed using ANOVA, and optimization was done by fitting experimental data. After 8 hours dissolution, the minimum and maximum cumulative drug release were observed to be 75.90% and 85.91%, and 77.32% and 88.74% for SPEIM-2 and SPEIM-1 and CHEIM-2 and CHEIM-1; 80.24% and 86.11% and 80.11% and 90.72% for SPTFH-2 and SPTFH-1, and CHTFH-7 and CHTFH-1, respectively. Data obtained from *in-vitro* dissolution tests were fitted to different kinetic models. FTIR and DSC studies revealed the absence of significant drug-polymer interaction. The SEM and FTIR studies were used to confirm of round and smooth surface and no interaction along with drug and excipient. *In-vitro* and *ex-vivo* permeability study was also done. From the analysis, it can be concluded that olmesartan-loaded niosomes are potential candidates for getting sustained drug release by improving solubility and bioavailability.

INTRODUCTION: Non-ionic surfactant vesicles (noisome) are a novel drug delivery system in which the medication is encapsulated in a vesicles having amphiphilic bilayer structure by self-assembly of hydrated surfactant monomers [1]. It can ensnare both hydrophilic and lipophilic drugs in the aqueous layer and vesicular membrane respectively [2].

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Therefore a large number of drugs and other materials can be delivered using niosomes which have shown pronounced benefits of enhanced bioavailability, sustained release, targeted delivery, decreased side effects, high stability, etc. For the flexibility of their structural features (composition, fluidity, and size) and ease of storage and handling, these lipid vesicles can be designed for delivery of a wide variety of drugs for drug targeting, controlled release, and permeation enhancement [3]. Niosomes helps to maintain the required plasma and tissue drug levels in the body. It may avoid hepatic first-pass metabolism by promoting lymphatic transport [4, 5].

Moreover, it has been gained much attention as carrier for the delivery of drugs with poor water solubility. The formulation is in the form of the aqueous vehicle-based suspension having greater patient compliance when compared to oily dosage forms [6, 7].

Olmesartan is an anti-hypertensive drug having low solubility and bioavailability; its bioavailability is only 26%. It is modified into non-ionic surfactant vesicle so that its solubility and bioavailability can be increased [8].

In niosomes, non-ionic surfactants and their combinations in different molar ratios have been used to entrap a wide range of drugs with varying features like size [9]. In addition, the use of cholesterol, a waxy steroid metabolite, into non-ionic surfactants is usually added to increase niosomal stability and to enhance the fluidity of the membrane by providing rigidity and orientational order [10]. On the other hand, charge inducers increase the stability of the vesicles by induction of charge on the surface of the prepared vesicles. It performs by preventing the fusion of vesicles due to repulsive forces of the same charge and provides higher values of zeta potential. Most commonly negative charge inducer like diacetyl phosphate and positive charge inducer stearylamine are used [11].

Therefore, the present research work is aimed to prepare olmesartan loaded nonionic surfactant vesicles to achieve a sustained release with increase solubility as well as increase bioavailability.

MATERIALS AND METHODS:

Materials: Olmesartan medoxomil (API) was a gift sample by Square Pharmaceuticals Ltd. Bangladesh. Span 60, methanol, stearylamin, and diethyl ether (MERCK, Germany), Cholesterol (ALFA Aesar, Great Britain, UK). Cellulose dialysis membrane was purchased from Spectrum lab. All other ingredients used throughout the study were of analytical grade.

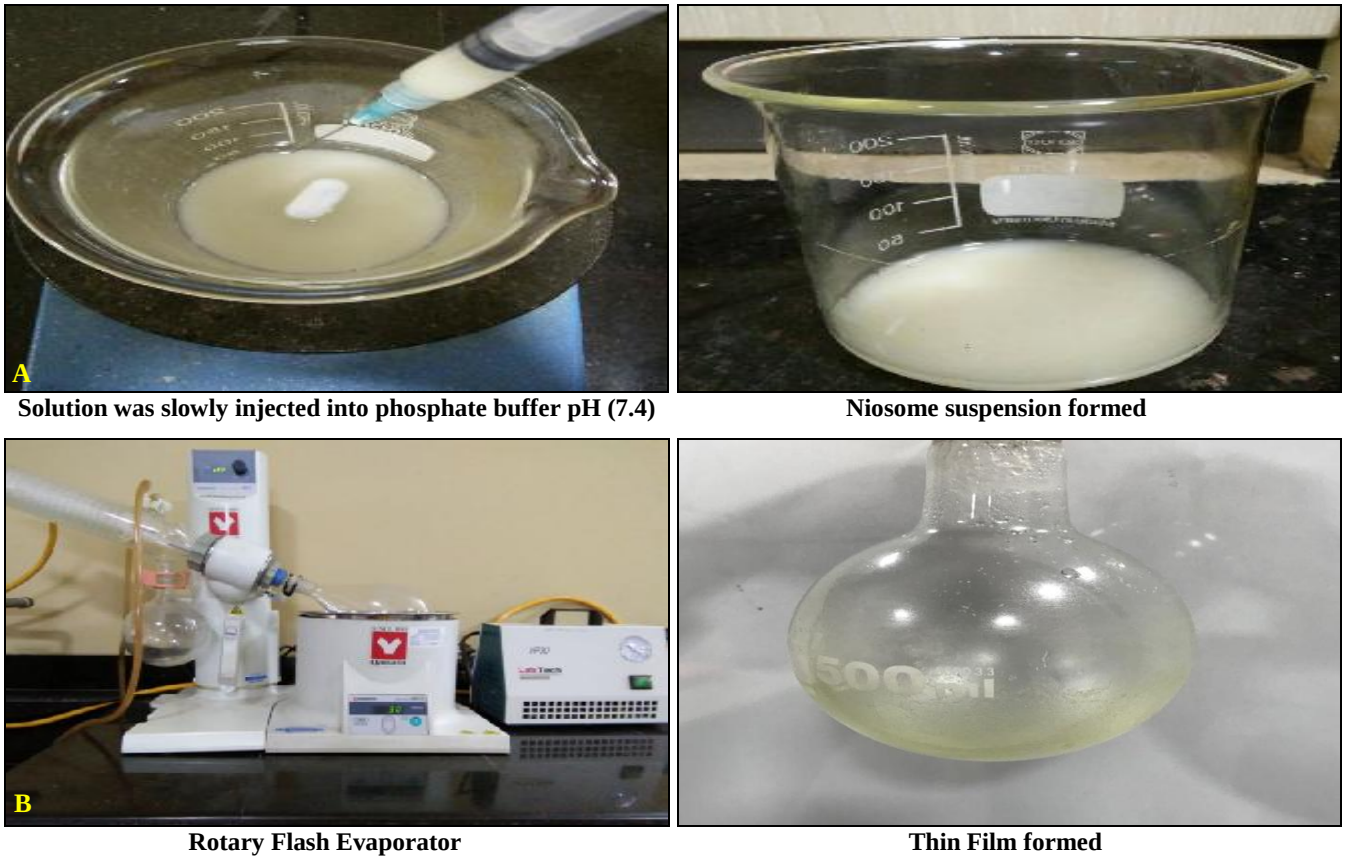
Methods:

Formulation Design: For the preparation of olmesartan loaded niosomes using randomized factorial designs were adopted to optimize the formulation parameters and to study the influence of independent formulation variables on dependent variables X1 (amounts of span 60), X2 (amount of cholesterol) and X3 (amount of chitosan/ PEG-6000) on two dependent variable Y1 (% DEE), and Y2 (% CDR), respectively shown in **Table 1** and **2**. The amount of drug (20 mg) was kept constant for each batch.

Preparation of Non-Ionic Surfactant by Ether Injection Method (EIM): According to **Table 1** and **Table 2**, cholesterol and span 60 was dissolved in 8 ml diethyl ether mixed with 2 ml methanol containing a weighed quantity of olmesartan medoxomil. The resulting solution was slowly injected using a microsyringe at a rate of 1ml/min into 20 ml of hydrating solution phosphate buffer pH (7.4). The solution was stirred continuously on a magnetic stirrer, and the temperature was maintained at 60-65 ºC. As the lipid solution was injected slowly into the aqueous phase, the differences in temperature between phases cause rapid vaporization of ether, resulting in spontaneous vesiculation and formation of niosomes shown in **Fig. 1a**. A factorial design was employed to study the effect of the independent variable on dependable variables. All the formulations as per experimental design were prepared using a similar procedure by addition of various quantities of span 60, cholesterol, and chitosan/ PEG-6000, respectively.

Preparation of Olmesartan Niosome by Thin Film Hydration Method (TFHM): According to **Table 1** and **Table 2**, cholesterol and span 60 was dissolved in 8 ml diethyl ether. Weighed quantity of the drug was dissolved in 2 ml methanol. Then the two solutions were mixed together in a round bottom flask. Using the rotary flash evaporator, the organic solvents were removed at a temperature of 60-65 ºC that is shown in **Fig. 1b**.

The flask was rotated at 135 RPM, which leaves a thin layer of the solid mixture on the wall of the flask. The dried film is then rehydrated with 20 ml phosphate buffer pH (7.4) solution at the temperature of 60-65 ºC for a specified period of time (about 3 h) with gentle agitation. Finally, the prepared niosomal dispersion was stabilized by keeping at 2-8 ºC for 24 h. All the formulations as per experimental design were prepared using a similar procedure by addition of various quantities of span 60 and cholesterol.



Solution was slowly injected into phosphate buffer pH (7.4) | Niosome suspension formed

Rotary Flash Evaporator | Thin Film formed

FIG. 1: PREPARATION OF NIOSOMES BY A) ETHER INJECTION METHOD (EIM) AND B) THIN FILM HYDRATION METHOD (TFHM), RESPECTIVELY

TABLE 1: INDEPENDENT VARIABLES AND THEIR LEVELS IN EXPERIMENTAL DESIGN

Independent Variables	Levels (Actual Code)	
	Low(-1)	High(+1)
X1: Span 60	100	150
X2: Cholesterol	75	100
X3: Chitosan/ PEG-6000	15	25
Dependent variables	**Goals**	
Y1: Drug Entrapment Efficiency (%)	Maximize	
Y2: CDR at 8 hrs (%)	Minimize	

TABLE 2: DESIGN LAYOUT OF EXPERIMENTS AS PER RANDOMIZED FACTORIAL DESIGNS

		Coded Value			Actual Value (mg)		
Run	Drug (mg)	Span 60	Cholesterol	Chitosan/ PEG-6000	Span 60	Cholesterol	Chitosan/ PEG-6000
R1	20	-1	1	-1	100	100	15
R2	20	1	1	1	150	100	25
R3	20	-1	1	1	100	100	25
R4	20	1	-1	-1	150	75	15
R5	20	1	-1	1	150	75	25
R6	20	-1	-1	1	100	75	25
R7	20	1	1	-1	150	100	15
R8	20	-1	-1	-1	100	75	15

Evaluation of Olmesartan Medoxomil Loaded Niosomes:

Standard Curve of Pure Olmesartan Medoxomil in Phosphate Buffer pH (7.4): To make standard curve of olmesartan medoxomil, 20 mg pure drug is taken and dissolved in 100 ml) phosphate buffers pH (7.4). The solution is sonicated for 10 min. Then 10 ml solution was taken and diluted up to 100 ml. Standard stock solution is diluted with phosphate buffer to obtain varying concentrations of stock solutions (2, 4, 6, 8, 10, 12, 14, 16, 18, 20 µg/mL). Then the contents of each flask

immediately transferred to the spectrophotometric cell and the absorbance was recorded at 256 nm and the data were plotted in **Fig. 2**. Finally, the calibration curve was plotted with a concentration on X-axis and respective absorbance on Y-axis.

Analysis of Drug Entrapment Efficiency: Drug entrapment efficiency was measured by measuring the unentrapped free drug. The free drug was determined by subjecting the niosomal formulation to centrifugation at 4000 rpm for 2 hrs to separate the free drug. After centrifugation, the supernatant was collected. The collected supernatant was analyzed for the drug content spectrophotometrically at 256 nm. The percent drug entrapment efficiency was determined by following formula:

% Drug entrapment efficiency (DEE) = (Amount entrapped/ total amount) × 100

***In-vitro* Release Kinetic Model:** The dissolution studies of the niosomes were carried out in type II USP dissolution test apparatus (Paddle Method). At first, 1.5 ml formulated niosomes were taken and filled in the one-end enclosed dialysis tubing with help of a 5 ml plastic disposable syringe. The other end of tubing was also enclosed with thread, and subsequently the dialyze-filled tubing were placed in 900 ml of different dialysate (pH 7.4) medium at 37 ± 0.5°C with a rotating speed of 75 rpm. A 10 ml aliquot was withdrawn from the dissolution medium at pre-determined intervals of 1st h, 2nd h, 3rd h, 4th h, 5th h, 6th h, 7th h and 8th h. At each interval, the withdrawn medium was replaced with an equivalent amount of fresh dissolution medium to maintain ideal sink condition. The withdrawn samples were filtered through 0.45μ membrane filter, and analyzed in UV-VIS spectrophotometer at a wavelength of 256 nm. The percentage of drug release was plotted against time.

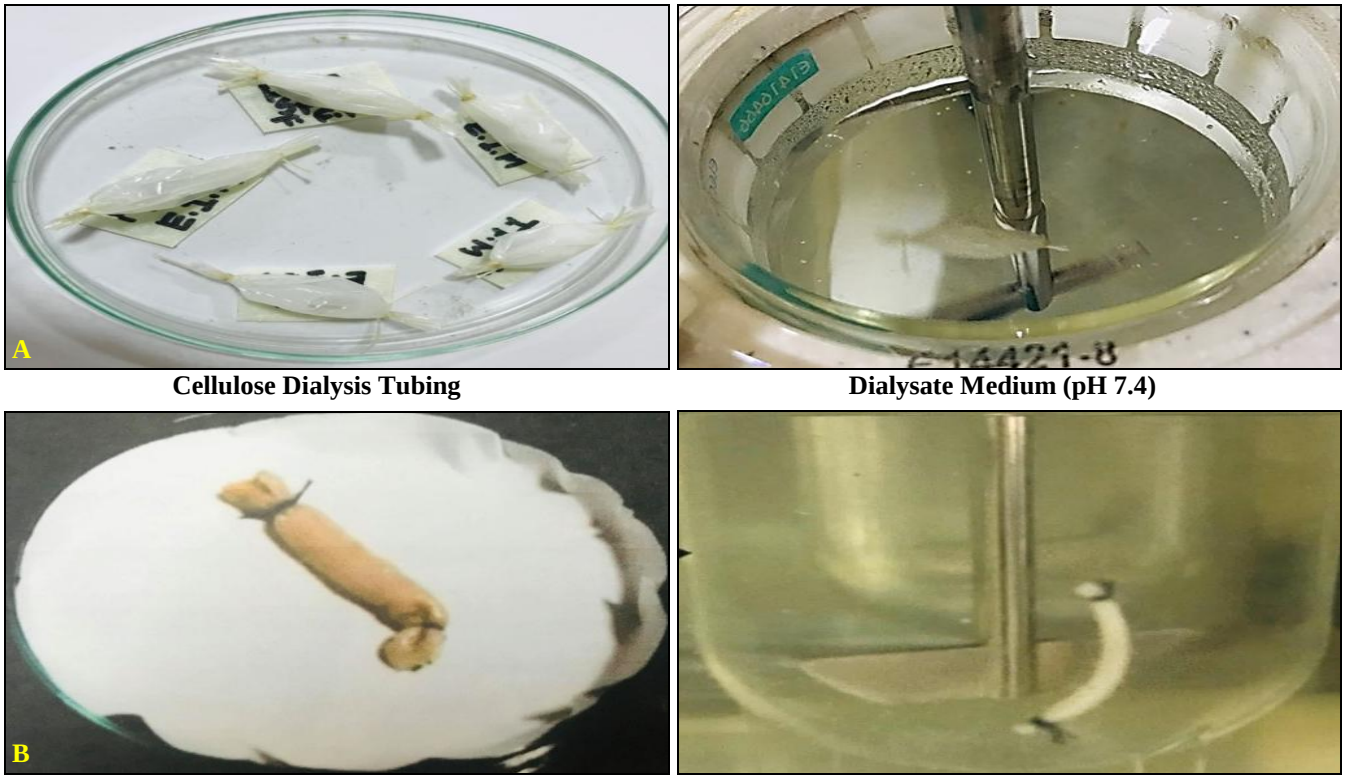
Each experiment was repeated three times. The average percentage of release was calculated for each batch to find a percentage of release. To study the release kinetics data obtained from *in-vitro* drug release studies were fitted to various kinetic equations. The kinetic models used zero-order kinetic, first-order, Higuchi kinetic and Korsmeyer-Peppas model [12, 13]. To find out the mechanism of drug release from different models coefficient of correlation (R^2) values were calculated for the linear curves obtained by regression analysis.

Successive Fractional Dissolution Time: To observe the characterization of the drug release rate in different experimental conditions, MDT, T_{25}%, T_{50}% and T_{80}% was calculated. Mean dissolution time (MDT) value is used to characterize the drug release rate from the dosage form and the retarding efficiency of the polymer. A higher value of MDT indicates a higher drug retaining ability of the polymer and *vice-versa*. The MDT value was also found to be a function of polymer loading, polymer nature and physico-chemical properties of the drug molecule [14].

Permeability Study of Non-Ionic Surfactant Vesicles:

***In-vitro* Permeability Study Using Cellulose Dialysis Tubing:** For permeability study the cellulose membrane was cut into small pieces (9 cm length) and soaked in large volume (1 L) distilled water at room temperature for 30 min after that rinsed membrane thoroughly. At first, 1.5 ml pure drug and formulated niosomes were taken and filled in the one-end enclosed dialysis tubing with the help of a 5 ml plastic disposable syringe. Then, other end of the tubing was also enclosed with thread and subsequently the dialyze filled tubing were placed in 900 ml of different dialysate (pH 7.4) medium. The sample tubing were allowed to rotate in the dissolution vessel of a USP II paddle dissolution test apparatus containing the dialyzing medium maintained at 37 ± 0.5 ºC and stirring speed 75 rpm. Then, 10 ml samples were withdrawn at predetermined time intervals with replacement of fresh dialyzing medium shown in **Fig. 2a**. Withdrawn samples were analyzed for drug content at 256 nm for olmesartan by UV spectrophotometer using the dialyzing medium as a blank after filtration [15].

***Ex-vivo* Permeability Study through Chicken Intestinal Sac:** At first, the chicken intestine sac was collected and rinsed with Krebs-Ringer solution. Then 6 cm long cutting sacs were prepared by tying up one end of the intestinal segments with thread. *Ex-vivo* diffusion study was conducted using chicken intestinal sac in the same manner used *in-vitro* studies shown in **Fig. 2b.** Finally, the permeability of pure drug and different formulations was analyzed by determining the permeability coefficient using Fick's Law of diffusion [16].



Cellulose Dialysis Tubing | Dialysate Medium (pH 7.4)

Chicken Intestinal Sac | Dialysate Medium (pH 7.4)

FIG. 2: PERMEABILITY STUDY OF OLMESARTAN LOADED NIOSOMES A) *IN-VITRO* AND B) *EX-VIVO* STUDY

Stability Studies: To confirm the stability of niosomes, ICH specifies the length of study and storage conditions. The best formulation of niosomes were stored at a different temperature at 25ºC ± 2ºC at 65% RH and 40ºC ± 2ºC at 75% RH for 3 months are storing at 2°C-8°C, room temperature 25°C/60% RH and elevated temperature 40°C/75% 90 days [16].

Particle Size and Size Distribution: For determination of niosome size, electron microscopy method is used to viewing of the possibility of the niosomes individually and obtaining the exact information about the niosome population over the whole range of sizes [17].

Zeta Potential Analysis: Zeta potential analysis is done for determining the colloidal properties of the prepared formulations. The suitably diluted niosomes derived from pronoisome dispersion were determined using a zeta potential analyzer based on electrophoretic light scattering and laser doppler velocimetry method. The temperature was set at 25°C. Charge on vesicles and their mean zeta potential values with standard deviation of measurements were obtained directly from the measurement. Often zeta potential values <-30 mV or >+30 mV are considered stable [17].

Drug-Excipient Compatibility Study:

Fourier Transform Infrared Spectroscopy (FTIR): Drug-excipient compatibility studies were done in order to evaluate any interaction between drug and excipients used in the preparation of niosomes. FTIR spectroscopy was carried out to check the compatibility between olmesartan medoxomil and span 60/cholesterol/chitosan/PEG-6000 used.

Scanning Electron Microscopy (SEM): Vesicle size of selected niosomal dispersion was determined by optical microscope, and vesicle size, shape, and surface property of the selected formula was studied using Scanning Electron Microscope.

Optimization using the Desirability Function: To optimize the multiple responses, they should be highly correlated with each other. In the present study, all two responses were simultaneously optimized by a desirability function that uses the numerical optimization method in the design expert software. Recently, the desirability function approach was reported in several articles for the optimization of multiple responses. Any response that falls outside the desired limit is considered completely unacceptable [18].

RESULTS AND DISCUSSION:

Standard Curve of Olmesartan: Absorbance was taken at 245 nm with the help of a UV-Visible spectrophotometer and the data were plotted in graph which has $y = 0.1456x + 0.0107$ and $R^2 = 0.999$ shown in **Fig. 3**.

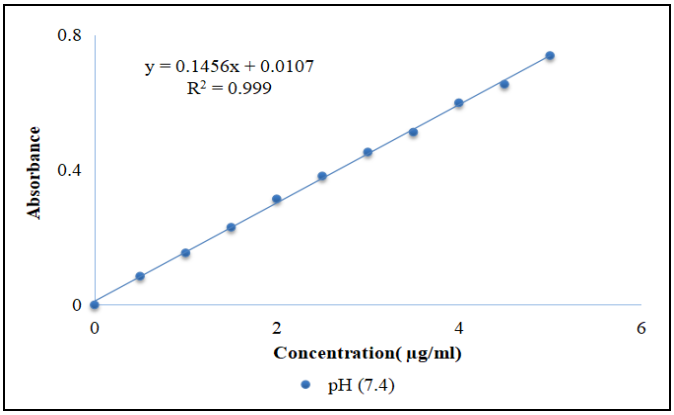


FIG. 3: STANDARD CURVE OF OLMESARTAN

Drug Entrapment Efficiency: Percent drug entrapment efficiency (% DEE) of olmesartan medoxomil loaded niosomes based on different excipients prepared by EIM and TFHM shown in **Fig. 4**.

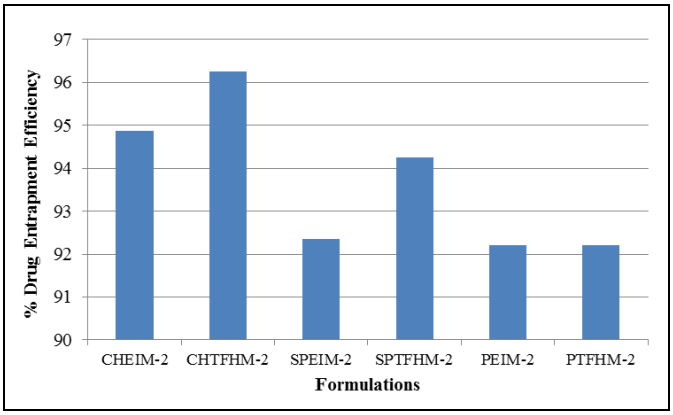


FIG. 4: % DEE OF NIOSOMES FORMED BY EIM AND TFHM

Drug entrapment efficiency analysis was carried out by using UV-spectrophotometer as described earlier. **Fig. 4** reflects that the relationship of excipients with drug entrapment efficiency of olmesartan loaded best formulations of niosomes. From the figure, it was observed that the maximum concentration of span 60 and cholesterol increase the drug entrapment efficiency. Cholesterol decreases the leakage of niosomes by increasing the fluidity. However, increasing the cholesterol beyond a certain concentration can disrupt the regular linear structures of the niosomal membranes, thereby reducing entrapment efficiency. In addition, it observed that the addition of PEG-6000 and chitosan also influence the drug entrapment efficiency. Moreover, the drug entrapment efficiency was increased by increasing the concentration of PEG-6000 and chitosan in formulations. This could be due to the presence of PEG-6000 and chitosan, placed on the outer surface of the lipid bilayer, causing an increase in drug entrapment within the bilayer.

***In-vitro* Drug Release Studies of Olmesartan Niosomes:** Drug release data from the olmesartan loaded niosomes were studied using different kinetic models, which include: Zero-order model, First-order model, Higuchi model, and Korsmeyer-Peppas model; results of the kinetic profiles graphically presented in **Fig. 5.**

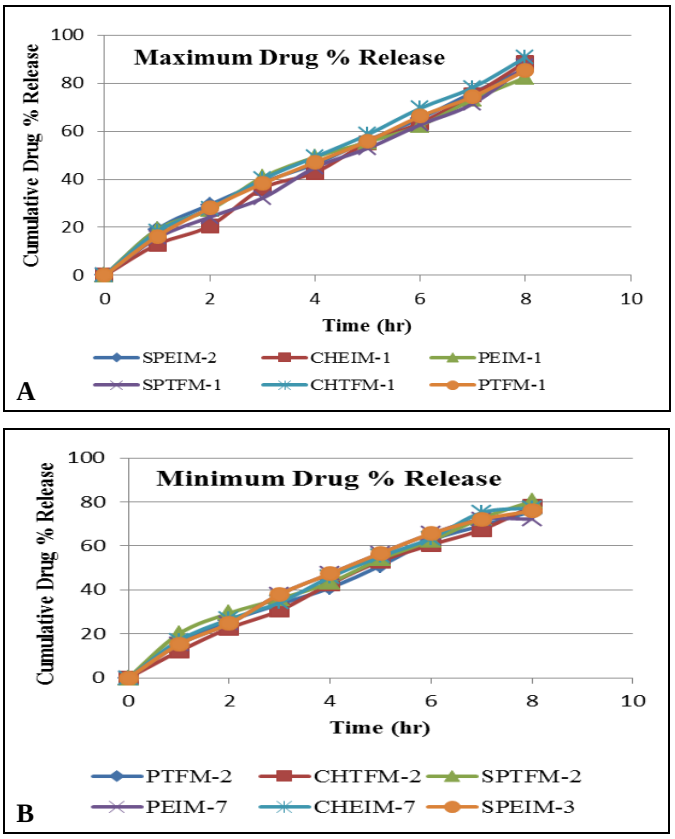


FIG. 5: RELEASE KINETIC PLOT OF A) MAXIMUM AND B) MINIMUM DRUG % RELEASE OF DIFFERENT FORMULATIONS, RESPECTIVELY

Fig. 5a and **5b** has been shown that the minimum and maximum cumulative drug release were observed to be 75.90% and 85.91%, 77.32% and 88.74% and 72.18% and 82.40% for SPEIM-2 and SPEIM-1, CHEIM-2 and CHEIM-1 and PEIM-2 and PEIM-1; 80.24% and 86.11%, 80.11% and

90.72% and 75.67% and 85.29% for SPTFH-2 and SPTFH-1, CHTFH-7 and CHTFH-1, PTFH-2 and PTFH-1, respectively after 8 hours dissolution. The overall results of *in-vitro* release studies reveal that formulated niosomes have the ability to sustain the drug release for a longer period.

From the figure, it can be said that with increasing the concentration of cholesterol and span, the drug release from the formulation decreases which may due to the increased rigidity of the vesicles. In addition, chitosan-based formulations had maximum percentage of drug release than span and PEG based formulations, which may be due to the presence of chitosan in formulations by decreasing the thickness of niosomal vesicles and increasing the drug solubility.

Moreover, PEG-based formulations had slow and minimum drug release than others which may occur due to PEG-6000 incorporation into vesicles.

Data demonstrated in **Fig. 5** showed the release pattern of olmesartan-loaded niosomes. Release data were treated with a statistical level of significance ($\alpha = 0.05$), and they were found statistically significant since in every case, "p" value was found less than 0.05 shown in **Table 5a**.

Studies of Release Rate Constants and Correlation Coefficient (R^2) Values: To find out the probable drug release mechanisms the release rate constants and correlation coefficient (R^2) values of different models were studied. **Table 3** has shown the release rate constants and correlation coefficient (R^2) values of fifteen formulations of olmesartan loaded niosomes from different release rate kinetics: Zero-order, First order, Higuchi model, and Korsmeyer-Peppas model. **Table 3** indicated that all the formulations were best fitted with zero-order models. The probable drug release mechanism could be fickian (class I) diffusion as the release exponent (n) was less than 0.43.

TABLE 3: INTERPRETATION OF RELEASE RATE CONSTANTS AND R^2 VALUES FOR DIFFERENT RELEASE KINETICS OF DIFFERENT NIOSOMAL FORMULATIONS

Formulation Code	Zero Order		First Order		Higuchi		Korsmeyer-Peppas	
	K_0	R^2	K_1	R^2	K_H	R^2	n	R^2
SPEIM-2	9.215	0.981	-0.170	0.976	27.844	0.964	0.325	0.940
CHEIM-1	10.769	0.997	-0.239	0.893	31.477	0.916	0.235	0.989
PEIM-1	9.808	0.986	-0.210	0.880	29.036	0.930	0.245	0.983
SPTFHM-1	9.823	0.983	-0.214	0.927	29.601	0.960	0.234	0.875
CHTFHM-1	10.745	0.993	-0.260	0.904	32.038	0.95	0.269	0.879
PTFHM-1	10.291	0.993	-0.228	0.923	30.69	0.95	0.266	0.929

Stability Studies: According to ICH guidelines, accelerated stability studies were carried out. For stability data formulation CHTFH-2 was taken and studied for 90 days. **Table 4** showed that the prepared niosomes pass stability studies with no much significant change in physical appearance, particle size, drug entrapment efficiency, and *in-vitro* drug release.

TABLE 4: STABILITY STUDIES OF NIOSOMES AT 40 ºC ± 2 °C / 75 ± 5 % RH

Parameter Studies	Initial (mean ± SD, % RH)	30 days (mean ± SD, % RH)	90 days (mean ± SD, % RH)
Physical appearance	Cloudy, off white color, odorless	No Change	No Change
Particle size (μm)	3.50±0.5	3.45±0.4	3.41±0.2
Drug entrapment efficiency	96.25±0.1	96.11±0.4	95.53±0.1
In-vitro drug release	90.72±0.2	89.54.1±0.1	88.21±0.3

Particle Size Analysis: Effects of excipients on particle size of niosomes have shown in **Fig. 6**. The particle size of all formulations was found within the range of 3.51 to 4.19 μm. It was found that cholesterol had direct effect on increasing niosomes particle size significantly. Particle size of niosomal formulations was increased on increasing the cholesterol content. At low cholesterol content, it is to be expected that the cholesterol and phospholipid are in close packing with increasing curvature and reducing flexibility and size. As the cholesterol content increases, it increases rigidity structure of bilayer membrane which also provides resistance to reduce size and results in vesicles with bigger size. The size of niosomes of span-based formulations larger than chitosan and PEG-based niosomes.

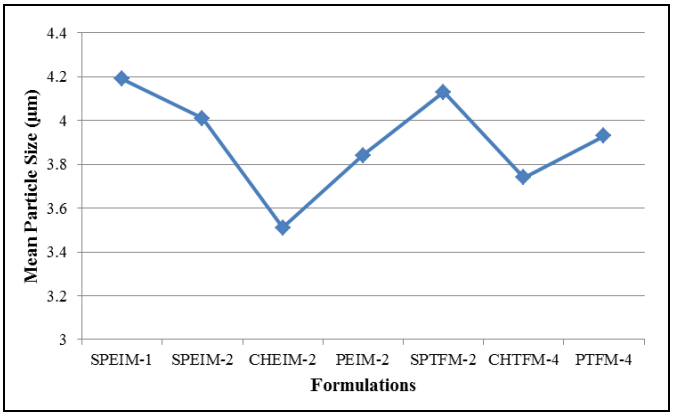


FIG. 6: PARTICLE SIZE OF NIOSOMES

Zeta Potential Study of Non-Ionic Surfactant Vesicles: A zeta potential value other than −30 mV to +30 mV is generally considered to have sufficient repulsive force to attain better physical stability. On the other hand, low values, smaller than 5 mV, can lead to agglomeration. A large positive or negative value of zeta potential of non-ionic surfactant vesicles indicates good physical stability of niosomal suspensions due to electrostatic repulsion of individual particles. **Fig. 7** showed that all the formulations were within -29.2 mV to -35 mV, no one is less than -5 or +5 mV.

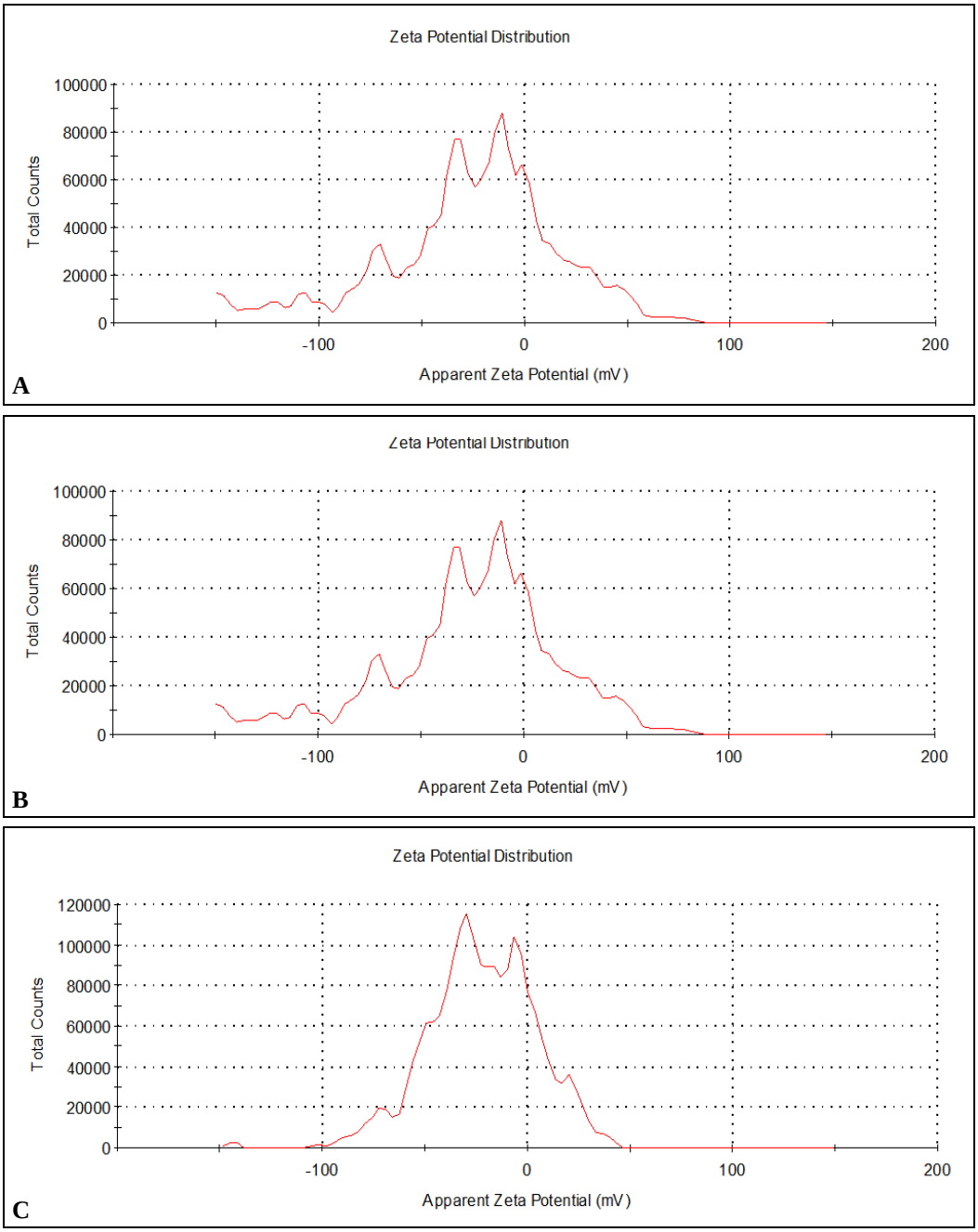


FIG. 7: ZETA POTENTIAL ANALYSIS OF OLMESARTAN LOADED NIOSOMAL FORMULATIONS A) CHTFM-2 B) SPEIM-2 AND C) PEIM-2, RESPECTIVELY

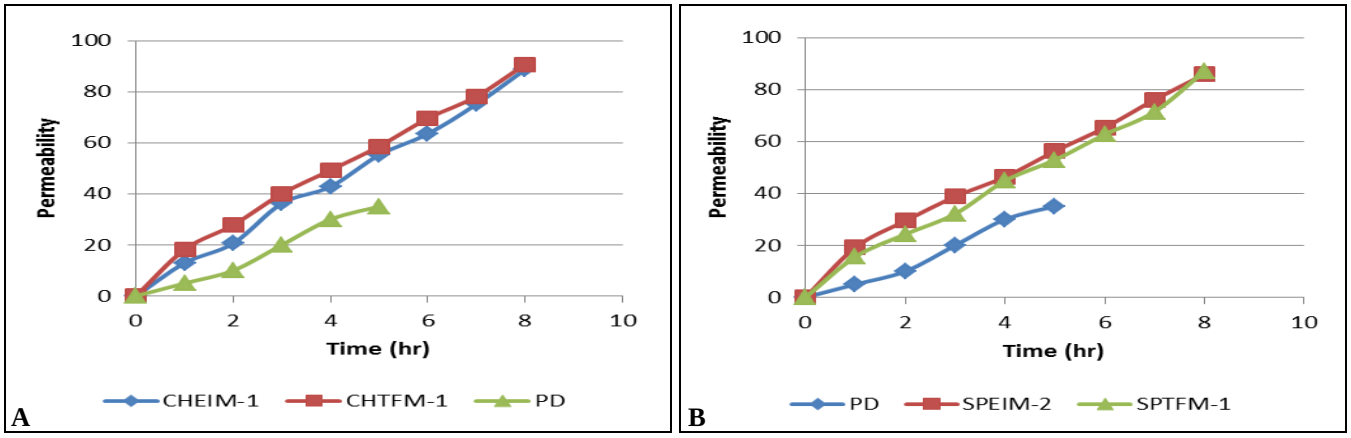
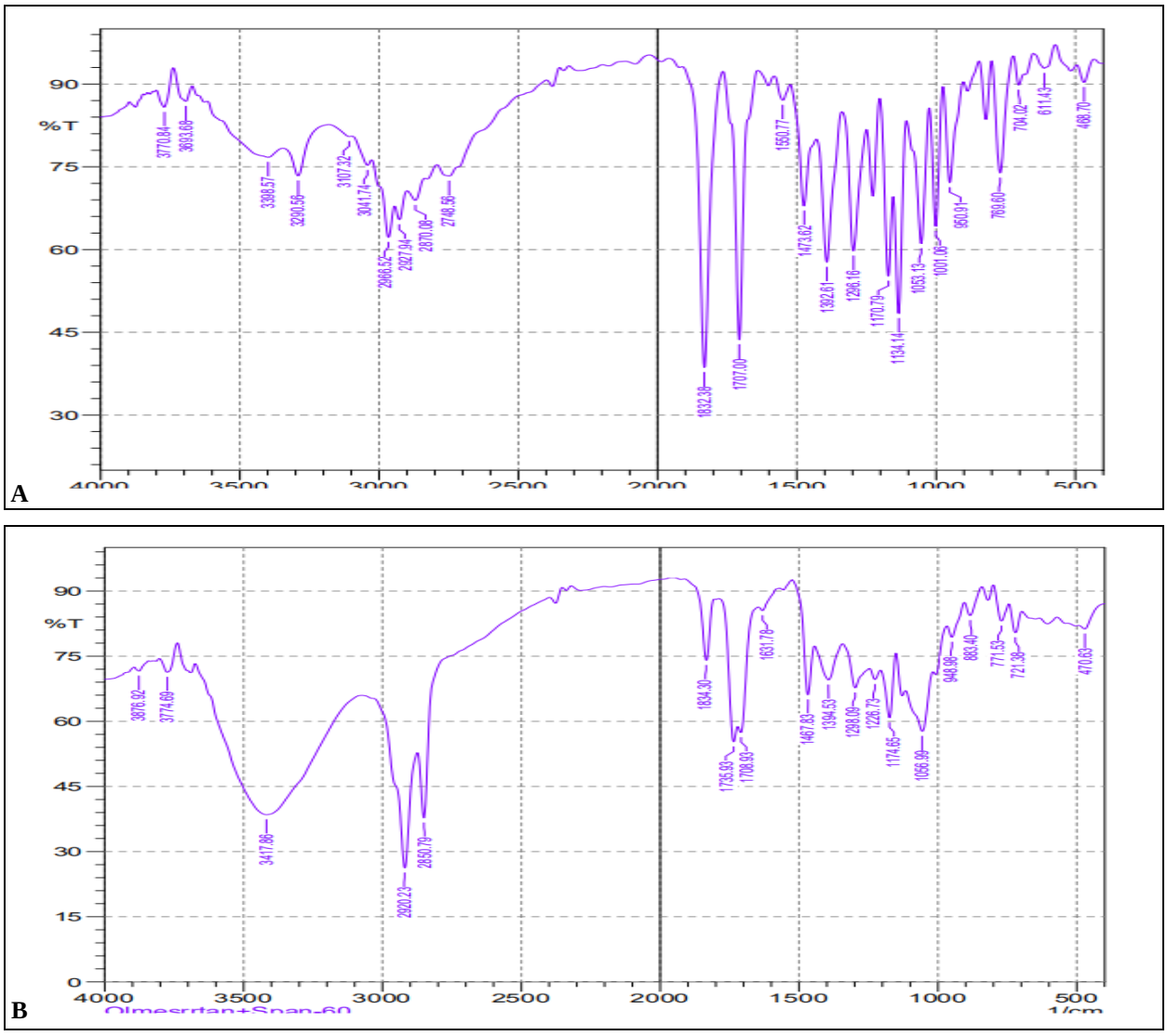


FIG. 8: PERMEABILITY STUDY OF OLMESARTAN LOADED NIOSOMES A) *IN-VITRO* RELEASE PLOT AND B) *EX-VIVO* RELEASE PLOT RESPECTIVELY

Drug Interaction Studies by FTIR: From **Fig. 9**, it was observed that the main functional group peaks are in the range of reported values in both the pure drug and the formulation, indicating no drug-polymer interactions. There was no appearance of any characteristic peaks that were shown in FTIR spectrum of pure drug, niosomal formulations SPEIM-1, CHEIM-6, and PEIM-2. This revealed that there was no interaction between the drug and excipients used in the preparation of niosomes.



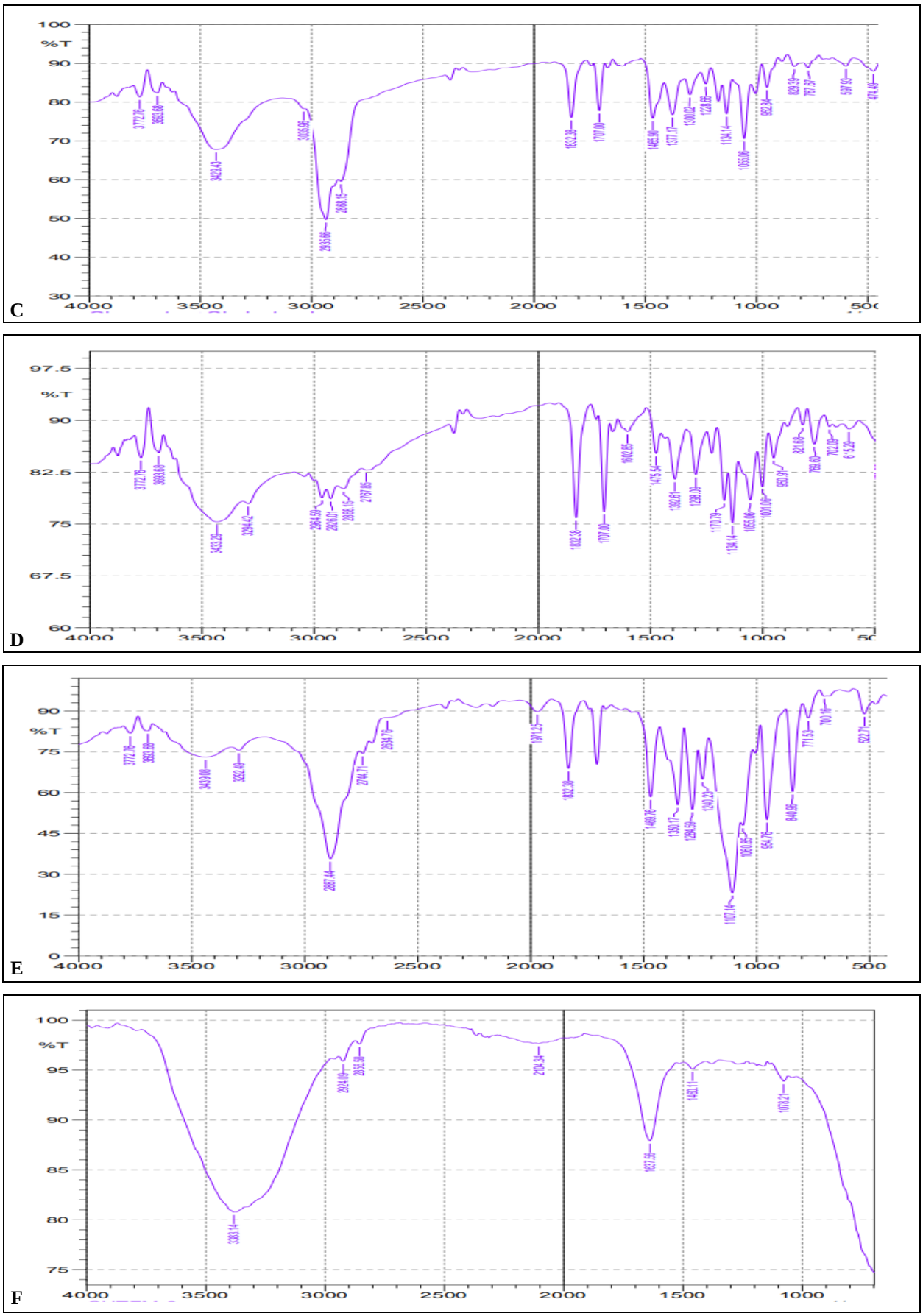


FIG. 9: FTIR IMAGE ON A) PURE DRUG B) DRUG+SPAN 60 C) DRUG+CHOLESTEROL D) SPEIM-1 E) CHEIM-6 F) PEIM-2, RESPECTIVELY

Scanning Electron Microscopy of Non-Ionic Surfactant Vesicles: Surface morphology of formulation CHEIM-2 and PEIM-1 shown in **Fig. 10** indicates that niosomal particles were appeared as discrete and round in shape with the irregular surface due to the presence of unentraped drug. SEM showed the morphology of the lipids and the arrangement of the lamellar structure the encore the drug molecules.

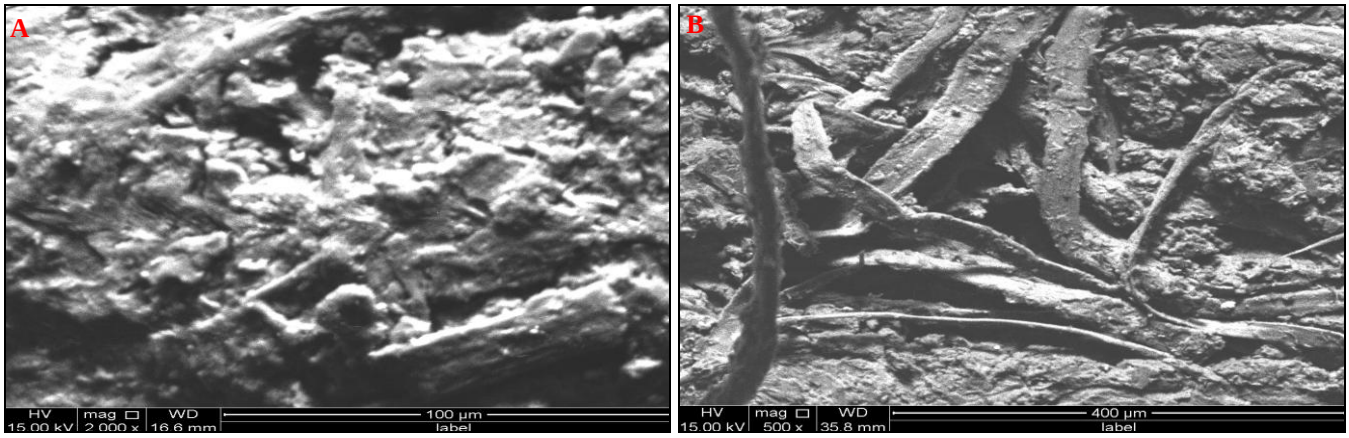


FIG. 10: SEM OF FORMULATION A) CHEIM-2 AND B) PEIM-1 RESPECTIVELY

Statistical Analysis using ANOVA: To evaluate statistical significance analysis of variance (ANOVA) was determined by using design of expert of the factorial design shown in **Table 5a** and **5b** and **Fig. 11** and **12**. In the ANOVA test, the *p* values of the model for all responses (Y1 and Y2) were less than 0.0001. Thus, it can be concluded that all the responses (Y1 andY2) fitted the quadratic model ($p<0.05$). Moreover, in the 'lack of fit' test, which is another good statistical parameter for checking the better fitness of the model, all the responses fitted in the quadratic model by showing a non-significant lack of fit ($p>0.1$). In this study, the R^2 values for the responses Y1 and Y2 were 96.6% and 89.8% for CHTFH-1 and 92.9% and 87.1% for CHEIM-1, respectively.

TABLE 6A: ANOVA AND LACK OF FIT TESTS OF THE QUADRATIC MODEL FOR THE RESPONSES (Y1 AND Y2) OF CHTFHM AND CHEIM -1, RESPECTIVELY

Run	Response		F Value	Probability > F	Comment
CHTFH-1	Y1	Model	85.33	< 0.0001[a]	Significant
		Lack of Fit	5.14	0.2095[b]	Not significant
	Y2	Model	166.19	< 0.0001[a]	Significant
		Lack of Fit	0.42	0.8539[b]	Not significant
CHEIM-1	Y1	Model	86.32	< 0.0001[a]	Significant
		Lack of Fit	1.12	0.4940[b]	Not significant
	Y2	Model	81.75	< 0.0001[a]	Significant
		Lack of Fit	0.29	0.9437[b]	Not significant

[a] Significance probability values (Probability>F) less than 0.05 implies that the model is significant; [b] Non-significant lack of fit (p value > 0.1) proves the adequacy of model fit.

TABLE 6B: SUMMARY OF THE REGRESSION ANALYSIS OF THE RESPONSES (Y1 AND Y2) OF DIFFERENT FORMULATIONS

Run	Quadratic Model	R^2	Adjusted R^2	Predicted R^2	Adequate Precision	SD	CV (%)
CHTFH-1	Y1	0.966	0.961	0.870	19.06	0.92	1.07
	Y2	0.898	0.959	0.942	27.53	2.60	0.56
CHEIM-1	Y1	0.929	0.839	0.737	15.97	1.09	1.21
	Y2	0.871	0.861	0.814	14.51	4.44	1.27

Response Surface and Contour Plot Analysis: Three-dimensional response surface plots and two-dimensional contour plots of the responses across the selected factors were constructed to further elucidate the relationship between the independent and dependent variables, as shown in **Fig. 11** and **12**. These plots are useful for studying the interaction effects between two factors and for

understanding how the effect of one factor will be influenced by the change in the level of another factor. As these types of plots can only express two independent variables at a time against the response, one independent variable must always be fixed.

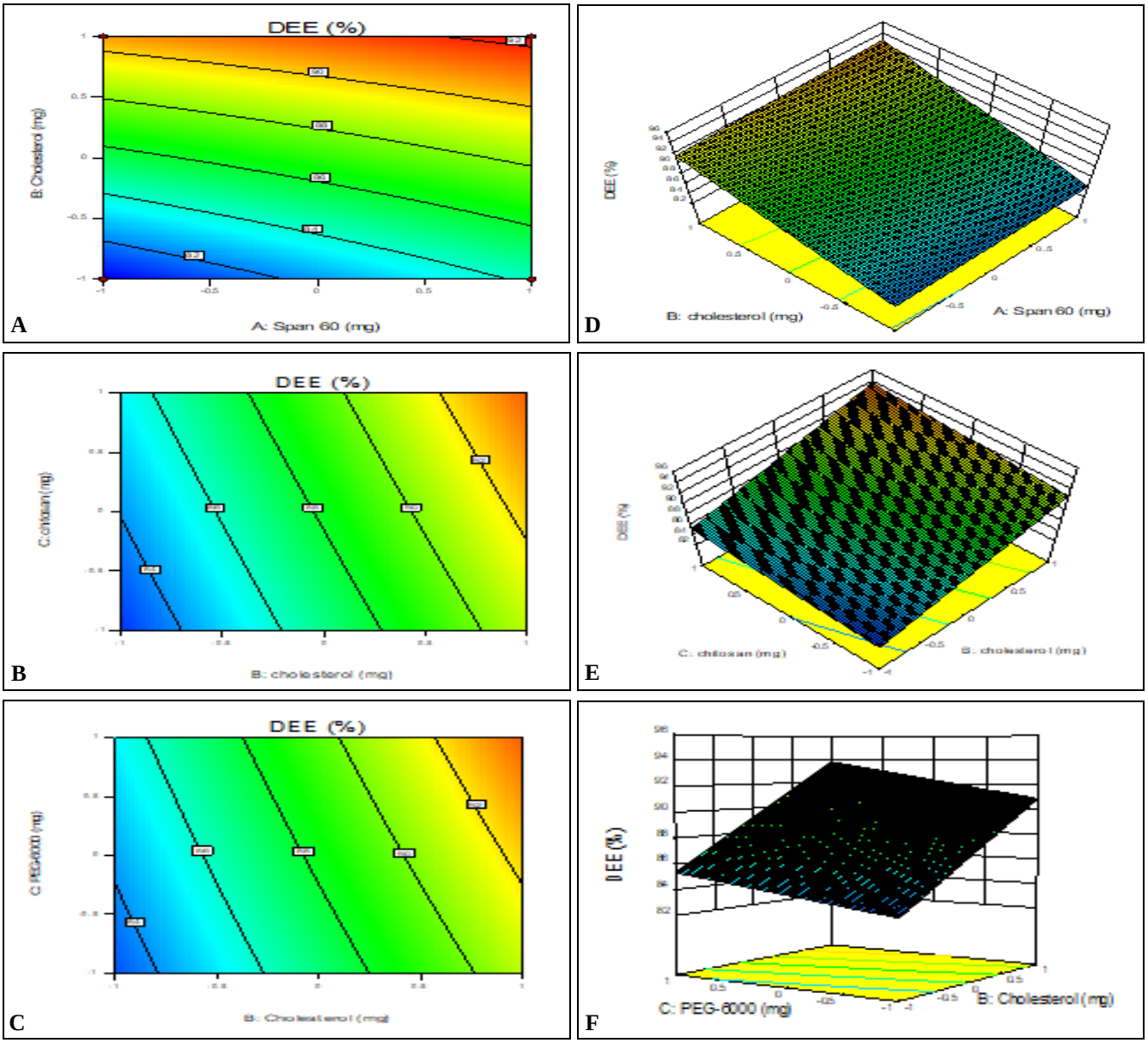
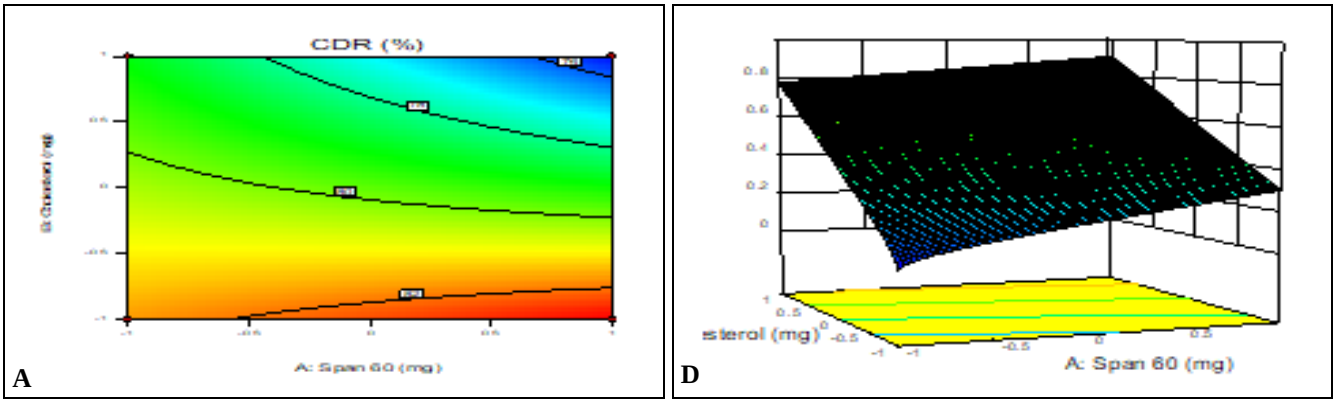


FIG. 11: CONTOUR PLOT SHOWING THE EFFECTS OF A) X1 AND X2 B) X2 AND X3 C) X2 AND X3 ON THE % OF DRUG ENTRAPMENT EFFICIENCY, RESPONSE SURFACE SHOWING THE EFFECTS OF D) X1 AND X2 E) X2 AND X3 F) X2 AND X3 ON THE % OF DRUG ENTRAPMENT EFFICIENCY, RESPECTIVELY



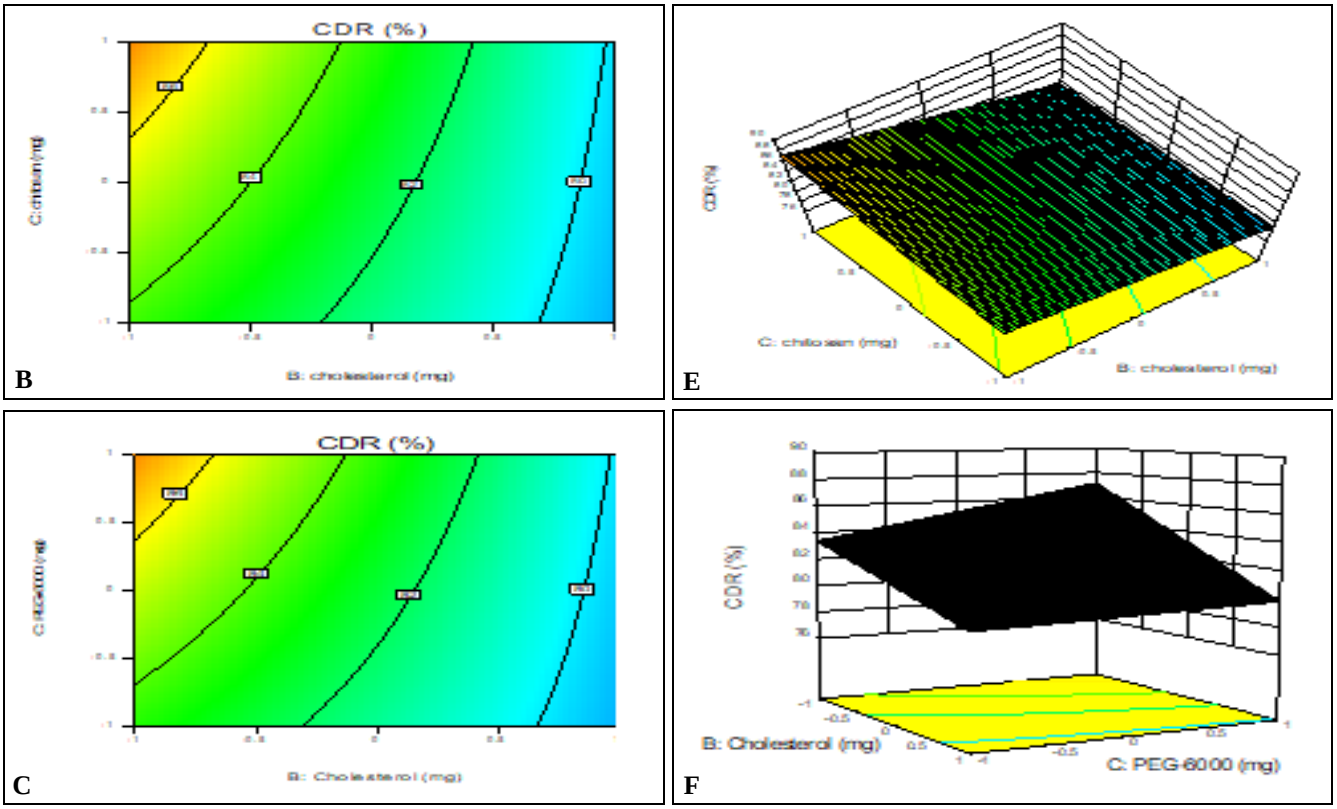


FIG. 12: CONTOUR PLOT SHOWING THE EFFECTS OF A) X1 AND X2 B) X2 AND X3 C) X2 AND X3 ON THE % OF CUMULATIVE DRUG RELEASE, RESPONSE SURFACE SHOWING THE EFFECTS OF D) X1 AND X2 E) X2 AND X3 F) X2 AND X3 ON THE % OF CUMULATIVE DRUG RELEASE, RESPECTIVELY

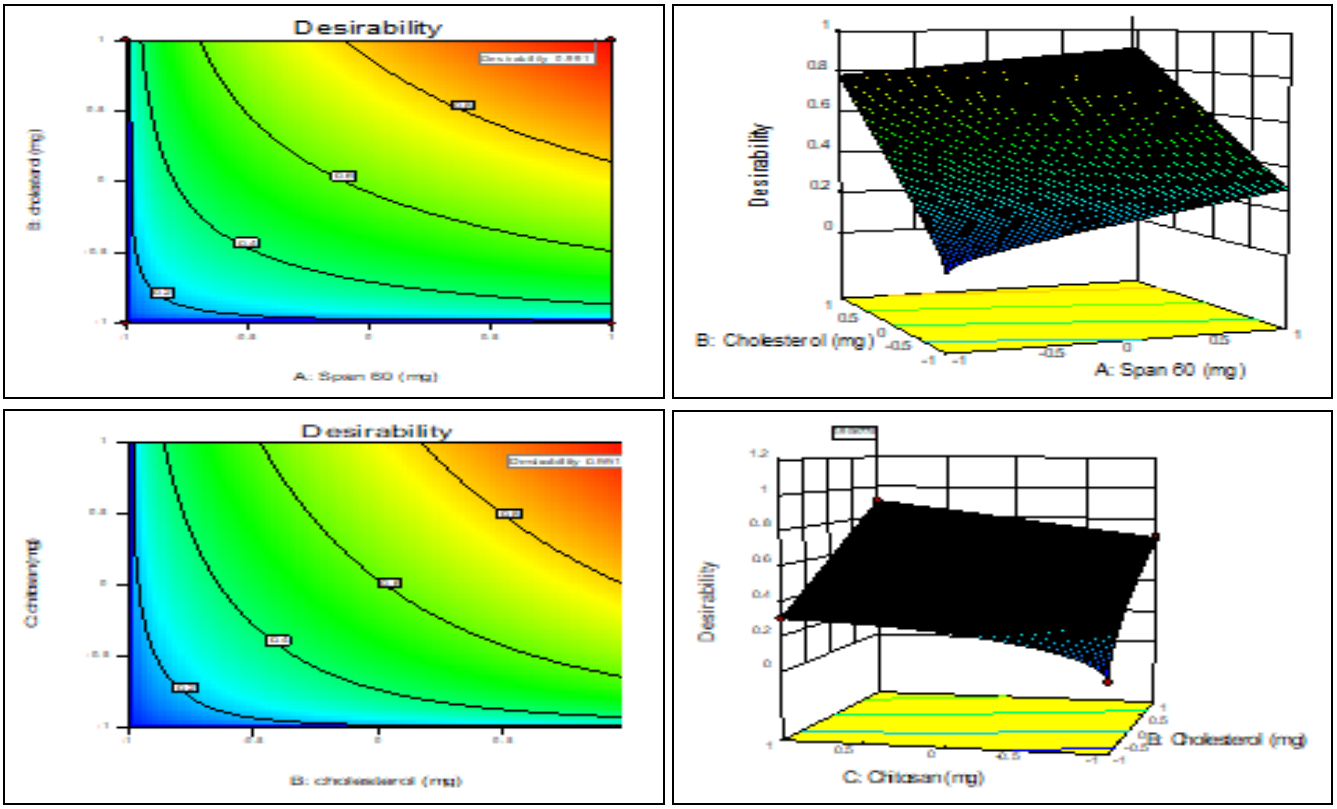


FIG. 13: CONTOUR PLOT SHOWING OVERALL DESIRABILITY (D) AS A FUNCTION OF X1, X2, X3 FOR A) SPAN BASED NIOSOMES AND B) CHITOSAN BASED NIOSOMES AND RESPONSE SURFACE PLOT SHOWING OVERALL DESIRABILITY (D) AS A FUNCTION OF X1, X2, X3 FOR C) SPAN BASED NIOSOMES AND D) CHITOSAN BASED NIOSOMES, RESPECTIVELY

Optimization using the Desirability Function: After studying the effects of the dependent and independent variables on the responses, the independent variables were simultaneously optimized for all three responses by using the desirability function. Responses Y1 and Y2 were transformed into individual desirability scales d1 and d2, respectively. Constraints were set against all of the responses. Among the responses, Y1 and Y2 were set to be maximized. Equal weight and importance were given to all of the responses. Finally, the global desirability value was calculated by combining the individual desirability function as the geometric mean by an extensive grid search, and feasibility search over the domain by the Design-Expert software (Stat- Ease Inc.) that are shown in **Table 7** and **Fig. 13** shows the response surface and counterplot for the desirability function holding the variable X1, X2, X3.

TABLE 7: CALCULATED VALUES FOR OPTIMIZED SOLUTION

Formulation	X1	X2	X3	Y1	Y2	Desirability
SPEIM-2	150	100	25	94.87	88.74	0.991
CHEIM-1	140	95	25	95.12	89.14	0.995

CONCLUSION: The present study was conducted to design olmesartan loaded controlled release niosomes by ether injection and thin-film hydration methods. Further study was conducted to characterize the effects of various excipients (span, PEG-6000, and chitosan) on the niosomal vesicles. The addition of span and chitosan to formulations showed an increase in entrapment efficiencies of the niosomes, and with an increasing amount of span/ PEG/chitosan, the percentage of drug entrapped into niosomes also increases. *In-vitro* dissolution study showed the sustained release of drug from the niosomes for 8 h. From the *in-vitro* dissolution data, it has been established that the drug dissolution profile could be slowed down by increasing the amount of cholesterol and span in the formulations and which can be improved by the addition of chitosan. Surface morphology indicates that niosomal particles were appeared as discrete and round in shape with the irregular surface due to the presence of the entrapped drug. FTIR data showed the absence of any new functional group and any other interaction between drugs and excipients. In addition, formulated niosomes can be chosen for *ex-vivo* study by the chicken intestine. Zeta potential data showed highly stable niosomal suspension. Formulated niosomes were successfully optimized by employing statistical tool ANOVA and response surface methodology (RSM). By changing concentration of polymer, and excipient evaluation parameter can be modified.

Finally, it can be said that anti-hypertensive olmesartan loaded niosomal drug delivery system might be the potential to sustain the drug release with enhanced solubility as well as bioavailability.

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