6.1. Introduction

Quality by Design (QbD) driven approach was used to optimize the formulation. The UDNVs formulation was designed and engineered for uterine targeting via intravaginal route. The experiments were scientifically designed for screening and optimization of the formulation using Design of Experiments (DoE). The systematic QbD approach and use of various statistical tools enabled exhaustive evaluation of the impact of material attributes and process parameters on the critical formulation attributes.

6.2. Materials and Equipment

Carboplatin (CBP) was obtained as a gift sample from Sun Pharma Advanced Research Centre, Vadodara, India. 1,2-Diacyl-sn-glycero-3-phosphocholine, hydrogenated (HSPC) and 1,2-Diacyl-sn-glycero-3-phosphocholine, Soy (SPC) were obtained as gift samples from Lipoid GmbH, Germany. Sodium Deoxycholate was purchased from Loba Chemie Pvt Ltd, Mumbai, India. HPLC grade methanol (MeOH) and acetonitrile (ACN) were procured from Fisher Scientific (Vadodara, Gujarat) to carry out chromatographic analysis. Double distilled water used in the study was filtered using 0.22 micron nylon filter, Nylon N66 membrane filters 47 mm, Rankem, India. All other reagents were purchased from S.D. finechem Ltd, India and were of analytical grade.

Equipment

- Electronic weighing balance (ATX 224, Shimadzu, Japan)
- Vortex Mixer (Spinix-Vortex Shaker, Tarsons, India)
- Ultrasonic Bath Sonicator (Ultrasonics Selec, Vetra, Italy)
- Rotary evaporator (IKA RV10, Karnataka, India)
- Probe Sonicator (LabsonicM, Sartorius Ltd, Mumbai, India)
- Zeta sizer (Nano ZS Malvern Instruments, UK)

6.3. Preparation and Optimization of CBP loaded UDNVs

6.3.1. Methods

6.3.1.1. Preparation of CBP loaded UDNVs

UDNVs were prepared by thin film hydration method [1]. Briefly, HSPC, SPC (total lipids 150 mg) and SDC were accurately weighed and dissolved in 10 ml of Methanol: Chloroform (1:9) mixture. The clear solution was transferred to the round bottom flask (RBF) and thin film was obtained using rotary flask evaporator under vacuum on a thermostatic water bath at 40 ± 2°C at the speed of 150 RPM. The film was hydrated using 10 ml double distilled water containing accurately weighed amount of CBP for 20 min at 55 °C. The residual hydration volume was achieved under vacuum at 55 °C. The nano vesicular dispersion was probe sonicated at 40% amplitude 0.8 second pulse rate for 5 second exposure time to obtain size reduction of the vesicles and visually clear dispersion.

6.3.1.2. Quality Target Product Profile (QTPP) of CBP loaded UDNVs and Identification of CQAs

The template for target product profile (TPP) has been provided by United States Food and Drug Administration (USFDA) guidance that portrays the parts of TPP for new drug applications. The target product quality profile is enlisted as the quality properties that a drug product ought to possess so as to fulfill the objectives set in TPP as quantitative attributes. The International conference of harmonization (ICH) Q8 (R2) recapitulates them as QTPP. The QTPP lays the foundation of design criteria for the product and ought to embody patient relevant product performance characteristics. It should furnish a quantitative surrogate to ascertain the aspects of clinical safety and efficacy. Thus it ought to form the basis for determining the critical quality attributes (CQAs), critical material attributes, critical process parameters, and control strategy.

The primary step in defining QTPP is to decide the type of dosage form, what is the purpose of your product, its key desired quality attributes, manufacturing methodology, etc. The ICH working definition of CQA was stated as: "A CQA is a quality attribute (a physical, chemical, biological or microbiological property or characteristic) that must be controlled (directly or indirectly) to ensure the product meets its intended

stability, safety, efficacy and performance". The CQAs relies on the type of formulation, dosage form designed, manufacturing or production methodology, etc. employed and selected amongst many possible options. Consequently, formulation and process development typically rely on empirical prior knowledge and small scale feasibility studies. The identification of a CQA from the QTPP was based on the severity of harm caused by the product falling outside the acceptable range for that attribute. Based on the prior knowledge, literature review and experiment trials, three response variables viz., % Entrapment Efficiency, vesicle size and Deformability Index (DI) were selected as critical quality attributes (CQA) for CBP loaded UDNVs.

6.3.1.3. Qualitative Risk assessment and Identification of Independent variables (factors)

Risk based compliance is an imperative FDA initiative for current Good Manufacturing Practice in the 21st century. ICH Q9 guidance document introduced the concept of quality risk management for evaluating, communicating, controlling and reviewing risks to the quality of drugs across product life cycle. After careful observation and cerebration of the development process of the CBP loaded UDNVs formulation, variables/factors involved were qualitatively categorized as "low, medium and high risk" based on their anticipated impact on CQA as described in Table.

Table 6-1: Measures of qualitative risk assessment

Low Risk	Factors with wide range of acceptability. No investigation required
Medium Risk	Acceptable risk. No adverse effect on product quality on small changes.
High Risk	Unacceptable risk. Acceptable range need to be investigated

The risk associated with the medium and low risk factors was mitigated by making them constant based on scientific knowledge gained during preliminary trials and literatures.

6.3.1.4. Quantitative risk assessment: Factors Screening Design

Screening Design refers to an experimental plan that is intended to find the few significant factors from a list of many potential ones. Even when the experimental objective is to finally fit a response surface model (an RSM Model), the primary

experiment run should be a screening design when there are many factors to study. Here, the factors at High risk were screened using 2-level Plackett-Burman design to statistically detect important factors and utilize them in main-optimization design to define the control limits (the design-space). The non-critical factors were also specified with their constant levels using the Screening design. Minitab® 16.1.1 was used to generate a Plackett-Burman design based on which experimental batches were prepared and the effect of the high risk factors on CQA were evaluated. Software generated Pareto charts were utilized to determine critical factors while the main effects charts were utilized to decide the optimum levels of non-critical factors. Following methods used for the estimation of response variables.

6.3.1.4.1. Entrapment Efficiency (%EE)

The vesicular dispersion was taken in centrifuge tubes and centrifuged at 20,000 rpm at 4 °C for 1hr. The pellet settles down while free drug remains in the supernatant. The pellet was separated from the supernatant and lysed using 1% Triton X100 solution. The contents were suitably diluted and analysed using the developed HPLC method. Supernatant was also diluted suitably and free drug amount was estimated to establish the mass balance.

$$\%\ Entrapment\ Efficiency\ = \frac{Estimated\ Entrapped\ drug}{Total\ drug\ added\ to\ formulation} \times 100$$

6.3.1.4.2. Vesicular size and size distribution

50 µl of the vesicular formulation was taken and added to 2 ml of distilled water in order to obtain proper vesicle density in the final dispersion for measurement of size of the vesicles. The dispersion thus prepared was filled in clear disposable sizing cuvettes and the globule size was measured using ZetaSizer (Nano ZS, Malvern Instruments, UK) equipped with a He-Ne laser at 633 nm and scattered light detector at an angle of 90°.

6.3.1.4.3. Deformability evaluation

The formulation was diluted using double distilled water to obtain final volume of 50 ml. The diluted formulations were extruded at constant pressure through the 25 mm diameter Isopore polycarbonate filter membrane having a pore diameter of 100 nm. The amount of vesicle dispersion that was extruded during 5 min was measured, and the vesicle was monitored before and after extrusion. The deformability index of the nano-vesicles was measured using following equation [2]:

$$DI = J \times \left(\frac{R_v}{R_p}\right)^2$$

Where, *DI*= Deformability Index;

J= Amount of dispersion extruded (ml);

R_v= Vesicles size after extrusion (nm); and

R_p= Pore size of the barrier (nm)

6.3.1.5. Formulation optimization using Box–Behnken Response Surface Design

Box-Behnken design is a spherical, revolving response surface methodology (RSM) design that consists of a central and middle points on the edges of the cube circumscribed on the sphere. RSM is a useful method for studying the effect of several variables influencing the responses; this method varies the variables simultaneously and carries out a limited number of experiments have reported that statistical methods are effective and powerful approaches for screening key factors rapidly from a multivariable system for the optimization of a particular process. Box–Behnken statistical design with 3-factors, 3- levels, and 17 runs was specifically employed for the optimization study using Design-Expert software (Design-Expert 7, State- Ease Inc., Minneapolis, USA). The suitable model for the experimental data set was suggested by the software. The polynomial equation was generated using software and the significant model terms were decided based on ANOVA and F-test. The insignificant model terms from the polynomial equation were later removed in order to simplify the equation for estimation of the CQAs. 3D response surface plots were generated in

order to understand and explore the effect of variations in the independent factors on the response variables. Desirability criteria was defined based on the set QTPP and the design space was also created in order to define range of the independent variables to achieve desired characteristics in the optimized formulation.

6.3.2. Results and Discussion

6.3.2.1. Quality Target Product Profile (QTPP) of CBP loaded UDNVs and Identification of CQAs

The parameters that will be focused in our study were chosen and enlisted as QTPP for CBP loaded UDNVs. QTPP for CBP loaded UDNVs is tabulated in **Table 6-2**. The depicted QTPP laid down the basis for determining CQA. % Entrapment Efficiency, vesicle size and DI were identified as critical factors governing the response variables.

Table 6-2: QTPP for CBP loaded UDNVs

QTPP Element		Target	Justification
Dosage form		Nano-vesicle formulation	Due to their small size and large surface area, nano-vesicles show enhanced bioavailability and additional ability to cross the biological membranes. Furthermore, in cancer therapy, nano-vesicles deliver the drug into the tumor tissue and avoid normal tissues and organs by accumulation in tumors by a passive targeting, furnishing higher therapeutic efficiency and less side effects
Formulation Design		Targeted Delivery	Vesicles accumulate in tumours by a passive targeting leading to higher therapeutic efficiency and less side effects
Route of administration		Intravaginal	Achieve passive targeting to uterus by first uterine pass effect (FUPE)
Quality attributes of the formulation	Vesicle size	Minimize	Most important factor influencing biodistribution and cellular uptake
	Zeta potential	>±30mV	Better colloidal stability of the

			dispersion
	Drug Entrapment	Maximize	A higher percentage of drug entrapment and Loading could reduce the manufacturing cost and increase drug concentration in the final formulation allowing greater flexibility in dosing. Higher drug concentration can result in increased dosing intervals and hence improved patient compliance
	Drug Loading	Maximize	
	Vesicle Deformability	Optimum	To enhance transmembrane permeability of vesicles along with the maintained pool of the encapsulated drug.
	Surface characteristics	Smooth and spherical	To achieve enhanced tissue permeation devoid of hindrance/ entanglement with biological polymers
	In-vitro Drug release	Prolonged release	To ensure controlled drug release for desired duration to minimize frequency of administration
	Biocompatibility	Lack of Haemolytic Activity	Incompatibility with blood components can results into complex clinical and/or pathological conditions
Ex vivo permeation		Maximum Transmembrane flux	Enhanced drug concentration at target site, Minimum tissue deposition
Stability		NLT 3 month at 2-8 °C (Refrigerated) and 25-30°C (Controlled room temperature) conditions	Minimum time period (at least 3 months initially) decided to study stability of final formulation

6.3.2.2. Qualitative Risk assessment and Identification of Independent variables (factors)

Based on QTPP, CQA were identified. An overall risk assessment of the drug product formulation components was performed to determine which formulation components

have a high risk of impacting the drug product attributes. Following table describes risk assessment of CBP loaded UDNVs.

Table 6-3: Qualitative Risk Assessment

Factors	Process step	Impact on CQA	Constant levels
API Source	Raw Materials Selection and Specifications	Low risk	Authentic Source
API Storage		Low risk	Stored at 2–8 °C and protected from the light as per recommended storage conditions
Selection of Lipid-Type I		Low risk	1,2-Diacyl-sn-glycero-3-phosphocholine, hydrogenated (HSPC)
Selection of Lipid-Type II		Low risk	1,2-Diacyl-sn-glycero-3-phosphocholine, Soy (SPC)
Lipid Source		Low risk	Authentic Source
Lipid Storage		Low risk	Recommended storage condition (-20°C)
Edge Activator Selection		Low risk	Sodium deoxycholate (SDC)
Edge Activator Source		Low risk	Authentic Source
Edge Activator Storage		Low risk	Recommended storage condition (RT)
Organic Solvent- Type I		Medium risk	Methanol
Organic Solvent- Type II		Medium risk	Chloroform
Source of Organic Solvents		Low risk	Authentic Source
Hydration Media		Low risk	Water
Source of Hydration Media		Low risk	In House
Hydration Media Standards		Low risk	Double distilled; filtered 0.2 µ
Dispensing area Temp/RH	Dispensing	Low risk	25 ± 3°C at NMT 45 % RH
Weighing balance type		Low risk	Digital weighing balance
Sensitivity and Calibration of Weighing balance		Low risk	Calibrated with 0.1 mg least count

Manufacturing Vessels	Manufacturing Setup	Low risk	Type I Borosilicate glass vessels
Temp/RH of area		Low risk	25±3°C, Ambient RH
Lipid: Surfactant Ratio	Organic Phase Preparation	High Risk	To be optimized
HSPC : SPC Ratio		High Risk	To be optimized
Evaporation Temperature	Evaporation of Organic Solvent and Thin film preparation	Low risk	40± 2°C (in thermostatic water bath)
Evaporation Time		Medium risk	3 hr
Speed of Rotation		Medium risk	150 RPM
Lipid:Drug Ratio	Aqueous Phase Preparation	High Risk	To be optimized
Hydration Temperature		High Risk	To be optimized
Hydration Time		High Risk	To be optimized
Hydration Media Volume		Low Risk	10 mL
Residual Volume of Hydration		High Risk	To be optimized
Type and MOC of the centrifuge tube	Removal of free drug	Low risk	Screw cap conical bottom standing centrifuge tube
Centrifugation Temperature		Low risk	4°C
Centrifugation Time		Medium risk	1 h
Centrifugation Speed		Medium risk	20,000 RPM
Analytical instruments	Analytical Setup and Storage	Low risk	Calibrated
Analytical methods		Low risk	Validated
Analytical Reagents/Solvents		Low risk	Analytical Grade
Formulation Storage vessel		Medium risk	20 ml glass vials with screw cap
Formulation Storage Temp.		Low risk	Refrigerated Conditions
Formulator	Personnel	Low risk	Common for entire experimental setup
Analyst		Low risk	

6.3.2.3. Quantitative risk assessment: Factors Screening Design

Factors with high risk were evaluated further using quantitative risk assessment. The statistical evaluation was done by 2-level Plackett-Burman screening design. High (+1) and low (-1) levels of the independent variables were determined based on the preliminary trials conducted as well as the literatures available. The experimental matrix was generated using Minitab® 16.1.1 statistical software. The data obtained by experiments conducted as per the matrix design were feed in the software to process for the results in terms of Pareto charts, Normal and main effect plot for all the CQA considering p-value < 0.05 as the level of significance.

Table 6-4: Independent variables and levels for Plackett-Burman screening design

Independent variables		Units	Levels	
			Low (-1)	High (+1)
A:	Lipid : Drug Ratio	By weight	5:1	7:1
B:	Lipid : Surfactant Ratio	By weight	85:15	95:5
C:	HSPC : SPC Ratio	By weight	70:30	90:10
D:	Residual Volume of Hydration	mL	2	6
E:	Hydration Temperature	°C	45	65
F:	Hydration Time	min	10	30

Table 6-5: Plackett-Burman design experimental matrix and results

Batch no.	Run order	Independent Variables						Response Variables		
		A	B	C	D	E	F	% EE	Size (nm)	DI
1	12	7	85	90	2	45	10	44.02	340.9	76.32
2	5	7	95	70	6	45	10	55.34	346.7	63.42
3	14	5	95	90	2	65	10	41.83	348.4	49.74
4	10	7	85	90	6	45	30	41.15	344.4	79.75
5	6	7	95	70	6	65	10	58.81	335.0	59.97
6	3	7	95	90	2	65	30	52.74	319.4	41.51
7	4	5	95	90	6	45	30	39.67	336.7	43.77
8	1	5	85	90	6	65	10	35.85	305.6	71.08
9	11	5	85	70	6	65	30	42.77	313.7	85.47
10	2	7	85	70	2	65	30	48.77	335.6	89.34

11	13	5	95	70	2	45	30	46.68	345.6	66.19
12	8	5	85	70	2	45	10	44.39	331.0	82.71
13	15	6	90	80	4	55	20	47.75	305.7	66.12
14	7	6	90	80	4	55	20	47.39	349.0	64.97
15	9	6	90	80	4	55	20	46.89	347.3	65.22

Pareto charts and Normal Plot charts were generated to screen the factors that significantly influence the response variables. The Pareto chart shows the absolute values of the standardized effects from the largest effect to the smallest effect. The chart also plots a reference line to indicate which effects are statistically significant. The reference line for statistical significance depends on the significance level (denoted by α or alpha). The magnitude of factors if crosses the reference line signifies its effect on the response. The normal plot of the standardised effect also depicts significantly affecting factors.

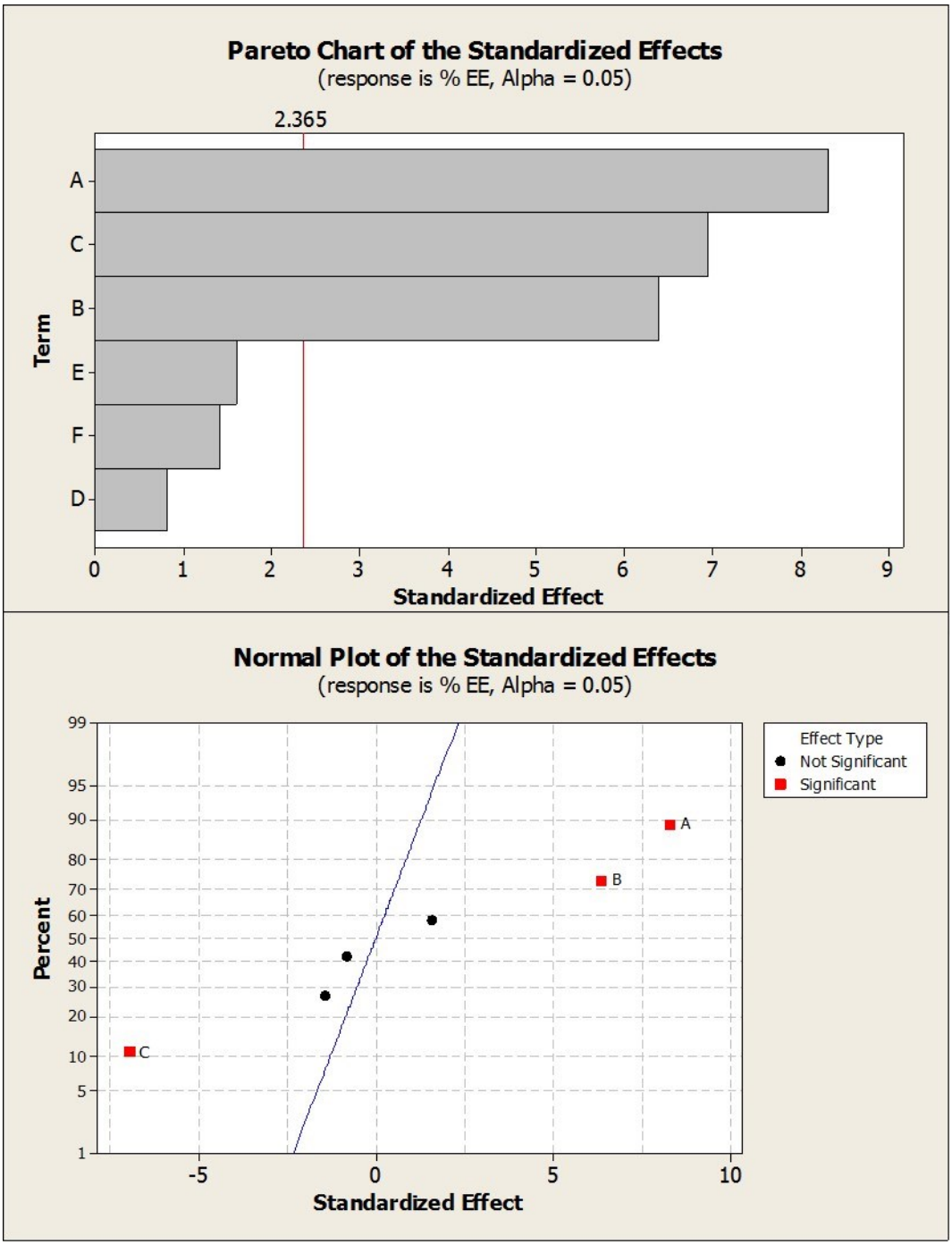

Fig. 6-1: Pareto and Normal plots for %EE

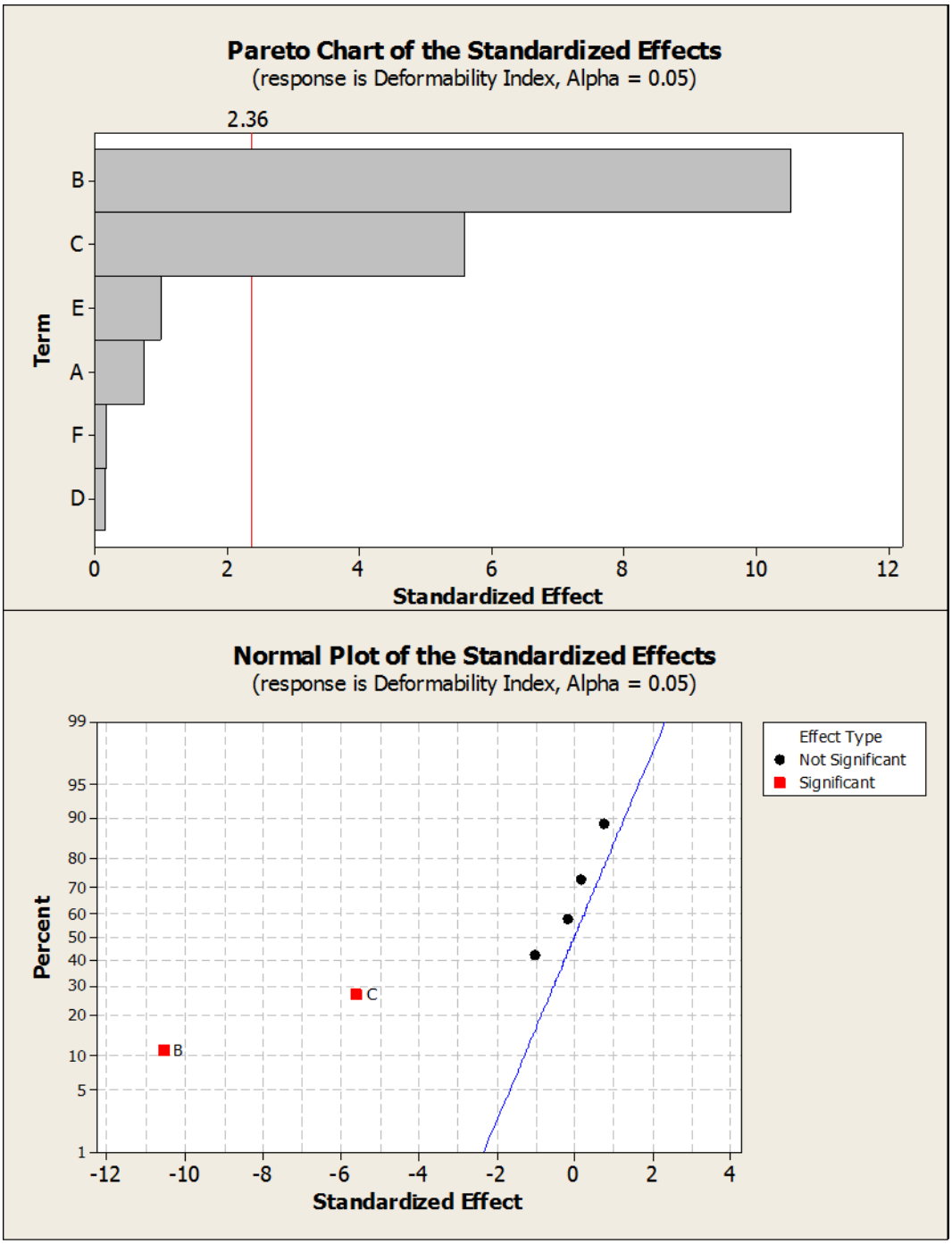

Fig. 6-2: Pareto and Normal plots for DI

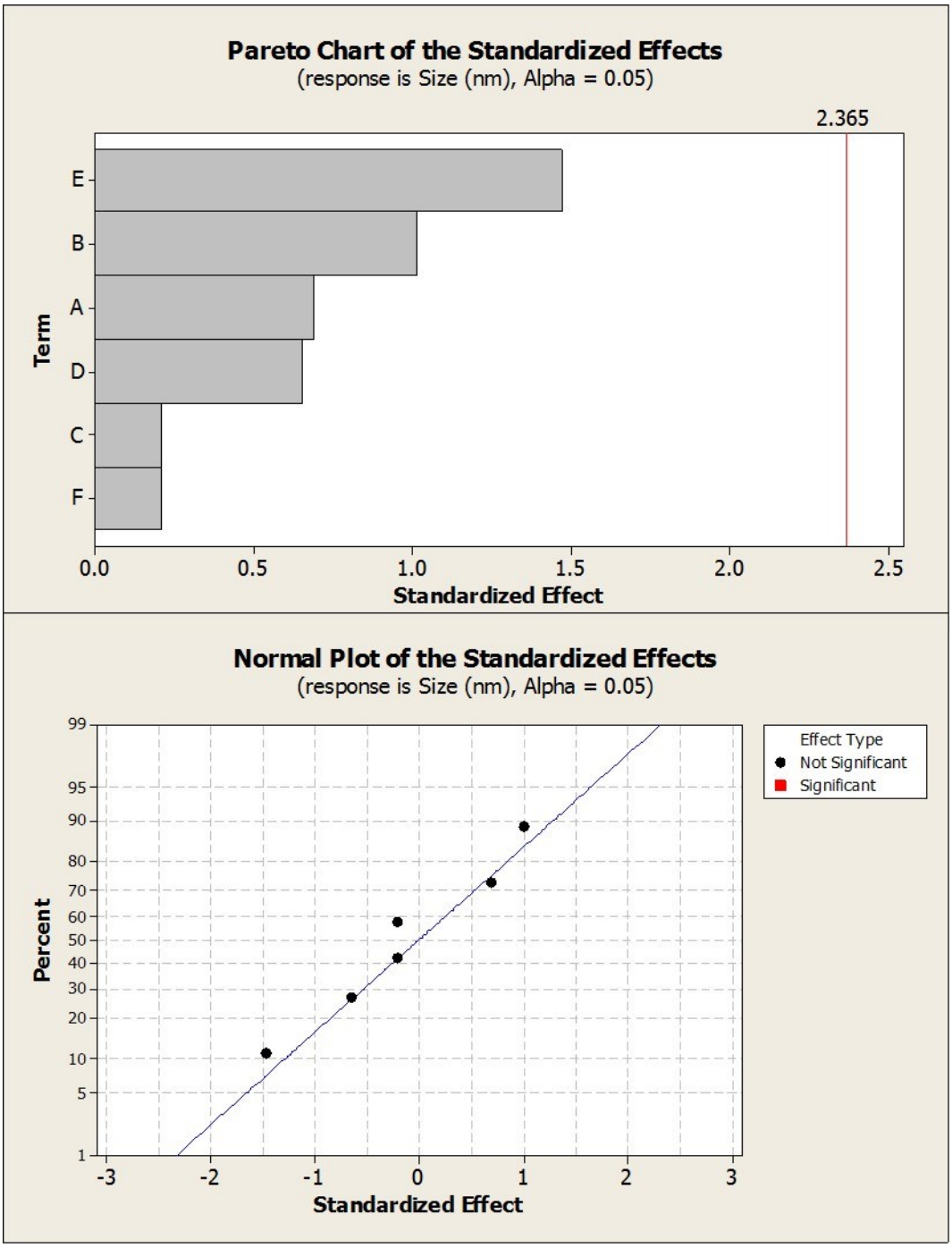

Fig. 6-3: Pareto and Normal plots for Vesicle Size

%EE was found to be significantly affected by Lipid : Drug Ratio, Lipid : Surfactant ratio and HSPC : SPC Ratio. The above fact can be evidenced from the Pareto Chart and Normal plot. All three material attributes were considered further for optimization design. The DI of the vesicles was found to be significantly affected by Lipid : Surfactant ratio and HSPC : SPC ratio. Both these material attributes were taken for final

optimization design. The vesicle size of the formulation was not found to be significantly affected by any of the process parameters and material attributes. In all the batches conducted for screening of the formulation it was observed that relatively insignificant variation was exhibited by the values of vesicle size. Residual volume of hydration and hydration temperature were made constant to 4 ml and 55 °C respectively by careful evaluation of the results of preliminary trials and screening batches of the formulation. Hydration time was not found to influence significantly to any of the CQAs of the study. Hence, after careful observation of the trials conducted during preliminary screening, the level of hydration time was fixed to 20 min for further optimization process.

6.3.2.4. Formulation optimization using Box–Behnken Response Surface Design

As suggested by the results of the screening design/preliminary trials, three critical material attributes were considered for the evaluation of their effects on critical quality attributes i.e. %EE and DI. The independent factors i.e. Lipid:Drug ratio, Lipid:Surfactant Ratio and HSPC:SPC Ratio were exhaustively studied using Box–Behnken Response surface statistical design. Selected independent variables and respective values were set at low (-1), medium (0) and high (+1) levels.

Table 6-6: Various critical material attributes along with their levels for optimization by Box-Behnken design

Independent variables (CMAs)		Units	Levels		
			Low (-1)	Medium (0)	High (+1)
A:	Lipid:Drug ratio	By weight	5	6	7
B:	Lipid : Surfactant Ratio	By weight	85:15	90:10	95:5
C:	HSPC:SPC Ratio	By weight	70:30	80:20	90:10

The 3-factor 3-level Box-Behnken experimental design matrix was generated using Design-Expert software. The batches presented in the design matrix were prepared and evaluated for CQA. The resulting values of the CQAs were used to analyse the respective experimental models. Analysis of variance (ANOVA) was used to establish

the statistical validation of the polynomial equations generated by Design Expert software. All the responses observed were simultaneously fitted to linear (first order), second order, and quadratic models. Various feasibilities were conducted over the experimental domain to find the compositions of the optimized formulation. 3D response surface plots were generated by the software, whereby intensive grid search performed over the whole experimental region. Checkpoint formulations were selected to validate the chosen experimental domain. Design space was created in order to identify area within which the deviations made in the independent factors will result into desired outcome. The resultant experimental values of the responses were quantitatively compared to that of the predicted values.

Table 6-7: Box-Behnken design experimental matrix and results

Batch no.	Run order	Independent Variables			CQA	
		A	B	C	% EE	DI
1	9	5	85	80	45.29	53.07
2	10	7	85	80	56.34	56.95
3	5	5	95	80	59.74	82.22
4	12	7	95	80	66.73	84.56
5	1	5	90	70	52.58	72.93
6	6	7	90	70	63.85	75.88
7	7	5	90	90	49.61	63.62
8	15	7	90	90	62.17	65.31
9	14	6	85	70	55.92	60.41
10	2	6	95	70	65.98	91.49
11	17	6	85	90	52.15	47.83
12	8	6	95	90	63.21	79.39
13	4	6	90	80	60.33	70.35
14	13	6	90	80	62.04	69.01
15	16	6	90	80	62.19	68.11
16	11	6	90	80	59.43	69.13
17	3	6	90	80	61.67	68.47

ANOVA- a multivariate analysis was applied to the results and the full as well as reduced models for %EE and DI were presented in below tables. ANOVA suggested a quadratic model as the best fit for the available data set since there were curvilinear

relationships amongst the multiple variables. The lack of fit was insignificant for the models selected which clearly indicates that there is existence of the good fit for the models selected.

Table 6-8: ANOVA of full as well as reduced quadratic model for %EE

Source	Full model					Backward Elimination Reduced model (α out - 0.1)*				
	DF	Adj SS	Adj MS	F-Value	P-Value	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	556.68	61.85	35.66	< 0.0001	4	544.55	4.00	136.14	67.33
A-Lipid:Drug	1	219.14	219.14	126.35	< 0.0001	1	219.14	1.00	219.14	108.37
B-Lipid:EA	1	264.04	264.04	152.25	< 0.0001	1	264.04	1.00	264.04	130.58
C-HSPC:SPC	1	15.65	15.65	9.02	0.0198	1	15.65	1.00	15.65	7.74
AB	1	4.12	4.12	2.38	0.1671					
AC	1	0.42	0.42	0.24	0.6393					
BC	1	0.25	0.25	0.14	0.7154					
A^2	1	42.71	42.71	24.62	0.0016	1	45.72	1.00	45.72	22.61
B^2	1	3.58	3.58	2.06	0.1939					
C^2	1	3.37	3.37	1.94	0.2059					
Residual	7	12.14	1.73			12	24.27	12.00	2.02	
Lack of Fit	3	6.37	2.12	1.47	0.3493	8	18.49	8.00	2.31	1.60
Pure Error	4	5.77	1.44			4	5.77	4.00	1.44	
Total	16	568.82				16	568.82	16.00		

* Shaded rows represent insignificant model terms removed during model reduction by backward elimination technique.

The ANOVA of %EE indicates that there are significant main effects of Lipid:Drug ratio, Lipid:Surfactant ratio and HSPC:SPC ratio with p-values significantly lower than 0.05 at 5% level of significance. Interaction effects amongst all three independent variables found insignificant while squared term of the Lipid:Drug ratio was found significant in full model. The reduced model was developed using backward elimination technique at alpha out level of 0.1 that selectively removed factor effects which were not found to be useful contributor for the prediction of the response. The polynomial equations were generated and represented below:

Full model equation:

$$\%EE = 61.13 + 5.23A + 5.75B - 1.40C - 1.02AB + 0.32AC - 0.25BC - 3.18A^2 - 0.92B^2 - 0.89C^2$$

Reduced model equation:

$$R1 = 60.32 + 5.23A + 5.75B - 1.40C - 3.29A^2$$

The number associated with the individual term indicates the magnitude of the effect contributed by that variable and the positive or negative sign indicative of the direct or inverse relationship of the model term.

Table 6-9: ANOVA of full as well as reduced quadratic model for DI

Source	Full model					Backward Elimination Reduced model (α out - 0.1)*				
	DF	Adj SS	Adj MS	F-Value	P-Value	DF	Adj SS	Adj MS	F-Value	P-Value
Model	3	2044.99	681.66	687.32	< 0.0001	3	2044.99	681.66	687.32	< 0.0001
A-Lipid:Drug	1	14.74	14.74	14.86	0.0020	1	14.74	14.74	14.86	0.0020
B-Lipid:EA	1	1782.05	1782.05	1796.85	< 0.0001	1	1782.05	1782.05	1796.85	< 0.0001
C-HSPC:SPC	1	248.20	248.20	250.26	< 0.0001	1	248.20	248.20	250.26	< 0.0001
Residual	13	12.89	0.99			13	12.89	0.99		
Lack of Fit	9	9.98	1.11	1.52	0.3627	9	9.98	1.11	1.52	0.3627
Pure Error	4	2.91	0.73			4	2.91	0.73		
Total	16	2057.88				16	2057.88			

ANOVA was also applied to the results obtained for the DI of the vesicles. The significant effects were indicated by the p-values lower than 0.05 at 5% level of significance. In the model generated all three main effects i.e. Lipid:Drug ratio, Lipid:Surfactant ratio and HSPC:SPC ratio were found significant. There were no interaction effects exhibited by the model. Moreover, backward elimination technique at alpha out level of 0.1 was applied to reduce the model which has resulted into no change in the full model generated since there were no factors to eliminate. Polynomial equations generated for full model/reduced model are presented below:

Full model/Reduced model equation:

R2= 69.34 +1.36A +14.93B -5.57C

Table 6-10: Model terms summary for full as well as reduced model of %EE and DI

Terms	%EE		DI	
	Full Model	Reduced Model	Full Model	Reduced Model
R-Squared	0.98	0.96	0.99	0.99
Adj R-Squared	0.95	0.94	0.99	0.99
Pred R-Squared	0.81	0.91	0.99	0.99
Adeq Precision	21.74	28.47	84.85	84.85

Std. Dev.	1.32	1.42	1.00	1.00
Mean	58.78	58.78	69.34	69.34
C.V. %	2.24	2.42	1.44	1.44
PRESS	110.89	49.83	23.68	23.68

The difference between adjusted R-Squared and predicted R-Squared was considerably decreased in reduced model for %EE. Also the value of predicted R^2 was increased in reduced model of %EE which clearly indicates that the prediction power of the reduced model was improved by model reduction. Model R^2 was not significantly affected by the backward elimination of the model terms. It was found near to 1 before and after model reduction. Standard deviation for %EE was not significantly affected by model reduction. The values for all the terms remained constant for full as well as reduced model of DI. Moreover, model R^2, Adj-R^2 and Pred R^2 values obtained near to 1.0 which shows good predictive capabilities of the developed model.

Table 6-11: Coded coefficients of full as well as reduced model for %EE

Term	Full Model					Reduced model (α out - 0.1)*				
	Coef	SE Coef	95% CI		VIF	Coef	SE Coef	95% CI		VIF
			Low	High				Low	High	
A-Lipid:Drug	5.23	0.47	4.13	6.33	1	5.23	0.50	4.14	6.33	1
B-Lipid:EA	5.75	0.47	4.64	6.85	1	5.75	0.50	4.65	6.84	1
C-HSPC:SPC	-1.40	0.47	-2.50	-0.30	1	-1.40	0.50	-2.49	-0.30	1
AB	-1.02	0.66	-2.57	0.54	1					
AC	0.32	0.66	-1.23	1.88	1					
BC	0.25	0.66	-1.31	1.81	1					
A^2	-3.18	0.64	-4.70	-1.67	1.006	-3.29	0.69	-4.79	-1.78	1
B^2	-0.92	0.64	-2.44	0.60	1.006					
C^2	-0.89	0.64	-2.41	0.62	1.006					

Table 6-12: Coded coefficients of full as well as reduced model for DI

Term	Full Model					Reduced model (α out - 0.1)*				
	Coef	SE Coef	95% CI		VIF	Coef	SE Coef	95% CI		VIF
			Low	High				Low	High	
A-Lipid:Drug	1.36	0.35	0.60	2.12	1	1.36	0.35	0.60	2.12	1
B-Lipid:EA	14.93	0.35	14.16	15.69	1	14.93	0.35	14.16	15.69	1
C-HSPC:SPC	-5.57	0.35	-6.33	-4.81	1	-5.57	0.35	-6.33	-4.81	1

The variance inflation factor (VIF) quantifies the extent of correlation between one predictor and the other predictors in a model. It is used for diagnosing co-llinearity / multi-co-llinearity. Higher values signify that it is difficult to assess accurately the contribution of predictors to a model. The higher the VIF, the more the standard error is inflated, and the larger the confidence interval and the smaller the chance that a coefficient is determined to be statistically significant. The value of the VIF was found 1 and near to 1 for all the model terms for both the response variables indicates that the predictor is not correlated with other variables [3]. Moreover, there was no significant change observed after model reduction in the limits of 95% confidence intervals and std error coefficients.

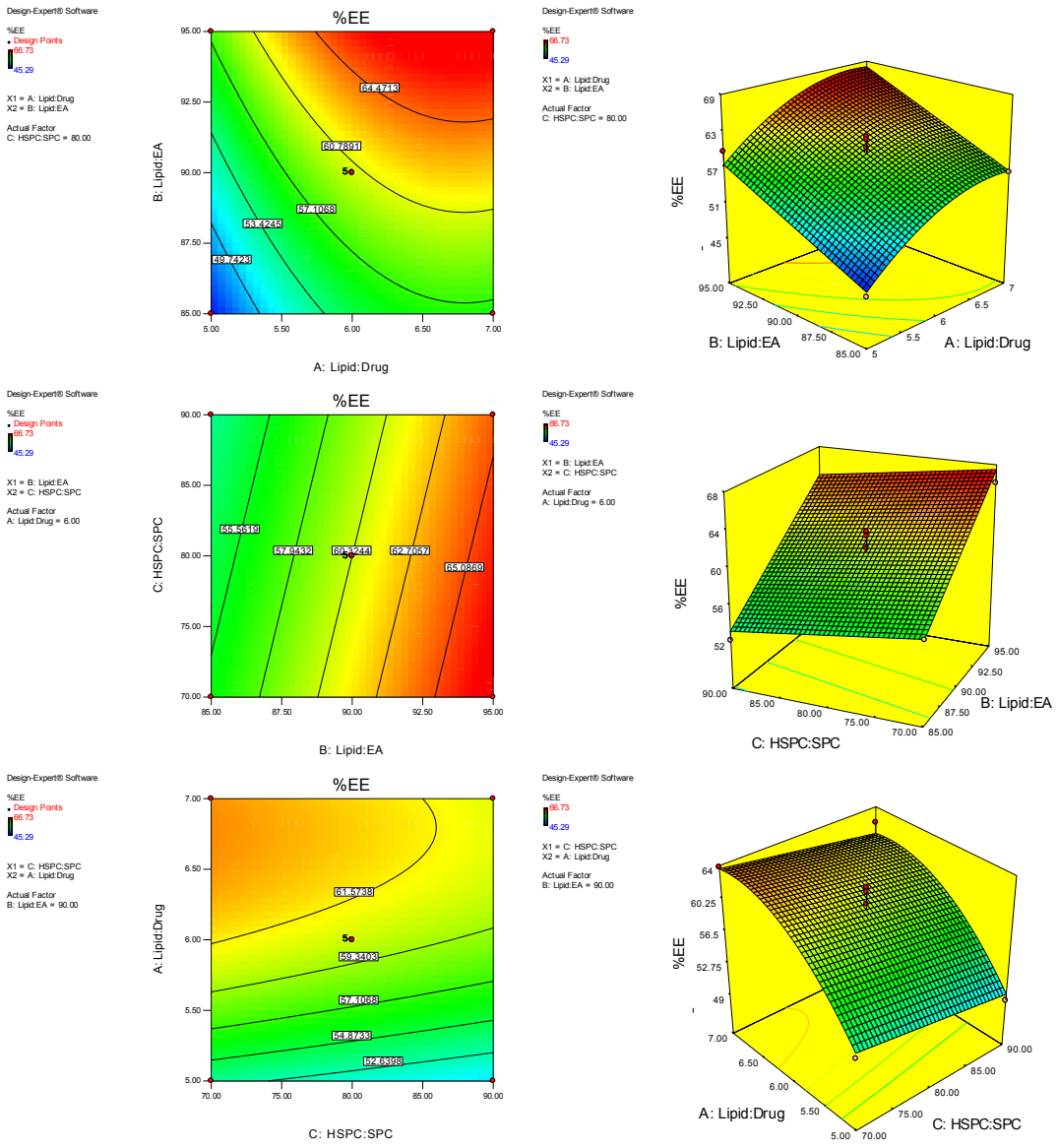

Fig. 6-4: Contour Plot and response surface plots for %EE

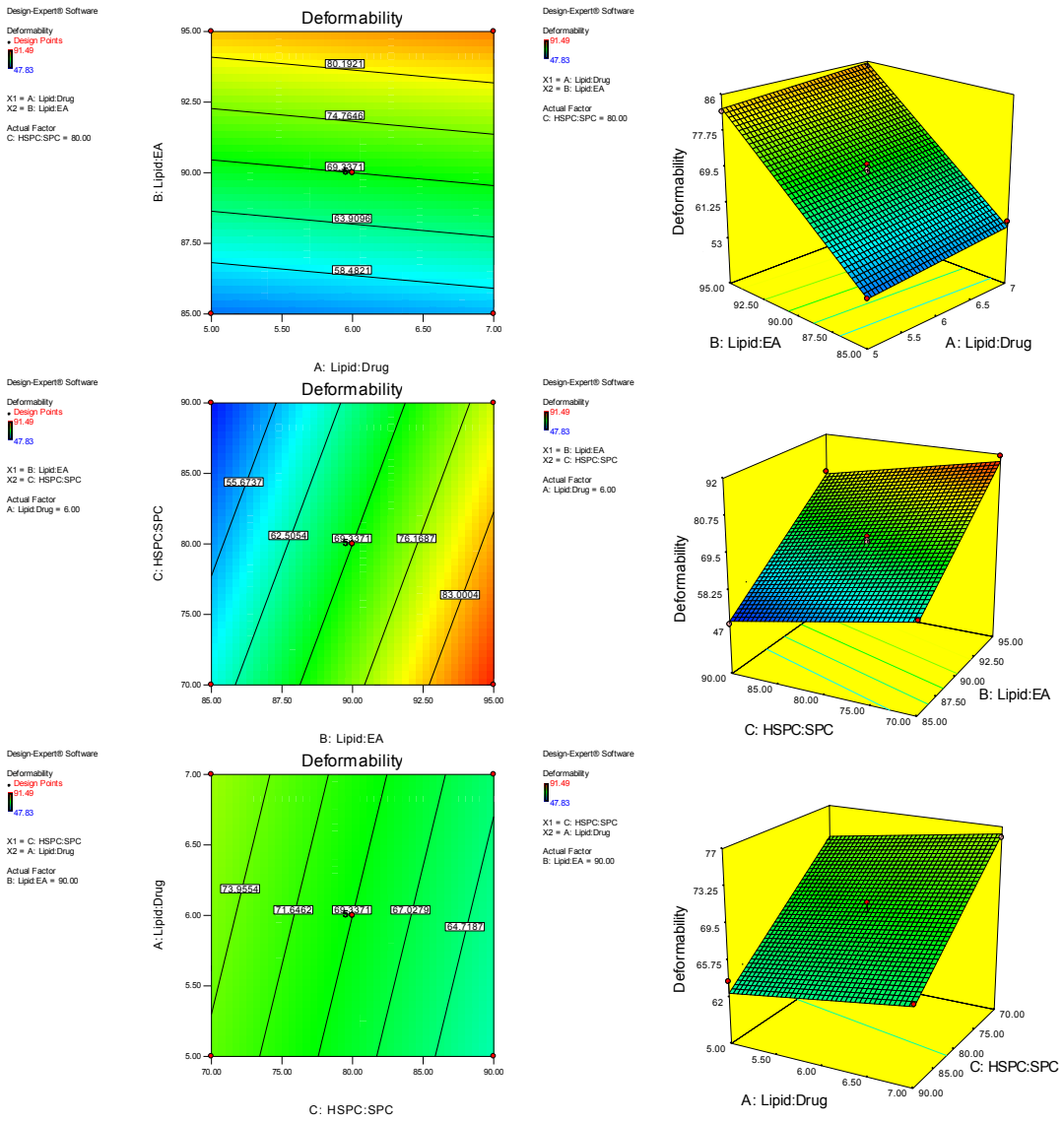

Fig. 6-5: Contour Plot and response surface plots for DI

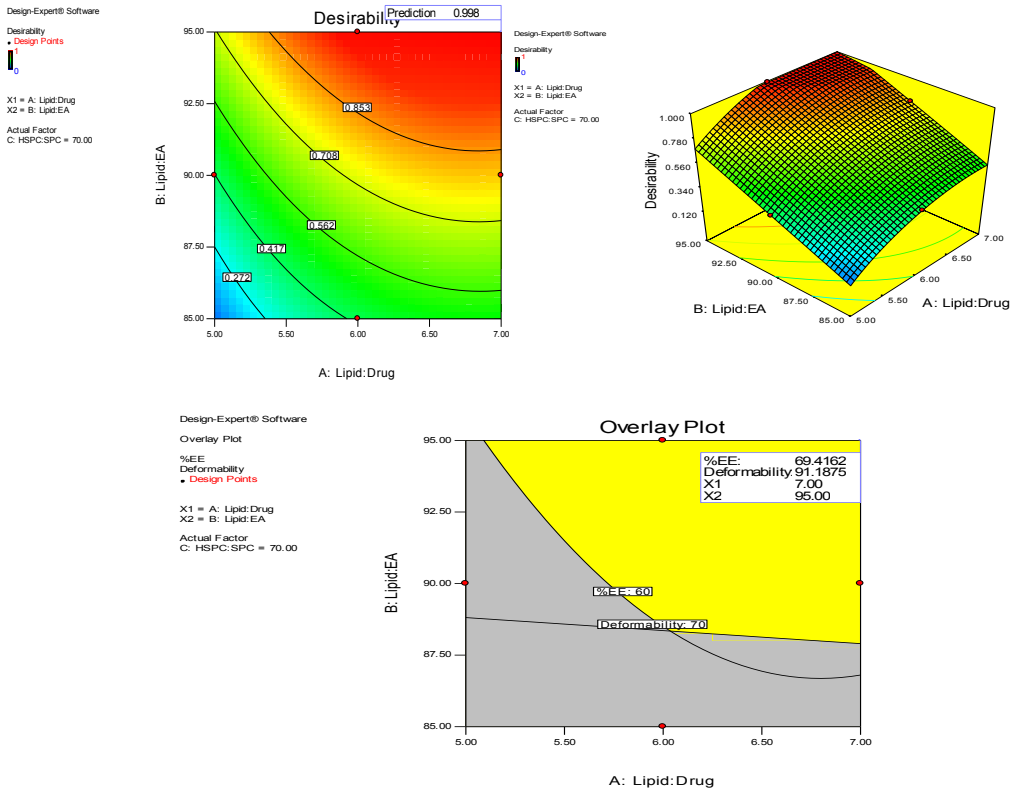

Fig. 6-6: Desirability Plot and Overlay plot with design space for the model

Numerical optimization was performed by the software for defined optimization criteria. The software was programmed to provide the optimization solution with maximum %EE and DI while keeping all the CMA within experimental range.

Table 6-13: Criteria for optimization of the CBP-UDNVs

Constraints name	Goal	Lower	Upper
Lipid:Drug	in range	5	7
Lipid:EA	in range	85	95
HSPC:SPC	in range	70	90
%EE	maximize	45.29	66.73
DI	maximize	47.83	91.49

The optimization process using design space resulted into a composite desirability of 0.998 for the solution provided by the software.

Table 6-14: Summary of the optimization solution

Multiple Response Prediction

Variable	Setting
Lipid:Drug	7
Lipid:EA	95.00
HSPC:SPC	70.00

Responses	Fit	SE Fit	95% Confidence interval		95% Prediction interval	
			Lower	Upper	Lower	Upper
%EE	69.42	1.01	67.23	71.61	65.62	73.21
DI	91.19	0.66	89.77	92.60	88.61	93.76

Table 6-15: Results of verification trials

Responses	95% Prediction interval		Results			
	Lower	Upper	Batch-1	Batch-2	Batch-3	Average
%EE	65.62	73.21	70.81	66.74	68.82	**68.79**
DI	88.61	93.76	89.00	85.76	92.63	**89.13**

The mean of both the quality attributes of the formulation found to fall within 95% confidence interval. The results are indicative of the statistical validity of the model.

6.4. Preparation of CBP-UDNVs loaded Intravaginal Rod Inserts

The past ten years has witnessed unprecedented advances in vaginal ring technology for the delivery of drugs, driven almost exclusively by the development of practical, long-acting and user-friendly devices [4]. Various innovative technologies for the designing of IVR have been studied by different scientists to suit desired formulation properties. The rod insert type of IVR has been studied here.

The rod inserts were prepared by lyophilisation of the CBP-UDNVs loaded gel matrix contained in silicon tubing. The lyophilized matrix swell in contact with vaginal fluid and release the formulation. Briefly, CBP-UDNVs dispersion was evaporated to 1 mL using a rotary vacuum evaporator at 500 mmHg vacuum and mixed with 4.2 % w/v of mannitol and 1 % w/v of gelatin and allowed to hydrate overnight at 2 to 8 °C to obtain a gel like consistency. This mixture was then mixed properly and inserted into medical

grade silicon tubing (3 mm ID, VWR International, UK) using a syringe. The tubing was then frozen at -20 C for 12 hr and then cut into 1 cm segments to obtain rods. These rods were kept for lyophilisation of the gel matrix in freeze dryer under ramping to -30 °C and hold for 6 h, followed by primary drying at -20 °C for 20 h and ramping to +20 °C over 60 min and holding for 10 h [5, 6]. The freeze dried rods were removed from the lyophilizer and stored at refrigerated conditions (2-8 °C) in moisture protective coverings until used. The CBP-UDNVs were further characterized for physical stability in IVR and in vitro drug release. The rods were used to study the efficacy of the developed formulations in endometrial cancer induced rabbit model.

References:

1. Wehbe, M., et al., *A simple passive equilibration method for loading carboplatin into pre-formed liposomes incubated with ethanol as a temperature dependent permeability enhancer.* Journal of Controlled Release, 2017. **252**: p. 50-61.
2. Singh, H.P., et al., *Elastic liposomal formulation for sustained delivery of colchicine: in vitro characterization and in vivo evaluation of anti-gout activity.* The AAPS journal, 2009. **11**(1): p. 54-64.
3. James, G., et al., *An introduction to statistical learning*. Vol. 112. 2013: Springer.
4. Malcolm, R.K., et al., *Microbicide vaginal rings: technological challenges and clinical development.* Advanced drug delivery reviews, 2016. **103**: p. 33-56.
5. Pattani, A., et al., *Characterisation of protein stability in rod-insert vaginal rings.* International journal of pharmaceutics, 2012. **430**(1-2): p. 89-97.
6. Gupta, P.N., et al., *Development of liposome gel based formulations for intravaginal delivery of the recombinant HIV-1 envelope protein CN54gp140.* European journal of pharmaceutical sciences, 2012. **46**(5): p. 315-322.