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Design and Optimization of Everolimus Drug Loaded Protein Nanoparticles to Treat Glioblastomas

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Abstract— *The experimental design is used to optimize the trials for Everolimus drug loaded with protein (Fibrinogen) nanoparticles in the design space in a way that produces reliable and consistent results with the fewest possible experiments in order to optimize the batches, the study was carried out to know the effects of independent variables by calculating their respective responses based on the trial runs performed. The design points are positioned at the middle of the subareas of the dimension k-1. The optimization studies were performed on 17 trial batches and the responses i.e., particle size and percent drug entrapment were evaluated. Out of the 17 batches it was observed that F14 batch depicted optimum results for the respective responses i.e., particle size and percent drug entrapment. The results of ANOVA along with response surface and contour plots were generated for response. Optimum results of particle size were observed to be 149.8±1.02 nm and 92.2±0.22 % drug entrapment.*

Keywords— *Box-Behnken design, Everolimus, Factorial design, Independent variables, Optimisation study*.

I. INTRODUCTION

The Box-Behnken designs are used to generate better order reaction surfaces the usage of fewer required runs than a everyday factorial technique. The Box-Behnken layout makes use of the twelve center side nodes and 3 middle nodes to suit a 2d order equation. The vital composite plus Box-Behnken turns into a complete factorial with 3 greater samples taken on the centre[1].

Box-Behnken designs location factors at the midpoints of the rims of the cubical layout region, in addition to factors on the centre. The Box–Behnken designs of experiments offer modeling of the reaction surface[2].

II. OPTIMIZATION STUDY

The experimental layout is used to prepare the rigors withinside the layout area in a manner that produces dependable and regular consequences with the fewest viable experiments so one can optimize the batches. It uses a famous statistical technique known as factorial layout, which proved a success in demonstrating the relative significance of a number of things and their interactions.

The Box-Behnken layout became decided on that's an impartial quadratic layout in that it does now no longer contain an embedded factorial or fractional factorial layout. In this layout the remedy combos are on the midpoints of edges of the system area and on the middle. These designs are rotatable (or close to rotatable) and require 3 ranges of every factor. Box-Behnken designs are used to generate better order reaction surfaces the usage of fewer required runs than a everyday factorial technique[3]. This and the vital composite strategies basically suppress decided on runs in a try to keep the better order floor definition. The Box-Behnken layout makes use of the twelve center side nodes and 3 middle nodes to suit a 2d order equation.

The vital composite plus Box-Behnken turns into a complete factorial with 3 greater samples taken on the middle. Box-Behnken designs location points[4] at the midpoints of the edges of the cubical layout region, in addition to factors on the middle.

The Box–Behnken designs of experiments offer modeling of the reaction floor. These designs are now no longer primarily based totally on complete or fractional factorial designs[5]. The layout factors are located on the center of the subareas of the measurement k-1. These designs require 3 ranges in keeping with factor. The Box–Behnken layout for 3 elements does now no longer follow the standards of iso-variance in keeping with rotation. However, the designs above, having extra than 3 elements, can meet the iso-variance standards if middle factors are added. These designs can also recognize the orthogonality criteria[6].

TABLE I. Optimization of Process Variable.

Independent Variables	Levels		
	Low (-1)	Medium (0)	High (+1)
Drug Conjugate (%)	0.5	1	1.5
Fibrinogen (%wt)	8	10	12
Soya Lecithin (%w/v)	0.5	1.25	2
Dependent Variables	Y1: Particle size (nm) Y2: Percent Drug Entrapment		

Box-Behnken have a look at layout for drug changed into evaluated to optimize the impact of dependent variables on independent variables. All the batches had been organized in line with the layout and analysed the usage of the layout professional design expert 11.0 software. The effects of ANOVA alongside with reaction floor and contour plots generated for response[7].

The optimization trials indicates that when the drug conjugate (%) 1, fibrinogen (%wt) 10 and soya lecithin (%w/v) 1.25 is used, the statistical analysis suggests optimum particle size in range of 149.8±1.02 and % drug entrapment efficiency as 92.2±0.22 as compared to other batches. For formulation parameters optimization Box-Behnken design was employed to study the effect of independent variables on

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dependent variable (Y1) particle size, (Y2) Percentage drug Entrapment. All the batches were prepared according to the design and analyzed using the design expert software.

TABLE II. Optimization of formulation variable using Box-Behnken Design.

Sr. No	Independent Variables			Responses	
	Drug Conjugate (%)	Fibrinogen (%Wt)	Soya Lecithin (%w/v)	Particle Size (nm)	% Drug Entrapment
F1	1.5	10	2	190.3±1.59	65.3±0.23
F2	0.5	12	1.25	276.9±0.56	71.2±0.71
F3	0.5	10	2	290±0.51	76.3±0.45
F4	1.5	10	0.5	179.6±0.37	42.3±1.82
F5	0.5	8	1.25	290.6±0.68	76.6±1.33
F6	1	10	1.25	165.3±0.94	89.3±0.49
F7	1	8	2	176.1±10.66	80.3±1.17
F8	1	10	1.25	157.8±6.65	89.3±0.92
F9	1	12	0.5	139.2±1.06	78±0.54
F10	0.5	10	0.5	288.8±0.11	69.1±0.43
F11	1	10	1.25	151.3±0.39	91.4±0.65
F12	1	8	0.5	157.2±0.51	60.9±0.32
F13	1.5	8	1.25	178.2±0.65	52.7±1.05
F14	**1**	**10**	**1.25**	**149.8±1.02**	**92.2±0.22**
F15	1	12	2	169.9±0.68	73.5±1.18
F16	1	10	1.25	159.8±0.47	86.6±0.93
F17	1.5	12	1.25	189.2±0.21	67.3±0.47

(n=3, Mean ±SD)

The consequences of ANOVA at the side of reaction floor and contour plots and three-D graphs generated for every reaction respectively.

III. Response Surface Study and Contour Plots

Response 1 - (Y1) Particle Size

Particle size of batches F1 to F17 was found to be considered minimum size as 149.8±1.02nm. Polynomial Equation for the Particle size was found to be:

$+156.74- 51.15*A +9.26*B +9.34*C +6.22*AB +2.38*AC +10.75*BC -94.90*A^2+17.97*B^2-14.47*C^2$.

TABLE III. Results of ANOVA for Particle Size.

Source	Sum of Squares	Degree of freedom	Mean Square	F Value	p-value	Decision
Model	62029.39	9	6892.15	37.69	< 0.0001	Significant
R^2	0.9798					

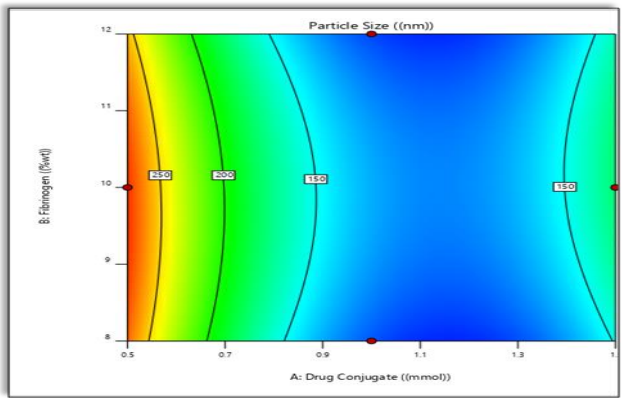

Fig. 1. Response surface plot of effect of Drug Conjugate (A) and Fibrinogen (B) on particle size.

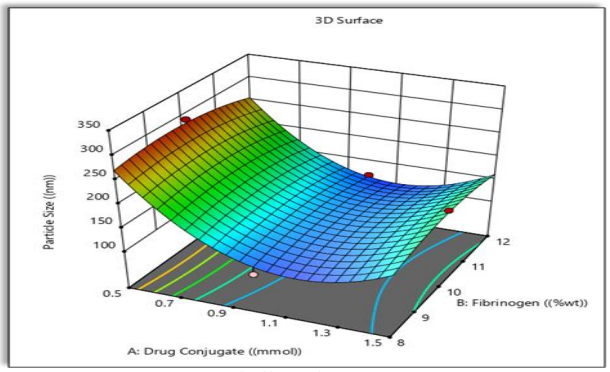

Fig. 2. Response contour plot of effect of Drug Conjugate (A) and Fibrinogen (B) on particle size.

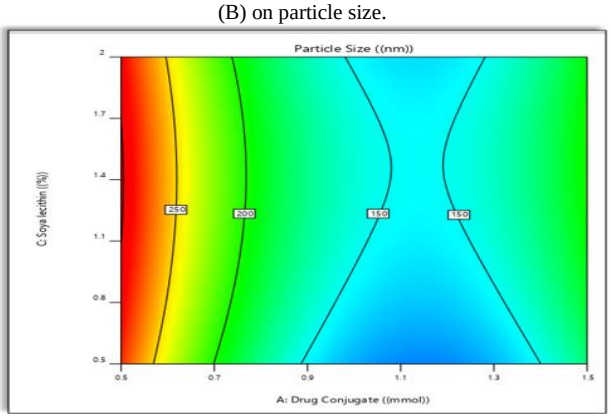

Fig. 3. Response surface plot of effect of Drug Conjugate (A) and Soya Lecithin (C) on particle size.

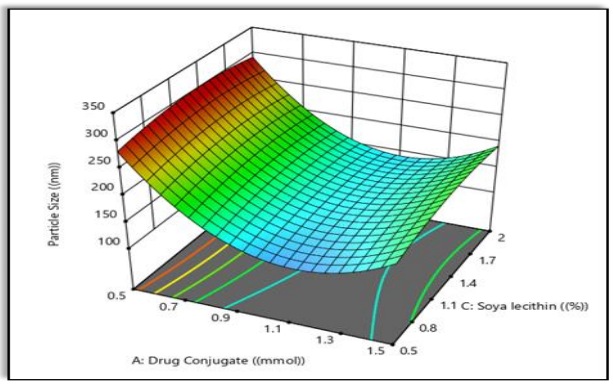

Fig. 4. Response contour plot of effect of Drug Conjugate (A) and Soya Lecithin (C) on particle size.

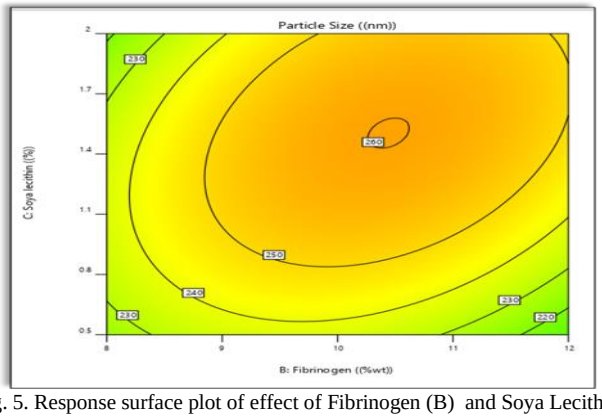

Fig. 5. Response surface plot of effect of Fibrinogen (B) and Soya Lecithin (C) on particle size.

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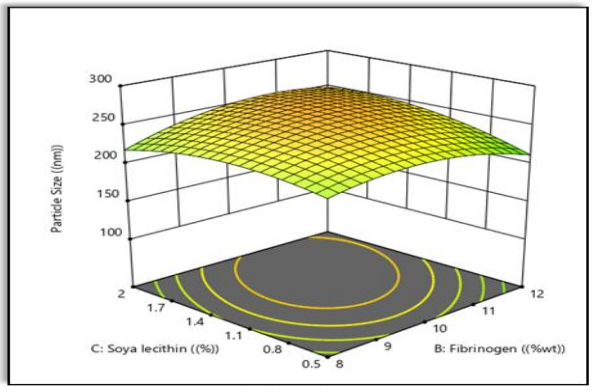

Fig. 6. Response contour plot of effect of Fibrinogen (B) and Soya Lecithin (C) on particle size.

From the ANOVA results the F value of the model was found to be 37.69 which implies the model was significant. The predicted R2 value 0.9798 was found to be close to the adjusted R2 value 0.9538, which indicate that there was no need of model reduction.

Response 2 - (Y2) % Drug Entrapment Efficiency

Drug Entrapment Efficiency of batches F1 to F17 was found to be consider maximum drug entrapment efficiency as 92.2±0.22.

Polynomial Equation for the Percentage Drug Entrapment Efficiency was found to be:

Percentage Drug Entrapment Efficiency,

*+91.76-8.20 *A +2.44*B +5.64*C +5.00*AB -3.95*AC -5.97*BC -17.37*A2-7.44*B2-11.14*C*^{2}.

TABLE IV. Results of ANOVA for %EE.

Source	Sumof Squares	Degree of freedom	Mean Square	F Value	p-value	Decision
Model	3373.76	9	374.86	40.39	< 0.0001	Significant
R²	0.9811					

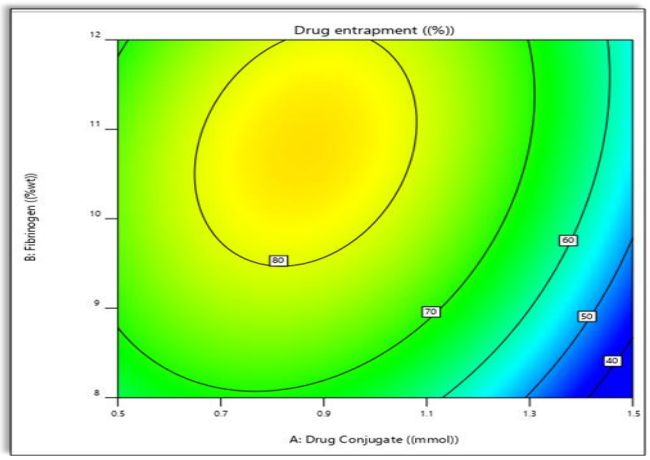

Fig. 7. Response surface plot of effect of Fibrinogen (B) and Soya Lecithin (C) on particle Percent drug entrapment.

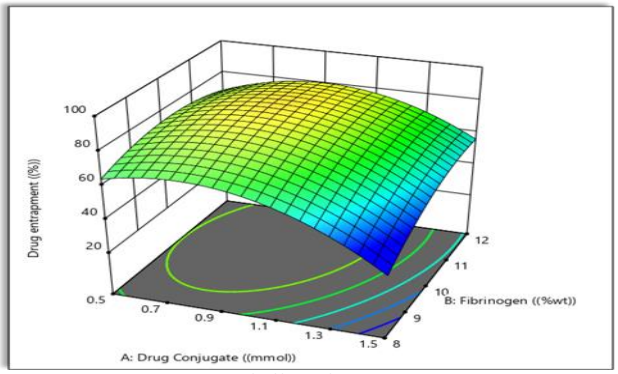

Fig. 8. Response contour plot of effect of Fibrinogen (B) and Soya Lecithin (C) on particle Percent drug entrapment.

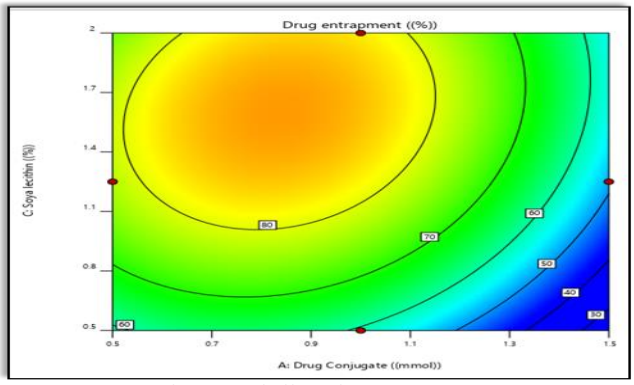

Fig. 9. Response surface plot of effect of Drug Conjugate (A) and (b) Soya Lecithin (C) on particle Percent drug entrapment.

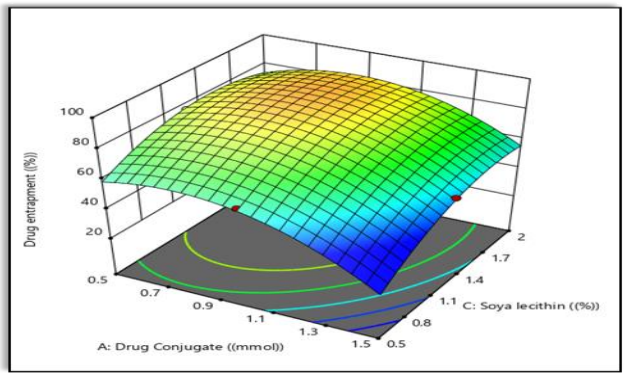

Fig. 10. Response contour plot of effect of Drug Conjugate (A) and (b) Soya Lecithin (C) on particle Percent drug entrapment.

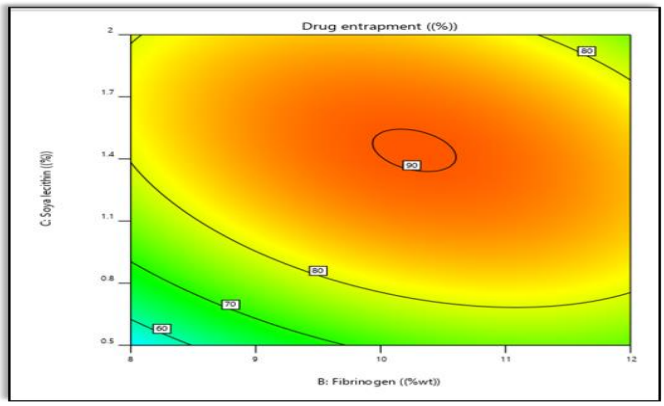

Fig. 11. Response surface plot of effect of Fibrinogen (B) and (b) Soya Lecithin (C) on particle Percent drug entrapment.

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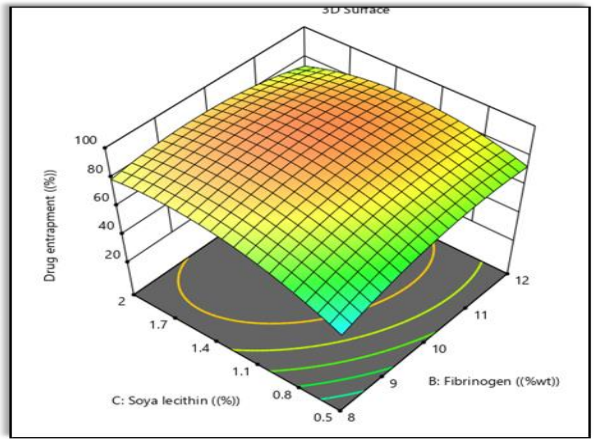

Fig. 12. Response contour plot of effect of Fibrinogen (B) and (b) Soya Lecithin (C) on particle Percent drug entrapment.

From the ANOVA results the F value of the model was found to be 40.39 which implies the model was significant.

The predicted R^2 value 0.9811 was found to be close to the adjusted R^2 value 0.9568, which indicate that there was no need of model reduction.

IV. Validation of Optimization Design

Numerical and graphical optimization was carried out using Design Expert software for optimization of final batch of Nanoscaffolds which should have following criteria. Selected criteria for independent and dependent variable for formula optimization:

Overlay plot for combined effect of Particle size, Drug Entrapment Efficiency are shown in Fig 13.

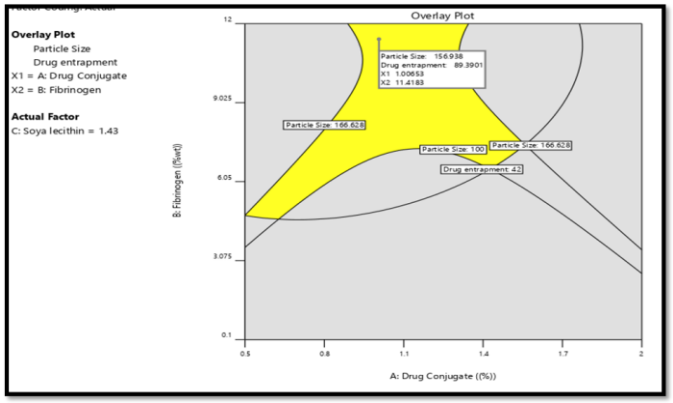

Fig. 13. Overlay plot showing combined effect of Particle Size and Percentage drug entrapment efficiency.

The overlay plot reflected that "yellow region" of the area shown in the figure is the area of interest (experimental region). Formulation having minimum particle size and maximum drug entrapment was found in experimental region of the overlay plot and having higher desirability than other check point batches. So, it was selected as optimized batch.

TABLE V. Results of Check Point Batch F14.

Batch Code	Parameter	Predicted Values	Experimental Values
F14	Particle Size (nm)	156.938	151.8±2.02
	Percentage Drug Entrapment (%)	89.3901	91.4±0.37

(Where n=3, Mean ± SD)

There was no significant difference between predicted value and experimental value. So, equation obtained for selected responses are validated in selected ranges of variables. The close resemblance between the observed and predicted response value assessed the robustness of predictions. These values indicate the validity of generated model.

IV. Conclusion

The results obtained for optimization studies that were carried out on 17 different batches reveals the relation of independent and dependent variables. Independent variables selected as Drug conjugate, Fibrinogen, Soya lecithin with low, medium and high levels indicates the effect on particle size and percent drug entrapment efficiency. Out of the 17 batches carried out as part of Box Behnken study batch F14 was considered to be optimum based on the results obtained for both of the responses. 149.8±1.02 nm particle size and 92.2±0.22 % drug entrapment was observed when the ratio of 1% Drug conjugate, 10% wt of fibrinogen and 1.25% w/v of soya lecithin was selected. Results of ANOVA suggested that the model was significant and there is no further need of model reduction.

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