Chapter 5B)
Formulation Development: Omiganan Liposomes

Dermal Delivery of Protein/Peptide Based Antimicrobial to Treat Secondary Infection in Psoriasis and Eczema

5B.1 Introduction

The aim of our present study was to formulate and characterize a novel liposomal formulation of Omiganan for dermal delivery. Several hydrophilic/amphiphilic molecules have been encapsulated in the liposomes by a reverse-phase evaporation method. Hence, this method was selected to prepare Omiganan loaded liposomes out of several available methods [1, 2]. A systematic Quality-by-design (QbD) approach employing statistical design of experiments was utilized to exhaustively assess the impact of material attributes and process parameters on the critical formulation attributes [3, 4].

5B.2 Materials and Instruments

5B.2.1 Materials

Table 5B.1 List of materials

Materials & Reagents	**Manufacturers**
Omiganan	S-Biochem, Kerala, India (Custom synthesis)
Methanol (A.R. & HPLC Grade)	Spectrochem Pvt. Ltd., Mumbai
Soya lecithin	Lipoid GmBH, Germany
Cholesterol	Sigma Aldrich, USA
Dipalmitoylphosphatidylcholine (DPPC)	Lipoid GmBH, Germany
Hydrogenatedsoyphosphatidylcholine (HSPC)	Lipoid GmBH, Germany
Sephadex G-50	Sigma Aldrich, USA
Distilled water	Prepared In-house

5B.2.2 Instruments

Table 5B.2 List of instruments

Equipment	**Manufacturer**
Digital Weighing Balance	Shimadzu, Japan
RP-HPLC with UV Detector (gradient)	Agilent OpenLab CDS EZChrom, India
Vortex mixer	Spinix, Japan
Magnetic stirrer	Remi equipments Pvt Ltd., India
pH meter	Lab India Pvt. Ltd, Mumbai

Bath Sonicator	Remi equipments Pvt. Ltd, India
Filtration assembly	Durga scientific Pvt. Ltd., Baroda
Centrifuge	Remi equipments Pvt. Ltd., India
Distillation assembly	Durga glassware, India
Probe sonicator	Labsonic M, Sartorius, Mumbai, India
Particle size analyzer (Nano-ZS)	Malvern Instrument, UK
Transmission electron microscope	FEI Tecnai, USA

5B.3 Methodology

5B.3.1 Preparation of Omiganan loaded liposomes

Liposomes encapsulating Omiganan were prepared by the reverse-phase evaporation technique [1, 2]. Briefly, the organic phase was prepared by dissolving all lipids (HSPC, DPPC, soya-lecithin, and cholesterol – ratio: 5.5:3:1:0.5) in 2 ml chloroform in a glass vial under continuous stirring at 1200 rpm on a magnetic stirrer at room temperature. Subsequently, an accurately weighed Omiganan (1%w/w) was dissolved in PBS pH 5.5 and added to the organic phase under continuous stirring (rate of addition was 1 ml/min) on a magnetic stirrer at 1200 rpm to form a water-in-oil emulsion. Later, the organic solvent was removed by continuous stirring (2 hr), and the resultant liposomal vesicles were then subjected to probe sonication for size reduction. Subsequently, this liposomal dispersion was passed through the Sephadex G-50 column to separate the free Omiganan and liposomes.

5B.3.2 QbD approach for the formulation development

Before developing any formulation, there is a strong need to identify formulation variables, process variables, and environmental variables expected to affect the product characteristics. Ishikawa diagram (Fig. 5B.1) was used to determine all the probable variables, i.e., formulation, process, and environment variables associated with the development of Omiganan loaded liposomes by a reverse-phase evaporation method (Table 5B.3).

Table 5B.3 List of variables mainly affecting Omiganan liposomal formulation

Formulation variables	Process variables	Environment variables
Drug (Omiganan)	Mixing of components	Temperature
Concentration of Lipids	The volume of organic phase	
Drug: lipid ratio	Stirring speed	
Organic phase volume	Stirring time	
pH of solution	Rate of addition of organic phase	
	Bead size	

Quality Target Product Profile (QTPP)

For the development of an accurate, precise, and reproducible manufacturing method as a quality target product profile, the following attributes were considered that will ensure desired product quality in all aspects of QTTP:

- ✓ % Entrapment Efficiency and % drug loading: high
- ✓ Vesicle Size: 200-300 nm
- ✓ Drug release from liposomes: NLT 60% in 6 hr

5B.3.2.1 Optimization of Formulation by BBD

The selected variable values for BBD i.e., independent variables and dependent variables (response parameters) are shown in Table 5B.4.

Table 5B.4 Selected values of variables for BBD

Variables	Levels (-1, 0, 1)
Independent variables	
A: Lipid concentration (mg/ml) (X1)	70,105,140 (Drug: lipid ratio - 1:2, 1:4, 1:6)
B: Sonication time (sec) (X2)	30s, 60s, 90s
C: Sonication amplitude (%) (X3)	60, 70, 80
Constant parameters	
Rate of organic phase addition (ml/min)	1 ml/min
The volume of organic solvent (ml)	2 ml
Stirring time (hr)	2 hr

Stirring speed (rpm)	1200 rpm
Temperature (°c)	25-40°C (room temp.)
Dependent variables (response parameters)	
% Drug entrapment (%) (Y1) Vesicle size (nm) (Y2)	

The selection of critical formulation variables was made according to the results obtained in the preliminary investigation. A BBD design matrix was generated using Stat-Ease Design-Expert Software 13.0. Total 17 experimental runs were obtained from the software. All the batches of Omiganan liposomes were prepared according to the design matrix while keeping all other process variables constant. % Drug entrapment and vesicle size of the formulated Omiganan liposomes were taken as response parameters (CQA).

5B.3.2.2 Vesicle size

The vesicle size of Omiganan liposomes were determined using Nano-ZS Zetasizer, Malvern Instruments Ltd., UK. Briefly, Omiganan liposomal dispersions were diluted with filtered distilled water (10 times) and filled into disposable sizing cuvette/folded capillary cells to measure vesicle size.

5B.3.2.3 % Drug entrapment

To determine the % drug entrapment, Omiganan liposomal dispersion was passed through the Sephadex G-50 column. The separated fraction of the liposomes was then dissolved in a mixture of water: methanol (2:8) and analyzed for Omiganan concentration by the developed gradient HPLC technique at λ_{max} of 220 nm. The supernatant was also analyzed to determine the free Omiganan content to ensure mass balance. To determine the % Drug loading, Omiganan liposomal dispersion was centrifuged at 18,000 rpm (4 °C for 60 min). % Drug loading was quantified by using the mass (weight) of the centrifuged pellet of liposomes. The % drug entrapment and % drug loading were found out by using the following equations:

$$\%\ \boldsymbol{Drug\ entrapment} = \frac{\boldsymbol{Amount\ of\ Entrapped\ drug}}{\boldsymbol{Total\ drug\ added}}\ \boldsymbol{x\ 100}$$

$$\%\ \boldsymbol{Drug\ loading} = \frac{\boldsymbol{Drug\ loaded\ (mg)}}{\boldsymbol{Total\ weight\ of\ liposomes\ (mg)}}\ \boldsymbol{x\ 100}$$

5B.3.2.4 Preparation of gel for Omiganan and Omiganan liposomes

The gel base formulation was developed and loaded with Omiganan (1% w/v) or optimized Omiganan liposomes (1% w/v) to increase the retention of formulation on the skin. Briefly, the weighed quantity of Carbopol 934P (1.2%w/v) was mixed and dispersed in Omiganan liposomal dispersion (1%w/v) using an overhead stirrer (2000 rpm for 1 h). Upon complete hydration, methylparaben (0.2%w/v) and propylparaben (0.02%w/v) in propylene glycol (4%w/v), was added under continuous stirring, and the pH adjustment (~6.5) were done by dropwise addition of 10% v/v Sodium hydroxide solution. The detailed formulation composition is showed in Table 5B.5.

Table 5B.5 Formulation components with their concentration used in the preparation of Omiganan liposomal gel

Formulation components	Concentration
Omiganan/Omiganan liposomes	1% w/v
Carbopol 934P	1.20% w/v
Propylene glycol	4% w/v
Methyl paraben and propyl paraben	0.2 and 0.02 %w/v
Sodium hydroxide solution (10%v/v)	…qs…to pH 6.5

5B.3.3 Characterization of optimized Omiganan liposomes and liposomal gel

5B.3.3.1 Zeta potential

The zeta potential of Omiganan liposomal dispersion was measured using a Nano-ZS zeta sizer equipped with a 5-mV He-Ne laser. Briefly, Omiganan liposomal dispersions were diluted with filtered distilled water (10 times) and filled into disposable sizing cuvette/folded capillary cells to measure the zeta potential. An average of 30 measurements of each sample was used to derive average zeta potential.

5B.3.3.2 Shape and surface morphology

The optimized Omiganan liposomes were evaluated using cryo-transmission electron microscopy for shape and surface characteristics (cryo-TEM, FEI Tecnai, USA). Briefly, liposomal dispersion was taken on a carbon film-coated copper grid and air-dried. Subsequently, the sample was stained with a 2% aqueous uranyl acetate

solution. The grid was placed in a sample probe, put in the microscope, and observed with a 20-200 kV operating voltage range with appropriate magnification.

5B.3.3.3 Viscosity of Omiganan liposomal gel

The viscosity of free Omiganan gel and Omiganan liposomal gel was determined using cone and plate rheometer (Bohlin C-VOR, Malvern Instruments Ltd., UK) at 25±1ºC. In brief, 200 mg of the sample was placed on the sample holder. After that, the spindle was lowered and kept for equilibrium for 5 min having a plate width of 20 mm and a cone angle of 4º. Subsequently, the spindle was rotated at a shear rate of 10/s, and viscosity (Pa.S) observed was reported [5].

5B.3.3.4 Spreadability of Omiganan liposomal gel

The spreadability of free Omiganan gel and Omiganan liposomal gel was evaluated by the previously reported method [6]. Briefly, 500 mg of sample was placed on a pre-marked circle with a 1 cm diameter on the glass plate over which a second glass plate was positioned. Subsequently, 500 g weight was applied on the upper glass plate for 5 min, and any change in diameter was reported.

5B.3.3.5 pH of Omiganan liposomal gel

The pH of free Omiganan gel and Omiganan liposomal gel was measured using a digital pH meter (Lab India Pvt. Ltd, Mumbai).

5B.3.3.6 Assay of Omiganan gel

The Omiganan content from the free Omiganan gel and Omiganan liposomal gel was determined by dissolving the 100 mg of sample in the PBS pH 7.4: methanol mixture (8:2 ratio). The amount of Omiganan was quantified by the developed gradient HPLC method.

5B.4 Results and Discussion

5B.4.1 Preparation and optimization of Omiganan liposomes

5B.4.1.1 Establishment of QTPP

All the variables linked with the development of Omiganan loaded liposomes by reverse-phase evaporation technique were identified into Process, Formulation, and Environment. Ishikawa diagram (Fig. 5B.1) was used to demonstrate the variables linked with the development of Omiganan loaded liposomes.

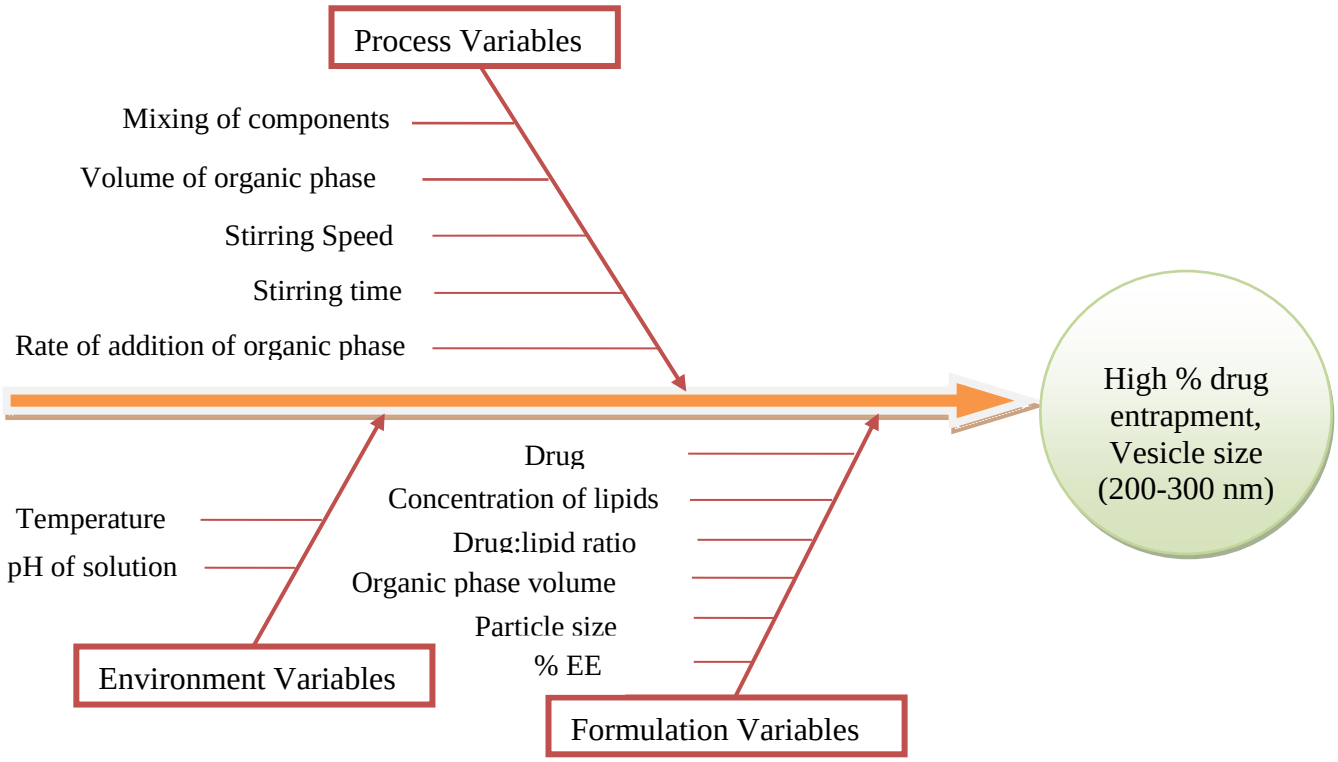

Fig. 5B.1 Ishikawa diagram showing probable variables that may influence CQA

5B.4.1.2 Formulation optimization by Box-Behnken Design

Based on the preliminary investigation, three CMA were identified, and their relationship with CQA was exhaustively investigated using Box-Behnken Design. A randomized matrix of 17 runs was generated by Design-Expert software and presented in Table 5.B.6.

Table 5B.6 Randomized BBD design matrix generated Design-Expert software

Run	Independent variables			Dependent variables (CQA)	
	Lipid Concentration (mg/ml)	Sonication time (second)	Sonication amplitude (%)	% Drug entrapment (%)	Vesicle size (nm)
1	105	60	70	70.78	115.8
2	140	60	80	63.48	148.7
3	105	30	60	66.82	132.5
4	70	60	60	52.95	106.2
5	140	30	70	62.31	153.4
6	140	90	70	65.92	137.1
7	105	90	60	67.09	112.4
8	105	60	70	72.81	117.7
9	105	90	80	61.25	106.8
10	105	60	70	71.65	121.5
11	140	60	60	66.31	149.7
12	105	60	70	73.64	123.1
13	105	30	80	61.68	136.8
14	70	90	70	46.02	97.2
15	105	60	70	72.04	118.9
16	70	30	70	53.27	127.8
17	70	60	80	49.25	100.8

5B.4.1.3 Effect analysis of critical variables on responses

5B.4.1.3.1 Influence of investigated parameters on % Drug entrapment

A) Statistical Analysis for % Drug entrapment

The statistical analysis of the design mentioned above is as follows:

Table 5B.7 Statistical analysis of design for % Drug entrapment

Source	Sequential p-value	Lack of Fit p-value	Adjusted R-Squared	Predicted R-Squared	
Linear	0.0913	0.0005	0.2382	0.0332	
2FI	0.9304	<0.0003	0.0510	-0.6804	
Quadratic	**<0.0001**	**0.4240**	**0.9822**	**0.9350**	**Suggested**
Cubic	0.4240		0.9834		Aliased

As shown in Table 5B.7, the best model to fit the experimental results of drug entrapment in liposomes is the quadratic model and was chosen for further evaluation.

B) ANOVA Analysis for % Drug entrapment

The ANOVA for % Drug entrapment is given in table 5B.8.

Table 5B.8 ANOVA for Response Surface Quadratic Model for % Drug entrapment

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1144.57	9	127.17	98.85	< 0.0001	significant
A-Lipid concentration	399.46	1	399.46	310.49	< 0.0001	
B-Sonication time	1.81	1	1.81	1.40	0.2749	
C-Sonication amplitude	38.33	1	38.33	29.79	0.0009	
AB	29.48	1	29.48	22.92	0.0020	
AC	0.1892	1	0.1892	0.1471	0.7127	
BC	0.1225	1	0.1225	0.0952	0.7666	
A^2	487.33	1	487.33	378.79	< 0.0001	
B^2	87.01	1	87.01	67.63	< 0.0001	
C^2	49.49	1	49.49	38.46	0.0004	
Residual	9.01	7	1.29			
Lack of Fit	4.22	3	1.41	1.17	0.4240	Not significant
Pure Error	4.79	4	1.20			
Cor Total	1153.58	16				

The Model F-value of 98.85 implies the model is significant. In this case, A, C, AB, A^2, B^2, C^2 are significant model terms. The Lack of Fit F-value of 1.17 implies the Lack of Fit is not significant relative to the pure error.

The value of ANOVA shows that the effects of factors were significant; hence, the model is significant for % drug entrapment. From ANOVA table 5B.8, we can observe that F value was high for Factor A (310.49) and Factor C (29.79) than Factor B (1.40), it indicates that all the factors affect the % drug entrapment, which can also be observed visually from the surface plots (contour plots and 3D plots). Among the variables affecting % drug entrapment, lipid concentration and sonication amplitude have maximum effect on % drug entrapment. In addition, the actual v/s

predicted plot for % drug entrapment shows an R^2 of 0.9922 which is a good correlation (Fig. 5B.2)

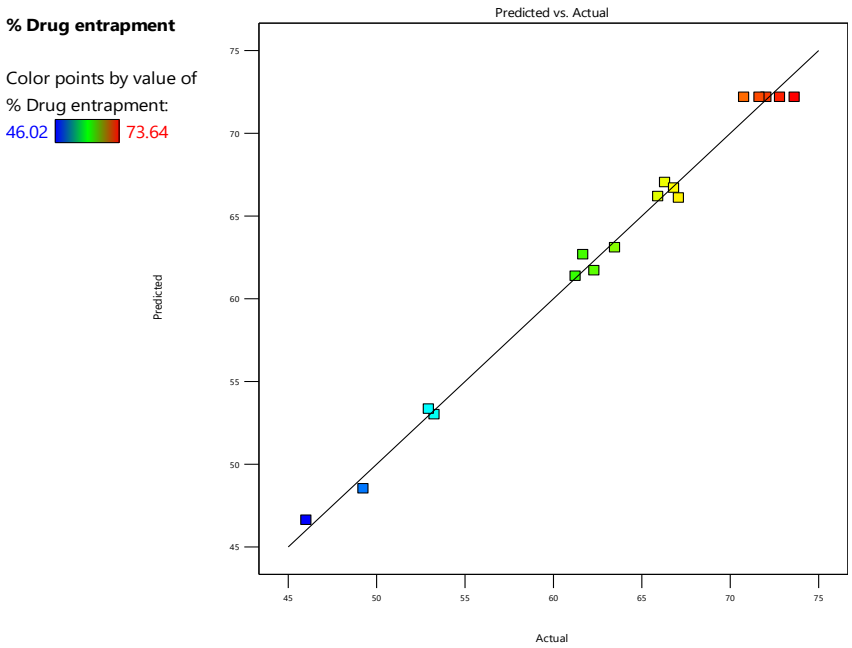

Figure 5B.2 Actual v/s Predicted plot for % Drug entrapment

Table 5B.9 ANOVA study results for % Drug entrapment

Parameters	**Results of Response**
Std Deviation	1.13
Mean	63.37
C.V.%	1.79
R-Squared	0.9922
Adjusted R-Squared	0.9822
Predicted R-Squared	0.9350
Adeq. Precision	29.3817

C) Mathematical Model for % Drug entrapment

To examine the effect of various factors on % drug entrapment, contour plots and the 3D plot were referred to along with the value of ANOVA. From Table 5B.8, we can observe that with change in the combination of various levels of factors, the final response, i.e., % drug entrapment confirming the effect of various factors. Looking closely at different factor involved provide us a better understanding of the extent of the impact. The equation talks about the type of effect that is positive or negative.

The final equation in terms of coded factors:

% Drug entrapment = +72.18+7.07*A-0.4750*B-2.19*C+2.71*AB+0.2175*AC-0.1750*BC-10.76*A^2-4.55*B^2-3.43

The final Equation in Terms of Actual Factors

% Drug entrapment	=
-197.32750	
+1.84752	Lipid concentration
-0.359600	Sonication time
-4.55042	Sonication amplitude
+0.002586	Lipid concentration * Sonication time
+0.000621	Lipid concentration * Sonication amplitude
-0.000583	Sonication time * Sonication amplitude
-0.008782	Lipid concentration²
-0.005051	Sonication time²
-0.034282	Sonication amplitude²

Additionally, from the above equation, we can observe that all the factors affected % drug entrapment to some extent. For example, an increase in lipid concentration increases % drug entrapment. An increase in % drug entrapment with an increase in lipid concentration was observed because of the partitioning of Omiganan into the lipidic phase [7, 8]. While the decrease in % drug entrapment was noted with an increase in sonication time and amplitude to some extent. Fig. 5B.3-5B.8 demonstrates the effects of independent variables on the % drug entrapment. The red area shows the maximum % drug entrapment, and the blue zone represents the area with the lowest % drug entrapment.

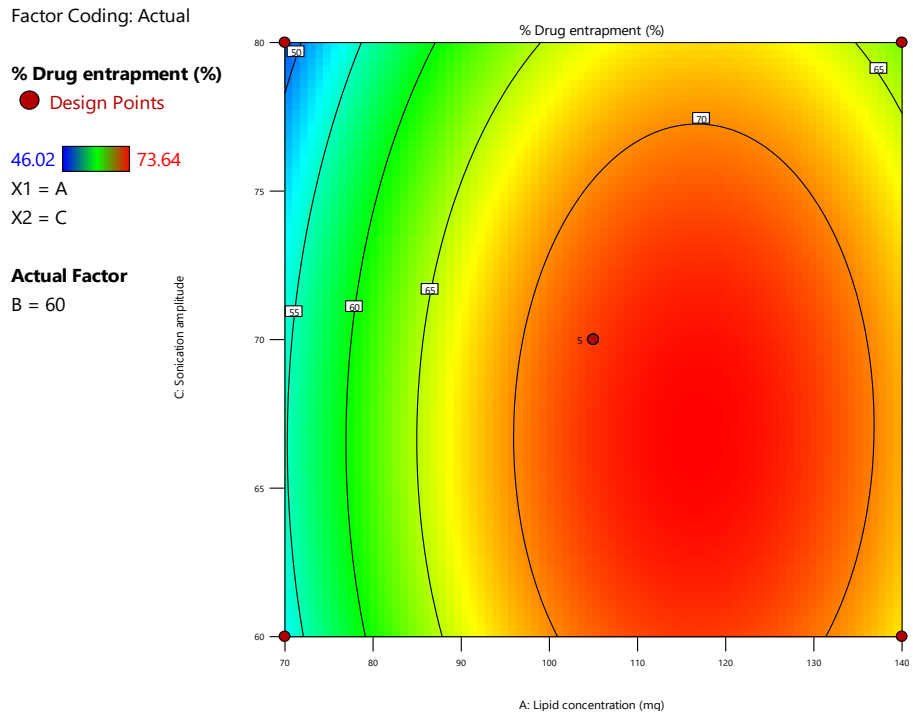

Figure 5B.3 Contour plot (2D) showing the combined effect of lipid concentration and sonication amplitude on % drug entrapment

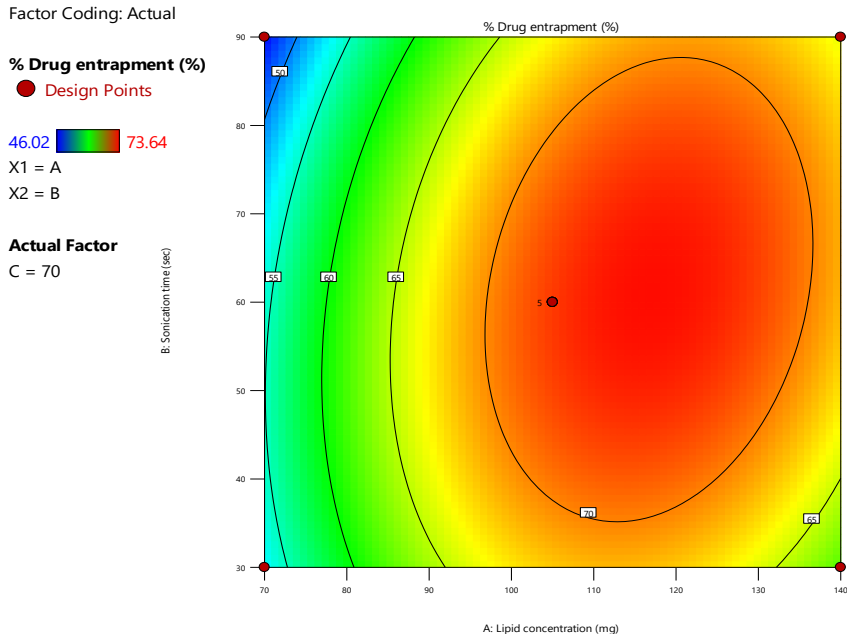

Figure 5B.4 Contour plot (2D) showing the combined effect of lipid concentration and sonication time on % drug entrapment

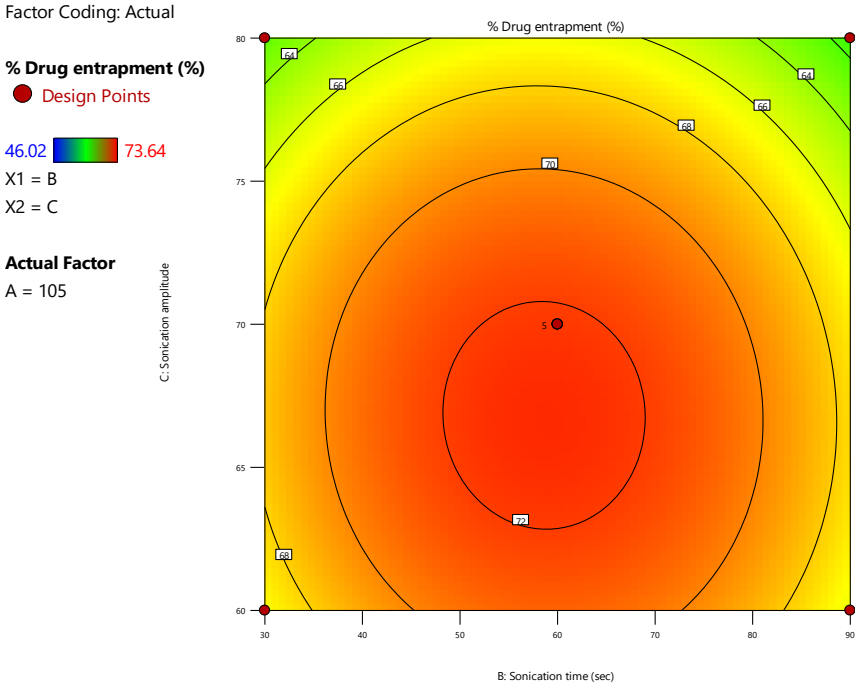

Figure 5B.5 Contour plot (2D) showing the combined effect of sonication time and sonication amplitude on % drug entrapment

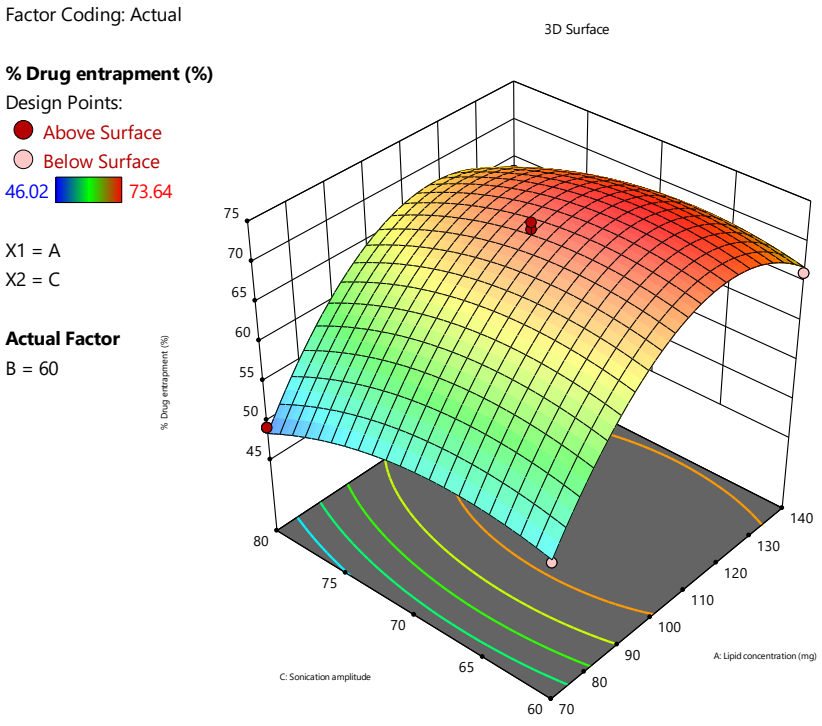

Figure 5B.6 Response surface (3D) showing the combined effect of lipid concentration and sonication amplitude on % drug entrapment

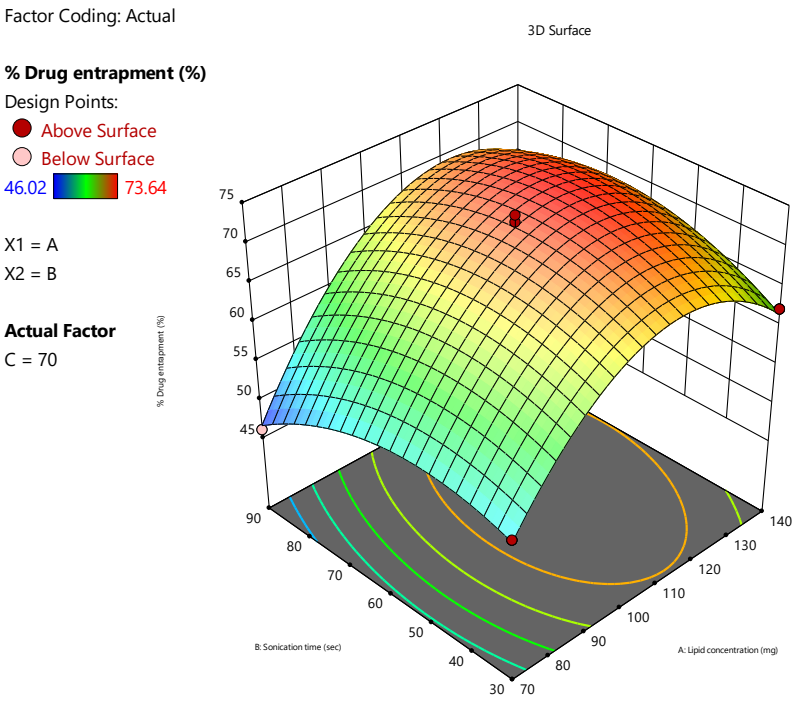

Figure 5B.7 Response surface (3D) showing the combined effect of lipid concentration and sonication time on % drug entrapment

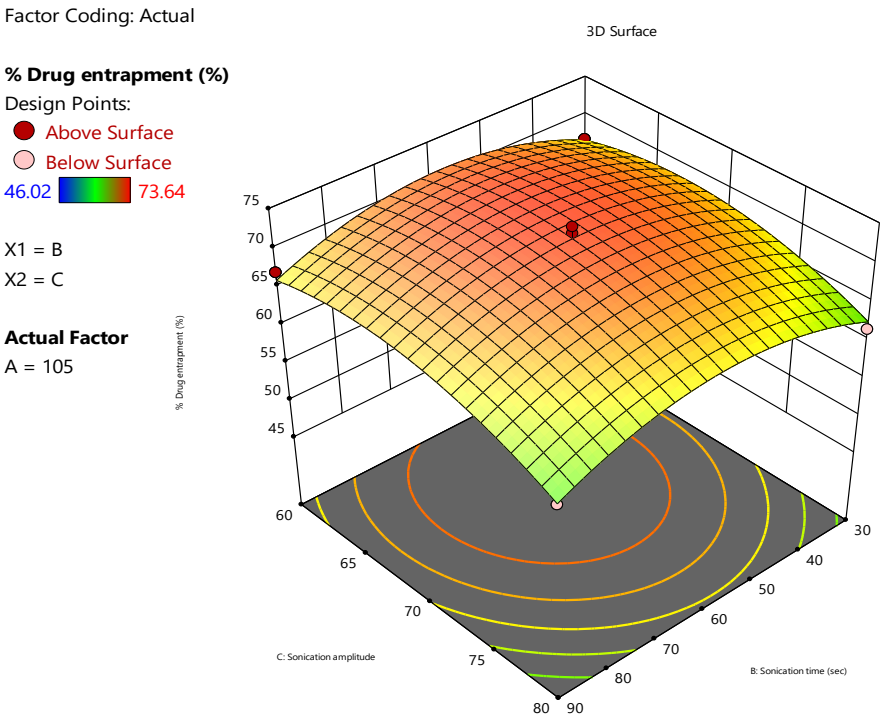

Figure 5B.8 Response surface (3D) showing the combined effect of sonication time and sonication amplitude on % drug entrapment

5B.4.1.3.2 Influence of investigated parameters on vesicle size

A) Statistical Analysis for vesicle size

The statistical analysis of the design mentioned above is as follows:

Table 5B.10 Statistical analysis of design for Vesicle size

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	< 0.0001	0.0622	0.8848	0.8319	
2FI	0.5488	0.0477	0.8777	0.7229	
Quadratic	**0.0462**	**0.1316**	**0.9404**	**0.6878**	**Suggested**
Cubic	0.1316		0.9709		**Aliased**

As shown in Table 5.21, the best model to fit the experimental results of vesicle size in liposomes is the quadratic model and was chosen for further evaluation.

B) ANOVA Analysis for Vesicle size

The ANOVA for vesicle size is given in Table 5B.11.

Table 5B.11 ANOVA for Response Surface Quadratic Model for Vesicle size

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	4578.07	9	508.67	29.05	< 0.0001	significant
A-Lipid concentration	3077.20	1	3077.20	175.72	< 0.0001	
B-Sonication time	1176.12	1	1176.12	67.16	< 0.0001	
C-Sonication amplitude	7.41	1	7.41	0.4232	0.5361	
AB	51.12	1	51.12	2.92	0.1313	
AC	4.84	1	4.84	0.2764	0.6153	
BC	24.50	1	24.50	1.40	0.2755	
A^2	197.57	1	197.57	11.28	0.0121	

B^2	29.01	1	29.01	1.66	0.2390	
C^2	0.0421	1	0.0421	0.0024	0.9623	
Residual	122.58	7	17.51			
Lack of Fit	88.38	3	29.46	3.45	0.1316	not significant
Pure Error	34.20	4	8.55			
Cor Total	4700.65	16				

The Model F-value of 29.05 implies the model is significant. In this case, A, B, A^2 are significant model terms. The Lack of Fit F-value of 3.45 implies the Lack of Fit is not significant relative to the pure error.

The value of ANOVA shows that the effects of factors were significant; hence, the model is significant for % vesicle size. From ANOVA Table 5B.11, we can observe that F value was high for Factor A (175.72) and Factor B (67.16) than Factor C (0.42), it indicates that all the factors affect the vesicle size to some extent which can also be observed visually from the surface plots (contour plots and 3D plots). Among the variables affecting vesicle size, lipid concentration and sonication time have maximum effect on vesicle size. In addition, the actual v/s predicted plot for vesicle size shows an R^2 of 0.9739 which is a good correlation (Fig. 5B.9).

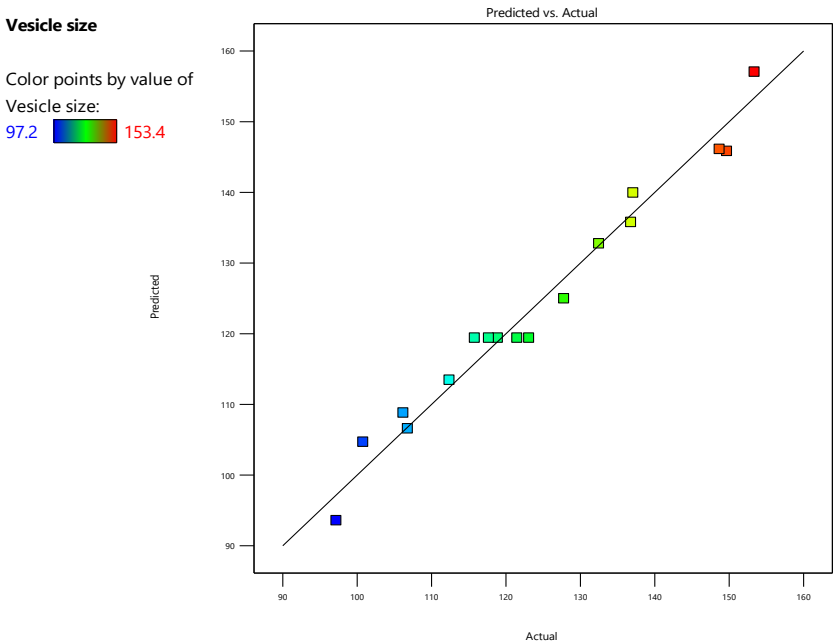

Figure 5B.9 Actual v/s Predicted plot for vesicle size

Table 5B.12 ANOVA study results for vesicle size

Parameters	Results of Response
Std Deviation	4.18
Mean	123.91
C.V.%	3.38
R-Squared	0.9739
Adjusted R-Squared	0.9404
Predicted R-Squared	0.8878
Adeq. Precision	19.7771

C) Mathematical Model for Particle Size

To examine the effect of various factors on vesicle size, contour plots and 3D plots were referred along with the value of ANOVA. From Table 5B.11, we can observe that with change in the combination of various levels of factors, the final response, i.e., vesicle size confirming the effect of various factors. Looking closely at different factor involved provide us a better understanding of the extent of the impact. The equation talks about the type of effect that is positive or negative.

The final equation in terms of coded factors:

Vesicle size = 119.4 + 19.6125 * A - 12.125 * B - 0.9625 * C + 3.575 * AB + 1.1 * AC + -2.475 * BC + 6.85 * A^2 + 2.625 * B^2 + 0.1 * C^2

The final equation in terms of Actual factors:

Vesicle size	=
+178.50000	
+1.03821	Lipid concentration
-0.534167	Sonication time
-0.071250	Sonication amplitude
+0.003405	Lipid concentration * Sonication time
+0.003143	Lipid concentration * Sonication amplitude
-0.008250	Sonication time * Sonication amplitude
+0.005592	Lipid concentration²
+0.002917	Sonication time²
+0.001000	Sonication amplitude²

Additionally, from the above equation, we can observe that all the factors affected vesicle size to some extent. For example, an increase in lipid concentration increases vesicle size. The increase in vesicle size was observed with increased lipid concentration and a decrease in the sonication time and amplitude. The viscosity of the lipidic phase will increase while increasing the lipid concentration and thus increase in the order of lamellarity, which may be the probable reason behind the increase in vesicle size. In contrast, the increase in sonication time and amplitude breaks the vesicles and reduces dispersion size [9, 10]. Fig. 5B.10-5B.15 demonstrates the effects of independent variables on the vesicle size. The red area shows maximum vesicle size and blue zone represents the area with the lowest vesicle size.

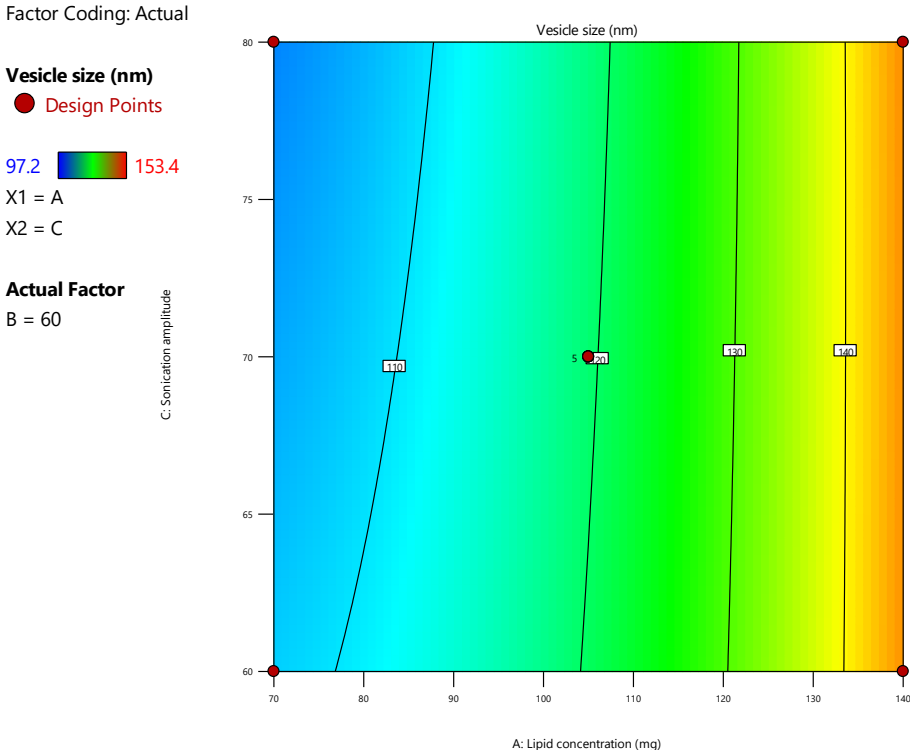

Figure 5B.10 Contour plot (2D) showing the combined effect of lipid concentration and sonication amplitude on vesicle size

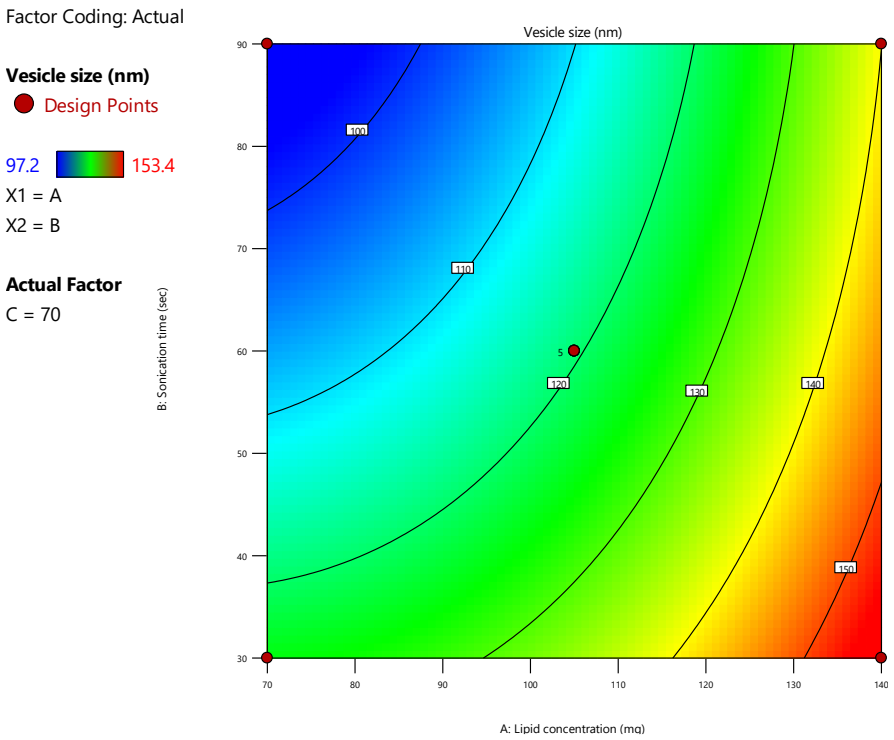

Figure 5B.11 Contour plot (2D) showing the combined effect of lipid concentration and sonication time on vesicle size

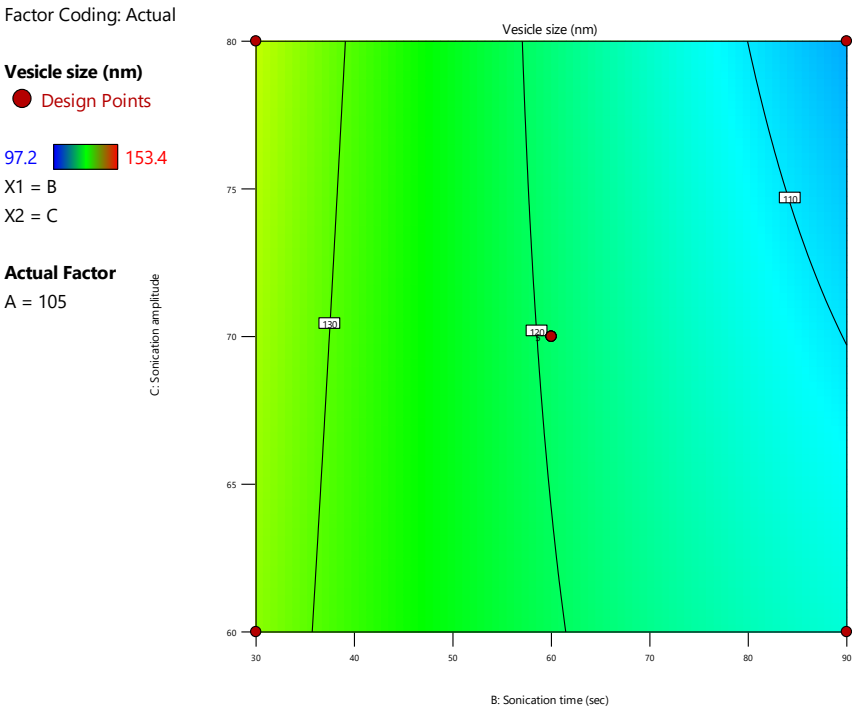

Figure 5B.12 Contour plot (2D) showing the combined effect of sonication time and sonication amplitude on vesicle size

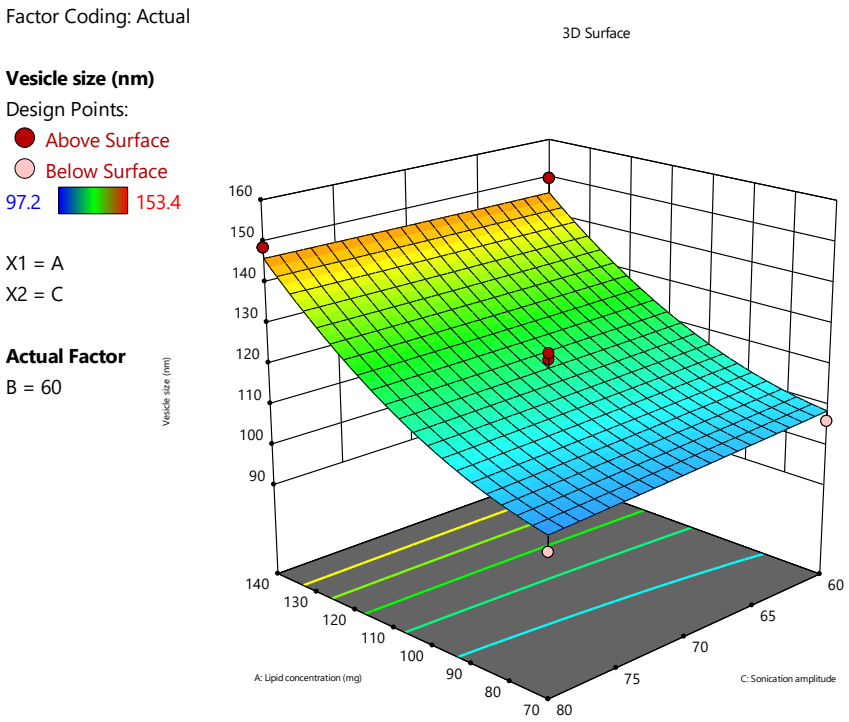

Figure 5B.13 Response surface (3D) showing the combined effect of lipid concentration and sonication amplitude on vesicle size

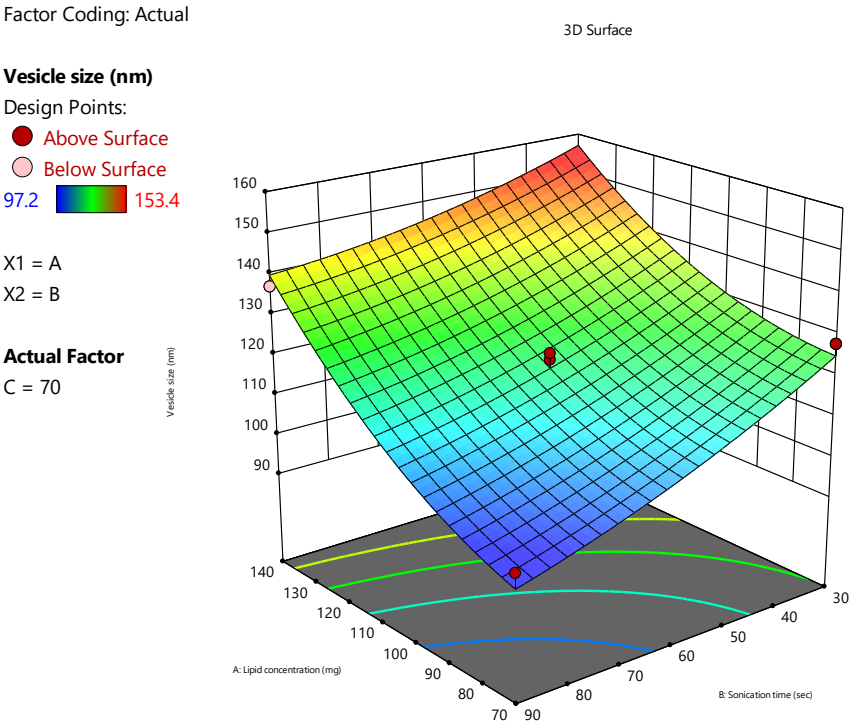

Figure 5B.14 Response surface (3D) showing the combined effect of lipid concentration and sonication time on vesicle size

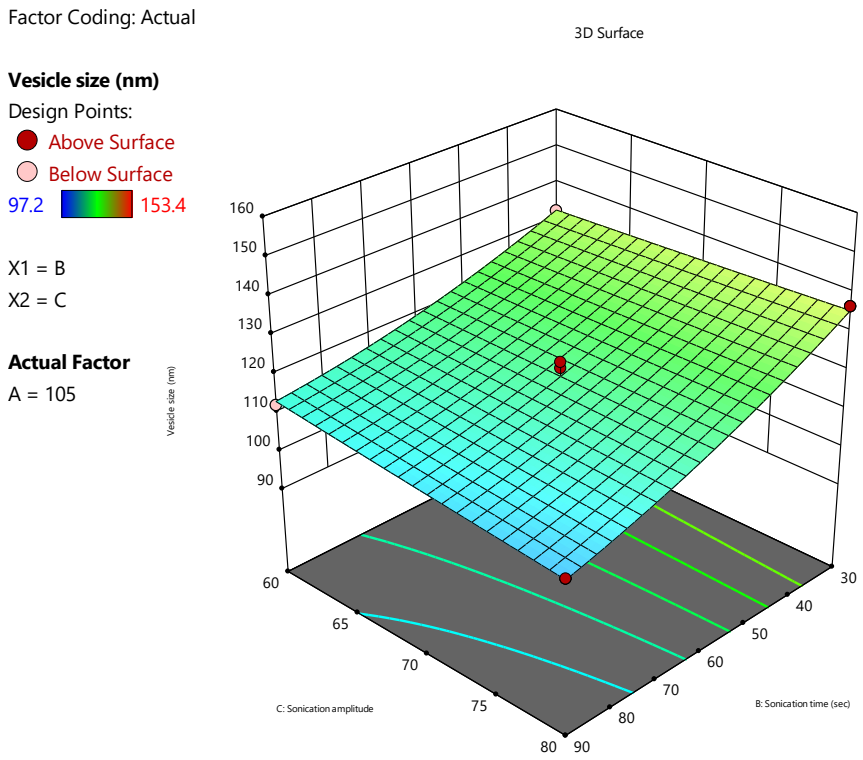

Figure 5B.15 Response surface (3D) showing the combined effect of sonication time and sonication amplitude on vesicle size

5B.4.1.3.3 Optimization using Desirability plot

A desirability plot gives the optimum value of variables to be obtained desired responses. A desirability plot was generated (Fig. 5B.16) using Design Expert 13.0. Parameters for the desirability batch were shown in Table 5B.13 and the evaluation of the desirability batch in Table 5B.14.

Table 5B.13 Variables for desirability plot and goals for response

Name	Goal	Lower limit	Upper limit
A: Lipid concentration (mg/ml)	In range	0.3	0.5
B: Sonication time (sec)	In range	0.2	0.3
C: Sonication amplitude	In range	3	9
% Drug entrapment	Maximize	49.68	69.7
Vesicle size (nm)	Minimize	152.1	262.4

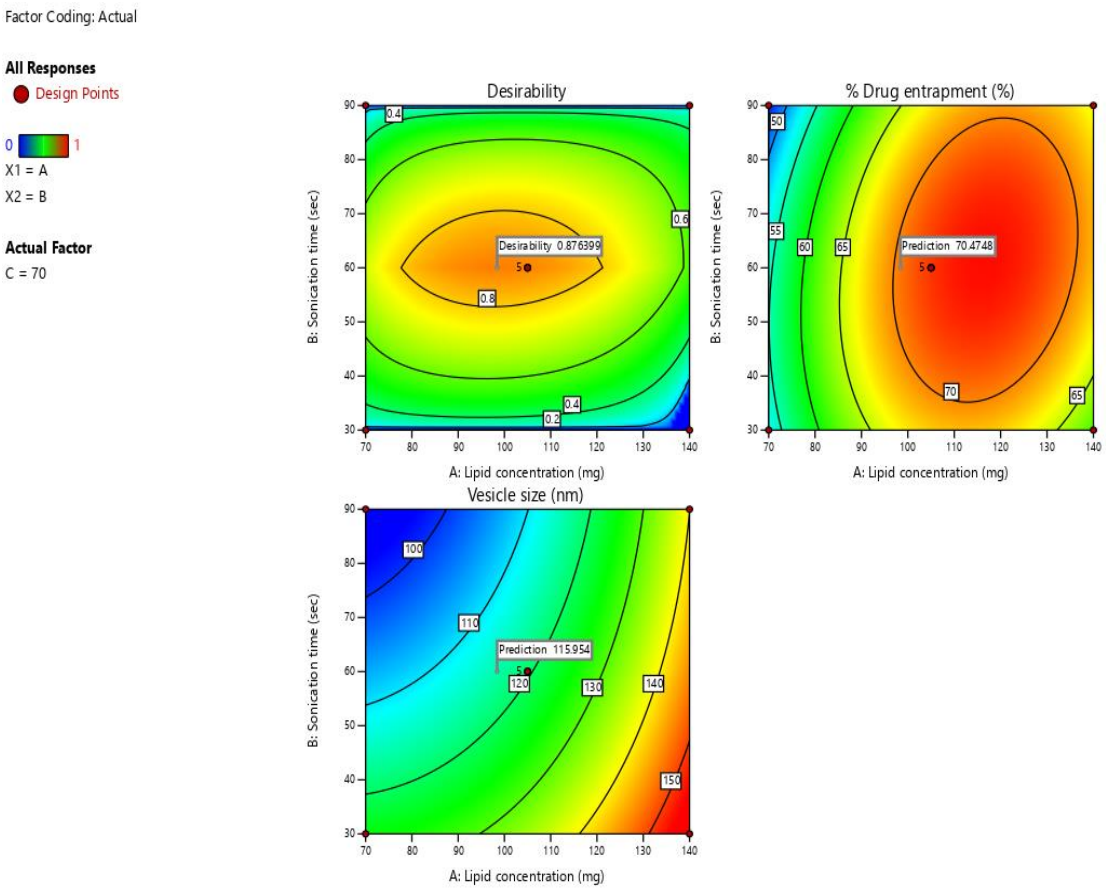

Figure 5B.16 Desirability plot

Table 5B.14 Desirability Plot for Optimization solution for Omiganan liposomes

Exp. Run	Amount of Lipid (mg/ml)	Sonication time (sec)	Sonication amplitude (%)	% Drug entrapment	Vesicle size (nm)	Desirability
1	98.418	60	70	70.475	115.95	0.876

Table 5B.15 Results of Evaluation of desirability batch

Response	Experiment value	Predicted value	Residual Difference
% Drug entrapment	71.77	70.475	1.295
Vesicle size (nm)	120.5	115.95	4.55

The obtained results demonstrate the suitability of the predicted desirability plot of the optimized liposomal formulation.

5B.4.1.3.4 Establishment of Design Space

ICH Q8 (2008) defines "Design Space" as a "multidimensional combination and interaction input variables and process parameters that have been demonstrated to provide assurance of quality." The composite desirability function based on the set constraints was used to determine the conditions that would result in an optima formulation design.

5B.4.1.3.5 Overlay Plot for predicted design space

The experimental design was used for numerous responses: % Drug entrapment and vesicle size. Overlay plot (Fig. 5.17) can be obtained by superimposing contour plots of both responses, which displays possible response values in the factor space. The highlighted yellow region indicates that a slight variation in the critical variables won't affect the final response and it will be in the desired range. Areas that do not fit the optimization criteria are shaded gray, while design space is accepted colored yellow. Fig. 5B.18 shows an overlay plot based on the desirability criteria.

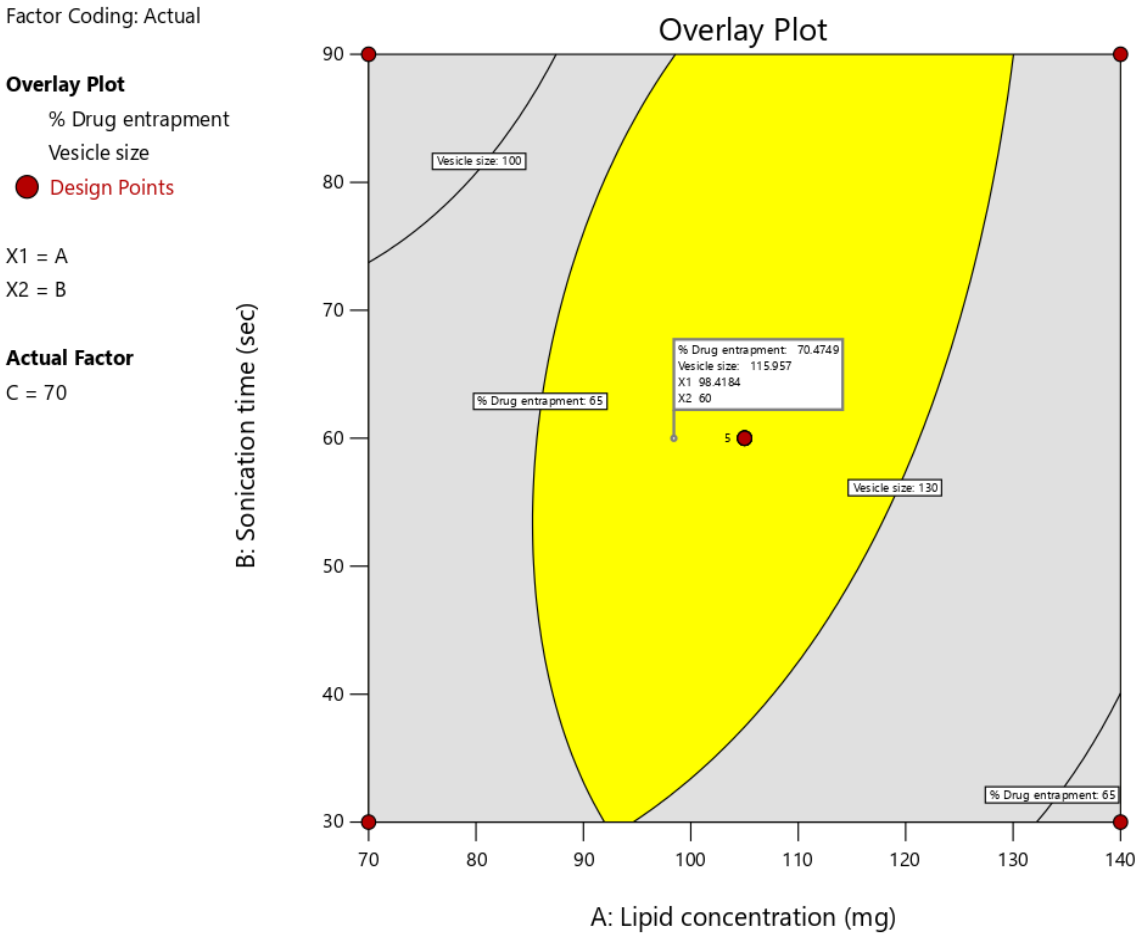

Figure 5B.17 Overlay plot

Table 5A.16 Composition of optimized batch of Omiganan liposomes and gel

Formulation components	Concentration
Omiganan/Omiganan liposomes	1% w/v
Amount of Lipid	98.42 mg/ml
Sonication time	60 sec
Sonication amplitude	70 %
Carbopol 934P	1.20% w/v
Propylene glycol	4% w/v
Methyl paraben	0.2 %w/v
Propyl paraben	and 0.02 %w/v
Sodium hydroxide solution (10%v/v)	…qs…to pH 6.5

5B.4.2 Characterization of optimized Omiganan liposomes and liposomal gel

5B.4.2.1 Zeta potential

The zeta potential graph of optimized Omiganan liposomes (Fig. 5B.18) showed a net negative charge on the liposome surface with a Z-avg value of -17.2 mV. The charge was found sufficient enough to keep the particles dispersed via repulsive forces.

5B.4.2.2 Shape and surface morphology

Cryo-Transmission electron microscopy of optimized Omiganan liposomes was performed, and the image is represented as Fig. 5B.19. The image showed a spherical shape with the smooth surface of liposomes. The size of liposomes seen in the image was found in line with the results of vesicle size data obtained from the Malvern zeta sizer (Fig. 5B.20).

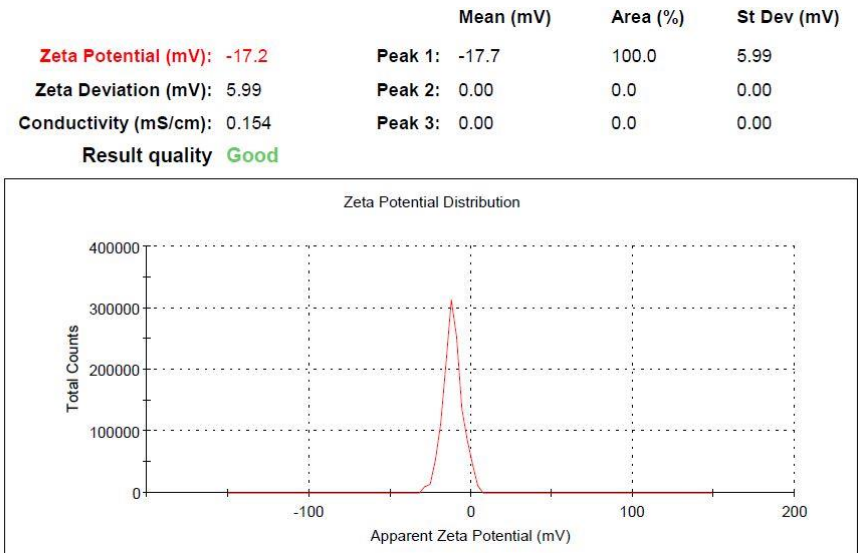

Figure 5B.18 Zeta potential of the developed Omiganan liposomes

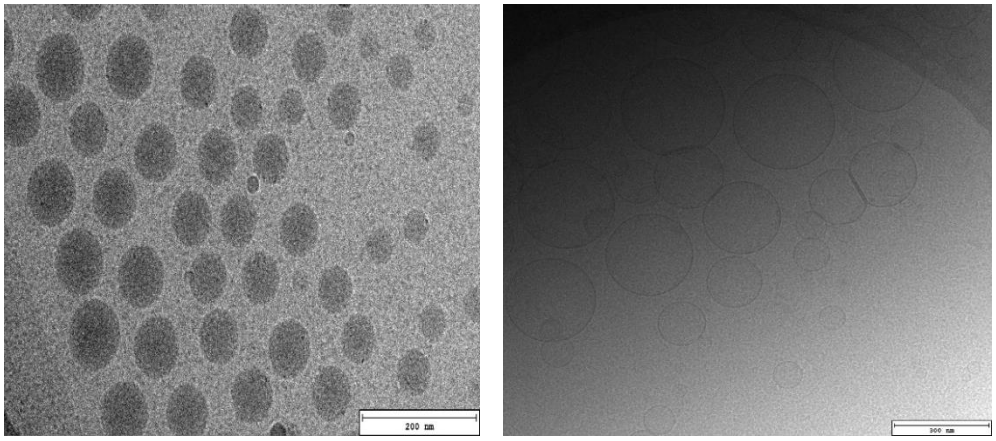

Figure 5B.19 Cryo-TEM images of the developed Omiganan loaded liposomes

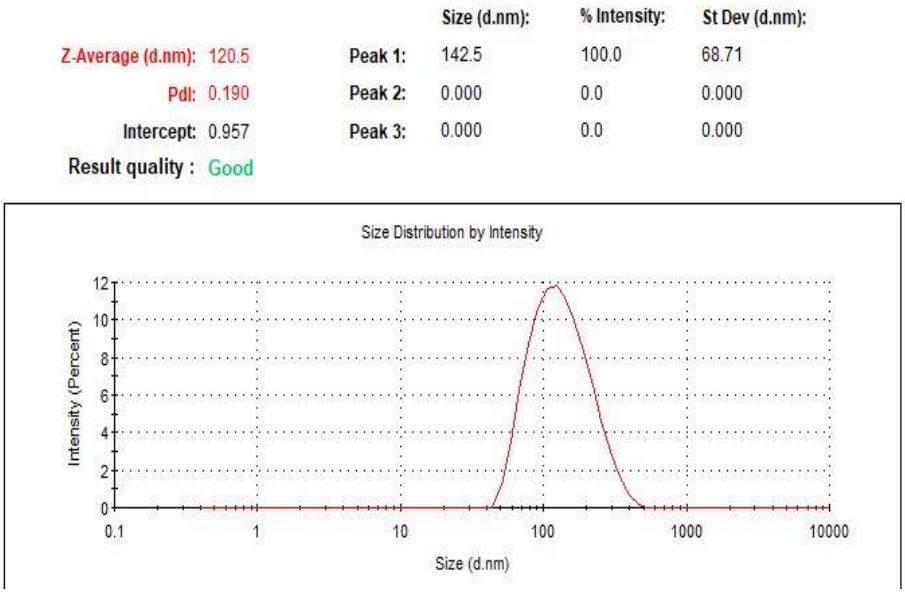

Figure 5B.20 Vesicle size of the optimized Omiganan liposomes

5B.4.2.3 % Drug entrapment and % drug loading

% Drug entrapment and % drug loading of the optimized Omiganan liposomes were found to be 71.89 ± 1.05 % and 7.8 ± 0.3 %w/w, respectively.

5B.4.2.4 Viscosity of Omiganan liposomal gel

The viscosity of free Omiganan gel and Omiganan liposomal gel was found to be 12.49 ± 0.367 Pa.S and 14.05 ± 0.420 Pa.S, respectively. The increase in viscosity of liposomal gel as compared to free Omiganan gel further leads to higher retention of liposomal gel on the skin.

5B.4.2.5 Spreadability of Omiganan liposomal gel

The spreadability of free Omiganan gel and Omiganan liposomal gel was found to be 6.21 ± 2.34 cm^2 and 7.39 ± 2.49 $cm^{2,}$ respectively, demonstrates the good spreadability of the formulated gel. The spreadability of free Omiganan gel was lesser than Omiganan liposomal gel, which may be attributed to the increased solid content of the gel after the addition of liposomal formulation.

5B.4.2.6 pH of Omiganan liposomal gel

The pH of free Omiganan gel and Omiganan liposomal gel was found to be 6.4±0.3 and 6.5±0.4, respectively, clearly resembles the pH of the skin thereby preventing irritation.

5B.4.2.7 Assay of Omiganan gel

The Omiganan content in free Omiganan gel and Omiganan liposomal gel was found to be 99.20 ± 1.10 % and 98.32 ± 1.77 %, respectively.

5B.5 References

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