

Development And Optimization Of 5-Fluorouracil Loaded Nanosponges Gel Using Box–Behnken Design

Deepak Mishra ^{1*}, Jitendra Singh Rajawat ², Bhupal Nobles³

¹Bhupal Nobles' College of Pharmacy, Udaipur, Rajasthan, India

²Bhupal Nobles' College of Pharmacy, Udaipur, Rajasthan, India

³University, Udaipur, Rajasthan, India

Corresponding author

Email id: deepak13mishra@gmail.com

Cite this paper as: Deepak Mishra , Jitendra Singh Rajawat , Bhupal Nobles (2024) Development And Optimization Of 5-Fluorouracil Loaded Nanosponges Gel Using Box–Behnken Design .*Frontiers in Health Informatics, Vol.13, No.8, 8327-8338*

ABSTRACT

5-Fluorouracil (5-FU) is an important antimetabolite drug widely used in the treatment of various cancers. However, conventional topical delivery of 5-FU may be associated with rapid drug release and limited control over drug residence at the site of application. Nanosponges provide a porous polymeric carrier system capable of incorporating drug molecules and modulating their release. The present study was undertaken to formulate and optimize 5-FU loaded nanosponges and develop them into a topical gel formulation for controlled drug delivery. A Box–Behnken experimental design was employed to investigate the effect of formulation variables on entrapment efficiency and particle size. The prepared nanosponges were evaluated for particle size, zeta potential, polydispersity index and entrapment efficiency. The optimized nanosponge formulation was incorporated into a gel and evaluated for physicochemical properties, homogeneity, grittiness, spreadability, extrudability, viscosity, content uniformity, in-vitro drug release and skin irritancy. The optimized formulation F13 exhibited an entrapment efficiency of 90.25% and particle size of 121.80 nm. The predicted values for particle size and entrapment efficiency were 122.58 nm and 87.75%, respectively. The optimized formulation showed a zeta potential of -38.45 mV, indicating a suitably charged nanosponge surface. The in-vitro drug release study of F13 nanosponges gel demonstrated a gradual release pattern, with cumulative drug release of $48.72 \pm 1.03\%$ at 4 h, $74.86 \pm 1.24\%$ at 8 h, $86.72 \pm 1.37\%$ at 12 h and $92.48 \pm 1.42\%$ at 24 h. The formulation also showed satisfactory physicochemical characteristics and was suitable for further evaluation. The findings demonstrate that the Box–Behnken design was useful for optimization of 5-FU loaded nanosponges. The optimized F13 formulation exhibited high drug entrapment, nanosized particles and prolonged drug release. Incorporation of the optimized nanosponges into a gel provided a promising approach for controlled topical delivery of 5-FU

Keywords: 5-Fluorouracil; Nanosponges; Topical gel; Box–Behnken design; Ethyl cellulose; PVA; Entrapment efficiency; Particle size; Controlled drug delivery; In-vitro drug release

INTRODUCTION

Cancer remains one of the major causes of morbidity and mortality worldwide and requires effective therapeutic approaches for controlling abnormal cellular proliferation. Chemotherapeutic agents are widely used in cancer management; however, conventional drug-delivery approaches may be associated with limitations such as nonspecific drug distribution, inadequate drug concentration at the target site, systemic adverse effects, and frequent administration (Mattiuzzi and Lippi, 2019). These limitations have encouraged the development of advanced drug-delivery systems capable of improving drug localization, controlling drug release, and enhancing therapeutic effectiveness (Anand et al., 2008).

5-Fluorouracil (5-FU) is a pyrimidine analogue and antimetabolite widely used in the management of several cancers (Longley et al., 2003). It interferes with nucleic acid synthesis and cellular proliferation by inhibiting thymidylate synthase and affecting RNA and DNA synthesis. Despite its therapeutic importance, conventional administration of 5-FU can be associated with rapid drug elimination and systemic toxicity (Thomas and Zalberg, 1998). Topical delivery of 5-FU can provide localized drug administration and may reduce unnecessary systemic exposure; however, conventional topical formulations may exhibit rapid drug release and limited residence time at the application site...

Nanotechnology-based drug-delivery systems have attracted considerable attention for improving the delivery of therapeutic agents (Suri *et al.*, 2007). Nanosponges are porous, sponge-like, nanosized carrier systems capable of incorporating drug molecules within their internal cavities and polymeric structure (Sahu *et al.*, 2021). Their high surface area, porous architecture, and ability to modify drug-release characteristics make them attractive for controlled and sustained drug delivery. The release of a drug from nanosponges can be influenced by factors such as polymer concentration, particle size, porosity, and formulation conditions.

Ethyl cellulose is a hydrophobic polymer that can provide a diffusion barrier and thereby contribute to prolonged drug release, whereas polyvinyl alcohol (PVA) can act as a stabilizing polymer during nanosponge preparation (Wasilewska and Winnicka, 2019). Incorporation of optimized nanosponges into a suitable topical gel can further improve application, spreadability, residence at the skin surface, and controlled release of the incorporated drug (Harika *et al.*, 2012).

Optimization of pharmaceutical formulations is essential because multiple formulation and process variables can simultaneously influence critical quality attributes (Ferreira *et al.*, 2007). Box–Behnken experimental design, a response-surface methodology, provides a systematic approach for studying the effects and interactions of independent variables while reducing the number of experimental trials. In the present study, ethyl cellulose concentration, PVA concentration, and stirring time were selected as formulation/process variables, while entrapment efficiency and particle size were selected as critical responses.

Therefore, the present study was designed to formulate, optimize, and characterize 5-Fluorouracil loaded nanosponges using Box–Behnken experimental design and subsequently incorporate the optimized nanosponges into a topical gel formulation. The developed formulation was evaluated for its physicochemical characteristics, particle size, zeta potential, polydispersity index, entrapment efficiency, content uniformity, *in-vitro* drug release, and skin irritancy, with the objective of developing a controlled topical delivery system for 5-FU.

MATERIAL AND METHODS

Material

5-Fluorouracil (5-FU) was obtained from Sigma-Aldrich, Mumbai, India. Ethyl cellulose and Polyvinyl alcohol (PVA) were procured from Loba Chemie Pvt. Ltd., Mumbai, India. Carbopol was obtained from Merck Life Science Pvt. Ltd., Mumbai, India. All other solvents, reagents, and chemicals used in the study were of analytical grade and procured from standard commercial suppliers.

Methods

Formulation of nanosponges by Box-Behnken experimental design

Experimental design

The drug loaded nanosponges were formulated and evaluated for their entrapment efficiency and particle size. The nanosponges which showed maximum entrapment efficiency and minimum particle size was selected for incorporation of different polymers at different levels of concentration by three factor three level Box Behnken factorial design.

Factorial design

A three factor three level Box Behnken design (BBD) was employed in optimization of nanosponges containing 5 FU.

The Ethyl cellulose (mg), PVA (mg) and Stirring Time (hrs.) were selected as independent variables. These independent variables (factors) were selected at three different levels i.e. low (-1), medium (0), and high (+1). The levels of factors and the obtained responses are shown in Table 4.3. These levels selected were based on initial trials. The dependent variables (response) studied in this research work were entrapment efficiency (Y1, % EE), particle size (Y2, nm). Thirteen runs of the experiment were evaluated for responses (Y1) and (Y2). The amount of drug remains same in all formulations, the polynomial equation generated using Box-Behnken experimental design. The quadratic response surfaces plots, contour plots and polynomial equations obtained from Box Behnken design were drawn and studied for statistical validity to find the compositions of optimized formulations (Chukka, 2025).

Technique for formulation of nanosponges containing 5 FU

The dispersed phase, containing the specified amounts of drug and polymer, was dissolved in 20 mL of dichloromethane (Patel, 2014). The aqueous phase was prepared by dissolving a specified amount of polyvinyl alcohol in 100 mL of distilled water. The dispersed phase was added dropwise to the aqueous phase under continuous magnetic stirring. Probe sonication was applied at 10-min intervals during stirring for total durations of 0.5, 1.0, and 1.5 h. The resulting nanosponges were collected by centrifugation at 4000 rpm for 15 min and subsequently dried.

Table 1: Formulation variables and their levels in Box-Behnken experimental design

Sr. No.	Formulation Variables				
1	Independent variables		Level		
			Low (-)	Medium (0)	High (+)
	Drug (5FU) (mg)		100		
	1	A: Ethyl cellulose (mg)	250	500	750
	2	B: PVA (mg)	100	125	150
	3	C: Stirring time (hrs.)	0.5	1.0	1.5
2	Response variables				
	1	R1: Percentage Entrapment Efficiency (% EE)			Maximizing
	2	R2: Particle Size (nm)			Minimizing

Design matrix in Box-Behnken design for nanosponges containing 5 FU

The Box–Behnken design (BBD) was employed to systematically study the combined influence of formulation and process variables on the development of 5-Fluorouracil (5-FU)–loaded nanosponges. In the present work, three independent variables were selected based on preliminary trials and literature evidence: Factor A – Ethyl cellulose concentration, Factor B – Polyvinyl alcohol (PVA) concentration, and Factor C – stirring time. Each factor was evaluated at three coded levels (–1, 0, +1), representing low, medium, and high levels, respectively.

The design matrix comprised 13 experimental runs, including 12 edge points and one center point (Run 15). The inclusion of a center point allowed for the estimation of experimental error and evaluation of curvature in the response surface, ensuring the adequacy and reliability of the proposed quadratic model. The randomized run order minimized the effect of uncontrolled variables and experimental bias.

Ethyl cellulose (Factor A), serving as the polymeric matrix former, played a critical role in nanosponge formation and drug entrapment. Variations at –1, 0, and +1 levels enabled the evaluation of its influence on structural integrity and encapsulation efficiency. PVA (Factor B), used as a stabilizing and emulsifying agent, significantly affected particle stabilization and dispersion uniformity. Stirring time (Factor C), a key process parameter, governed the extent of emulsification and nanosponge size reduction through shear force application.

The interaction of these factors was efficiently captured by the Box–Behnken design, allowing evaluation of not only individual effects but also two-factor interactions (AB, AC, BC) and quadratic effects (A^2 , B^2 , C^2). The balanced nature of the design ensured that extreme experimental conditions were avoided, thereby enhancing formulation safety and feasibility.

Table 2: Design matrix in Box-Behnken design for actual values of nanosponges containing 5 FU

		Actual Values		
Std	Run	Factor A : Ethyl cellulose (mg)	Factor B: PVA (mg)	Factor C: Stirring time (hrs.)
6	1	750	125	0.5
4	2	750	150	1
1	3	250	100	1
5	4	250	125	0.5
15	5	500	125	1
12	6	500	150	1.5
8	7	750	125	1.5
10	8	500	150	0.5
9	9	500	100	0.5
11	10	500	100	1.5
2	11	750	100	1
3	12	250	150	1
7	13	250	125	1.5

Final equation in terms of coded factors

$$\text{Entrapment Efficiency} = 85.394 - 2.1938A + 0.6025B + 5.3263C + 0.3925AB + 0.5850AC - 1.4625BC - 1.9420A^2 - 4.1645B^2 - 2.6320C^2$$

Final equation in terms of actual factors

$$\text{Entrapment Efficiency (EE\%)} = -54.65200 + 0.009767 \text{ Ethyl cellulose} + 1.77550 \text{ PVA} + 43.99350 \text{ Stirring Time} + 0.000063 \text{ Ethyl cellulose} * \text{PVA} + 0.004680 \text{ Ethyl cellulose} * \text{Stirring Time} - 0.117000 \text{ PVA} * \text{Stirring Time} - 0.000031 \text{ Ethyl cellulose}^2 - 0.006663 \text{ PVA}^2 - 10.52800 \text{ Stirring Time}^2$$

Final equation in terms of coded factors

$$\text{Particle Size (nm)} = 137.22 + 23.25A - 6.47B - 0.275C - 3.00AB - 1.30AC - 0.30BC + 4.24A^2 - 1.01B^2 + 3.34C^2$$

Final equation in terms of actual factors

$$\text{Particle Size} = +90.51500 + 0.095560 \text{ Ethyl cellulose} + 0.409000 \text{ PVA} + 0.409000 \text{ Ethyl cellulose} + 0.409000 \text{ PVA} - 19.07000 \text{ Stirring Time} - 0.000480 \text{ Ethyl cellulose} * \text{PVA} - 0.010400 \text{ Ethyl cellulose} * \text{Stirring Time} - 0.024000 \text{ PVA} * \text{Stirring Time} - 0.024000 \text{ PVA} * \text{Stirring Time} + 0.000068 \text{ Ethyl cellulose}^2 - 0.001616 \text{ PVA}^2 + 13.36000 \text{ Stirring Time}^2$$

Characterization of 5 FU loaded nanosponges

Particle size, zeta potential and PDI

The particle size, zeta potential and PDI of the properly diluted aqueous drug-loaded dispersions were estimated with Zetasizer (Malvern Zetasizer Nano series, Worcestershire, UK), at $25 \pm 0.5^\circ\text{C}$. The dynamic light scattering (DLS) technique investigates the fluctuation in light scattering as a result of the Brownian motion of the particles as function of time, at an angle of 90° (Guo *et al.*, 2008).

Entrapment Efficiency

Entrapped 5 FU in the 5 FU laded Nanosponges were calculated by estimating the amount of unentrapped drug recovered in the supernatant after centrifugation of the resultant nanosuspension (Mishra *et al.*, 2021). Nanosponges suspension was centrifuged by cooling centrifuge at 15000 RPM for 10 min at 10°C and the unentrapped drug was estimated in the supernatant with the help of UV Vis. Spectroscopy at 266nm. Further, the total amount of the drug and the unentrapped drug in the supernatant was substituted in the following equation to calculate % Entrapment Efficiency

$$\text{Drug entrapment(\%)} = \frac{\text{Concentration of total drug} - \text{Concentration of unentrapped drug}}{\text{Concentration of unentrapped drug}} \times 100$$

In-vitro drug release study

The dialysis membrane was soaked in the receptor medium before the experiment. The receptor compartment of the Franz diffusion cell was filled with phosphate buffer pH 7.4 and maintained at $32 \pm 0.5^\circ\text{C}$ using a thermostatically controlled water bath. The receptor medium was continuously stirred using a magnetic stirrer to maintain uniformity throughout the study.

An accurately weighed quantity of 5-FU nanosponges gel equivalent to a predetermined amount of 5-FU was uniformly placed on the donor side over the pre-treated dialysis membrane. The donor compartment was properly secured to prevent evaporation. The receptor medium was maintained under sink conditions throughout the experiment (Priyanka *et al.*, 2018).

At predetermined time intervals, suitable aliquots of the receptor medium were withdrawn and immediately replaced with an equal volume of fresh phosphate buffer pH 7.4 to maintain a constant receptor volume. The withdrawn samples were suitably diluted and analyzed spectrophotometrically at the predetermined λ_{max} of 5-FU. The amount of drug released was calculated from the previously prepared calibration curve. The cumulative percentage drug release was plotted against time to evaluate the release profile of the optimized formulation.

RESULTS AND DISCUSSION

The formulation of 5-Fluorouracil (5-FU) loaded nanosponges was optimized using a Box–Behnken experimental design. The amount of ethyl cellulose (Factor A), PVA (Factor B), and stirring time (Factor C) were selected as independent formulation variables, while entrapment efficiency (% EE) and particle size were selected as the dependent responses. The drug amount was maintained constant at 100 mg throughout the formulation study. The selected formulation variables and their respective low, medium, and high levels are presented in Table 1.

The experimental design generated different combinations of formulation variables, and the actual levels used for preparation of the nanosponges are presented in Table 2. The prepared formulations were evaluated for entrapment efficiency and particle size to determine the influence of formulation and process variables on the characteristics

of 5-FU loaded nanosponges.

The entrapment efficiency of the prepared formulations ranged from 70.22% to 90.25%, as shown in Table 3. Formulation F1 exhibited the lowest entrapment efficiency (70.22%), whereas F13 showed the highest entrapment efficiency of 90.25%. The considerable variation in % EE among the formulations indicates that the concentrations of ethyl cellulose and PVA, together with stirring time, influenced the incorporation of 5-FU into the nanosponge matrix.

Ethyl cellulose acts as a matrix-forming polymer and can enhance drug entrapment by providing a suitable polymeric network for incorporation of the drug. Increasing polymer concentration may provide a greater amount of matrix material available for drug incorporation. PVA, used as a stabilizing/emulsifying agent, can influence the formation and stabilization of nanosponge particles and consequently affect drug entrapment. Stirring time can also influence particle formation, polymer deposition, and drug incorporation.

Among the formulations, F13 demonstrated the maximum % EE (90.25%), indicating efficient incorporation of 5-FU into the nanosponge system. The normal probability plot of residuals for entrapment efficiency (Figure 1) can be used to assess the distribution of residuals, while the predicted-versus-actual plot (Figure 2) demonstrates the relationship between experimentally observed and model-predicted values. The contour plot in Figure 3 illustrates the combined influence of ethyl cellulose and PVA on entrapment efficiency, while the corresponding three-dimensional response surface plot in Figure 4 provides a graphical representation of their interaction.

The particle size of the prepared 5-FU loaded nanosponges ranged from 114.30 to 172.60 nm, as presented in Table 3. Formulation F11 showed the largest particle size (172.60 nm), whereas F12 exhibited the smallest particle size of 114.30 nm. Formulation F13 showed a particle size of 121.80 nm, indicating the formation of nanosized particles with a relatively narrow size range across the optimized formulations.

Particle size is an important parameter for nanosponge-based drug delivery systems because it can influence surface area, drug release, physical stability, and overall performance of the formulation. Changes in polymer concentration can alter the viscosity of the formulation system and affect the formation and growth of particles. Similarly, PVA concentration may influence stabilization of the formed particles and prevent excessive aggregation. Stirring time can affect particle formation by controlling the extent of dispersion and polymer deposition.

The normal probability plot for particle size is presented in Figure 5, while the relationship between predicted and experimentally observed particle size is shown in Figure 6. The contour plot illustrating the combined effect of ethyl cellulose and PVA on particle size is presented in Figure 7, and the corresponding three-dimensional response surface plot is shown in Figure 8. These graphical representations demonstrate the influence and interaction of the selected formulation variables on particle size.

Considering both responses, F13 was selected as the optimized formulation based on the desirability of achieving high entrapment efficiency along with a comparatively low particle size. F13 exhibited an entrapment efficiency of 90.25% and a particle size of 121.80 nm (Table 3). Although F12 showed a slightly smaller particle size (114.30 nm), its entrapment efficiency was lower (81.14%). Therefore, F13 provided a more favorable balance between the two optimization criteria.

The experimental and predicted responses of the optimized formulation are presented in Table 4. For particle size, the experimental value was 121.80 nm, while the predicted value was 122.58 nm, showing a very small difference of 0.78 nm. For entrapment efficiency, the experimental value was 90.25%, compared with a predicted value of 87.75%. The close agreement between the experimental and predicted values supports the suitability of the developed optimization model for predicting the selected responses.

The optimized formulation F13 was further characterized for its particle size and zeta potential. The particle-size profile of F13 is presented in Figure 9, confirming the nanosized nature of the prepared formulation. The zeta potential of F13 was found to be -38.45 mV, as reported in Table 4. The zeta-potential profile is presented in Figure 10. The negative surface charge indicates electrostatic repulsion between particles, which may contribute to the physical stability of the nanosponge dispersion by reducing the tendency toward particle aggregation.

The *in-vitro* drug release study of the optimized 5-FU nanosponges gel (F13) was carried out to evaluate the release behavior of 5-FU from the nanosponge-based gel formulation. The cumulative percentage drug release at different time intervals is presented in Table 5.

The optimized F13 formulation exhibited a gradual and sustained drug-release pattern throughout the study period. An initial drug release of $8.42 \pm 0.51\%$ was observed at 0.5 h, which progressively increased to $27.35 \pm 0.82\%$ at 2 h and $48.72 \pm 1.03\%$ at 4 h. At 6 h, the cumulative drug release reached $65.94 \pm 1.18\%$, while $74.86 \pm 1.24\%$ of the drug was released at 8 h. Further, the cumulative drug release increased to $81.35 \pm 1.31\%$ at 10 h and $86.72 \pm 1.37\%$ at 12 h. At the end of 24 h, the formulation exhibited a cumulative drug release of $92.48 \pm 1.42\%$.

The gradual increase in drug release may be attributed to the diffusion of 5-FU through the polymeric nanosponge matrix and subsequent movement through the gel network. The presence of ethyl cellulose provides a polymeric

barrier that can retard drug diffusion, while PVA contributes to the formation and stabilization of the nanosponge structure. The combination of the nanosponge system with the gel matrix may therefore provide prolonged drug release compared with an immediate-release formulation.

The relatively low standard deviation values observed at the different sampling intervals indicate good reproducibility of the release measurements. The release profile demonstrated that F13 was capable of providing prolonged and controlled release of 5-FU over 24 h, supporting its suitability for further evaluation as a topical nanosponge gel formulation.

Table 3: Results of Entrapment Efficiency (%) and Particle Size (nm)

F. Code	Entrapment Efficiency (%)	Particle Size (nm)
F1	70.22	170.40
F2	79.12	154.60
F3	80.24	120.30
F4	77.36	121.10
F5	83.32	138.50
F6	81.12	132.70
F7	85.45	165.90
F8	76.80	132.50
F9	73.15	145.80
F10	83.32	147.20
F11	76.65	172.60
F12	81.14	114.30
F13	90.25	121.80

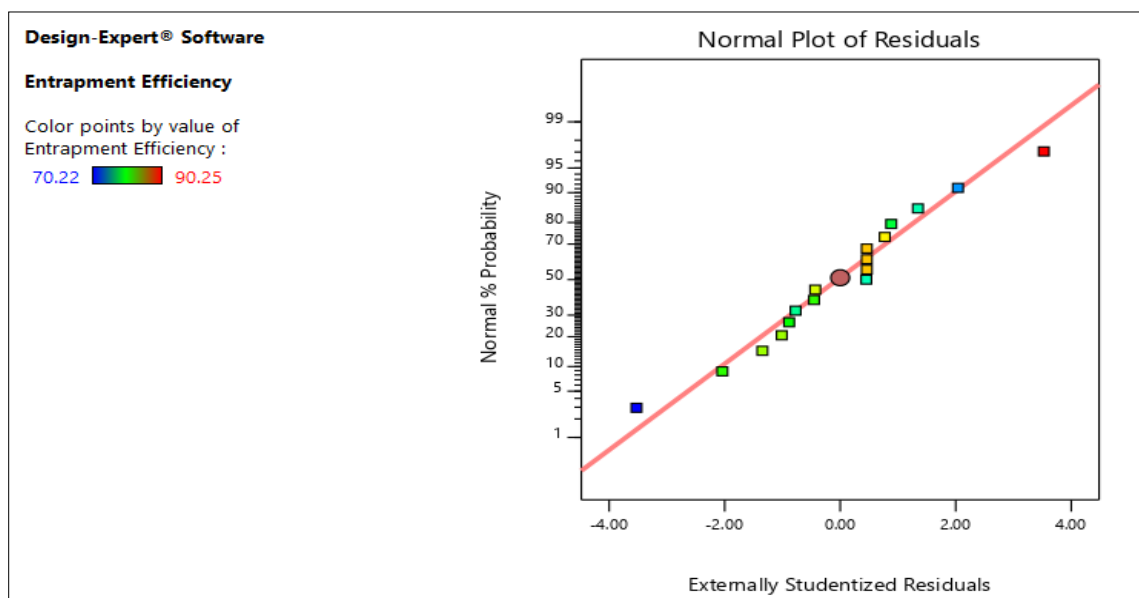


Figure 1: Graph for Entrapment Efficiency (Normal Plots for Residuals)

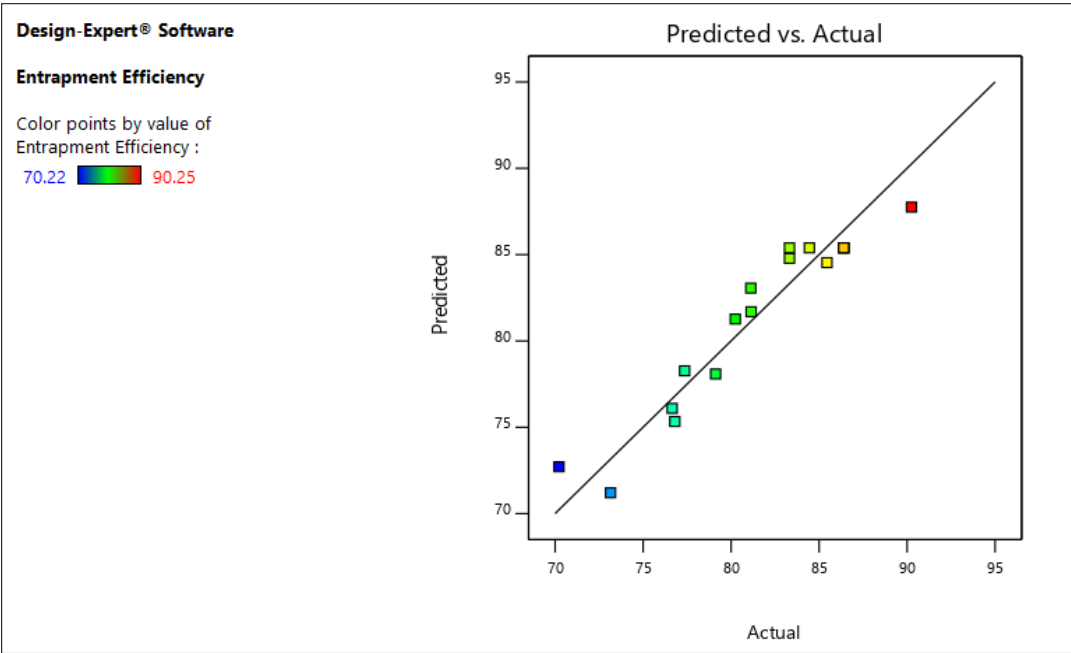


Figure 2: Graph for Entrapment Efficiency (Predicted vs. Actual)

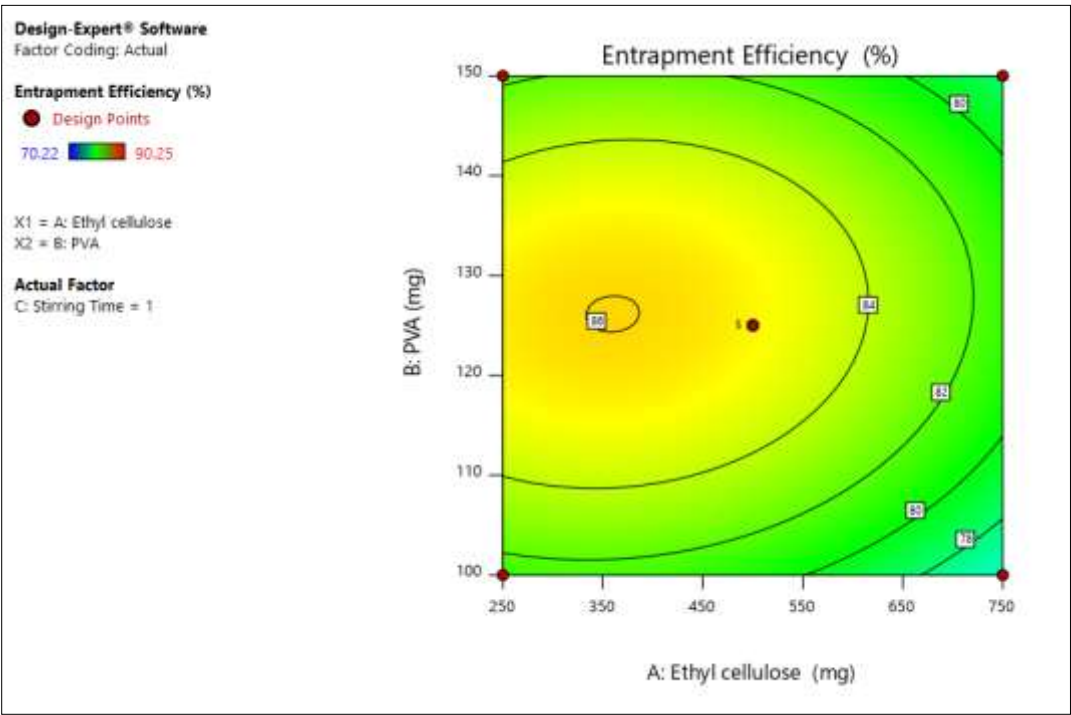


Figure 3: Contour plots for Entrapment Efficiency (between Ethyl cellulose vs. PVA)

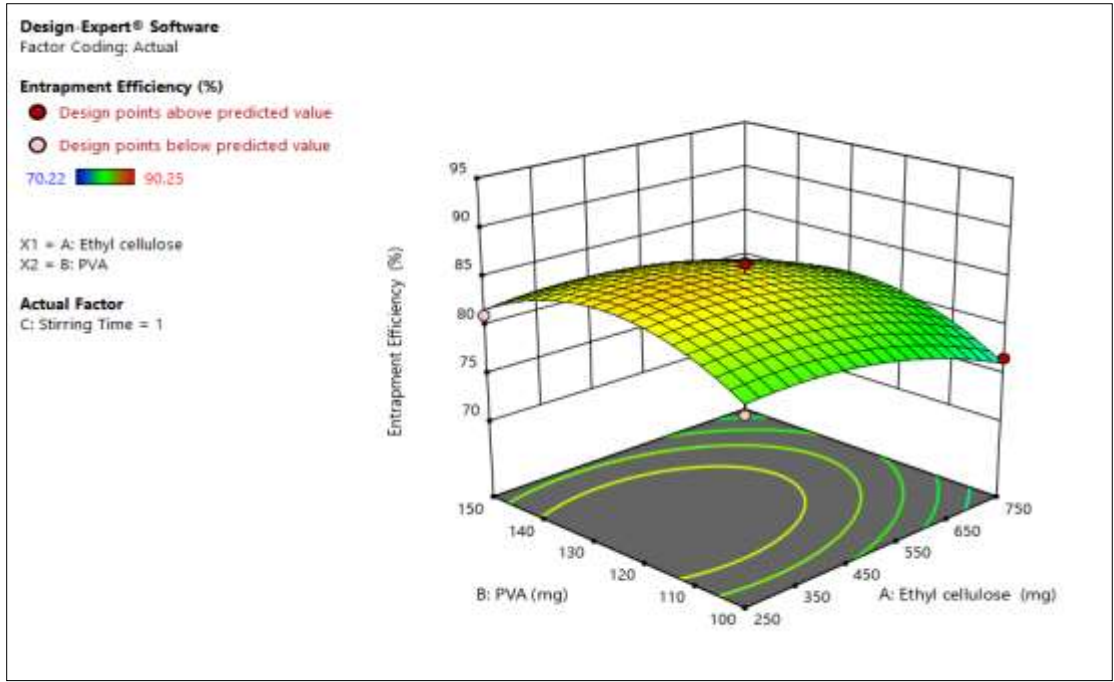


Figure 4: 3D surface plats for Entrapment Efficiency (between ethyl cellulose vs. PVA)

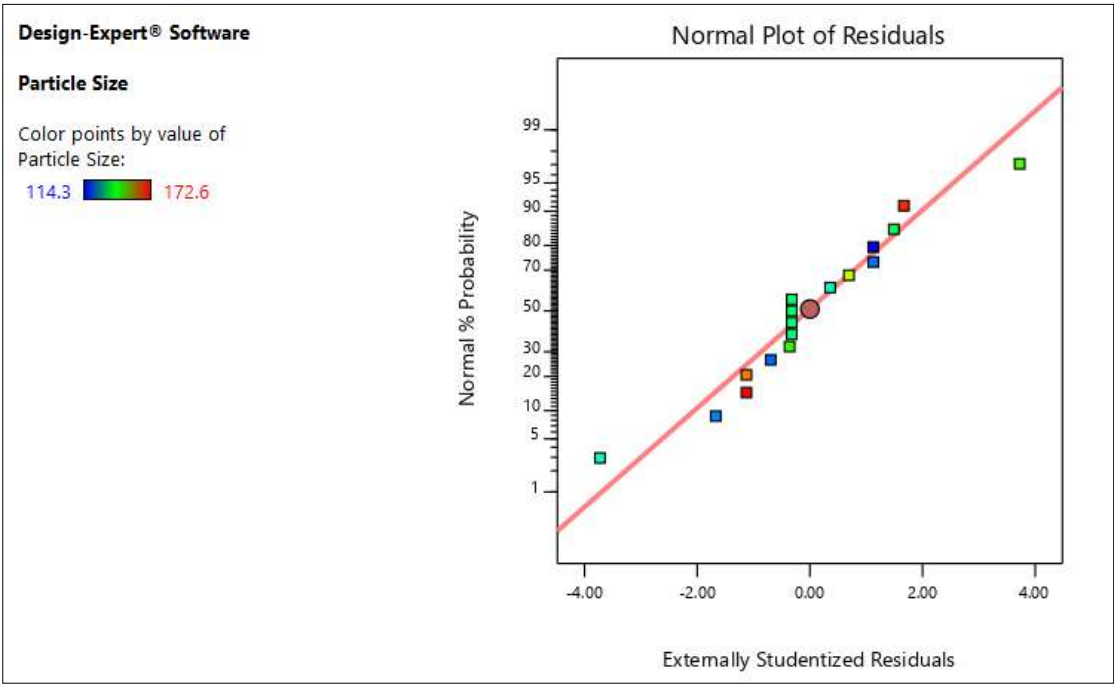


Figure 5: Graph for Particle size (Normal Plots for Residuals)

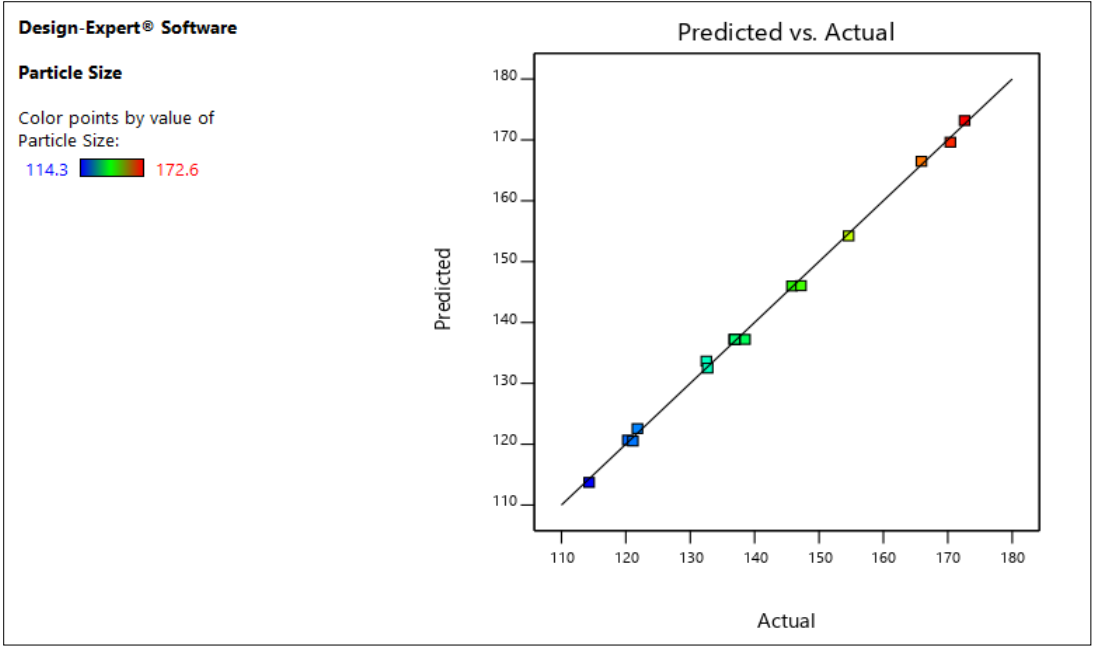


Figure 6: Graph for Particle size (Predicted vs. Actual)

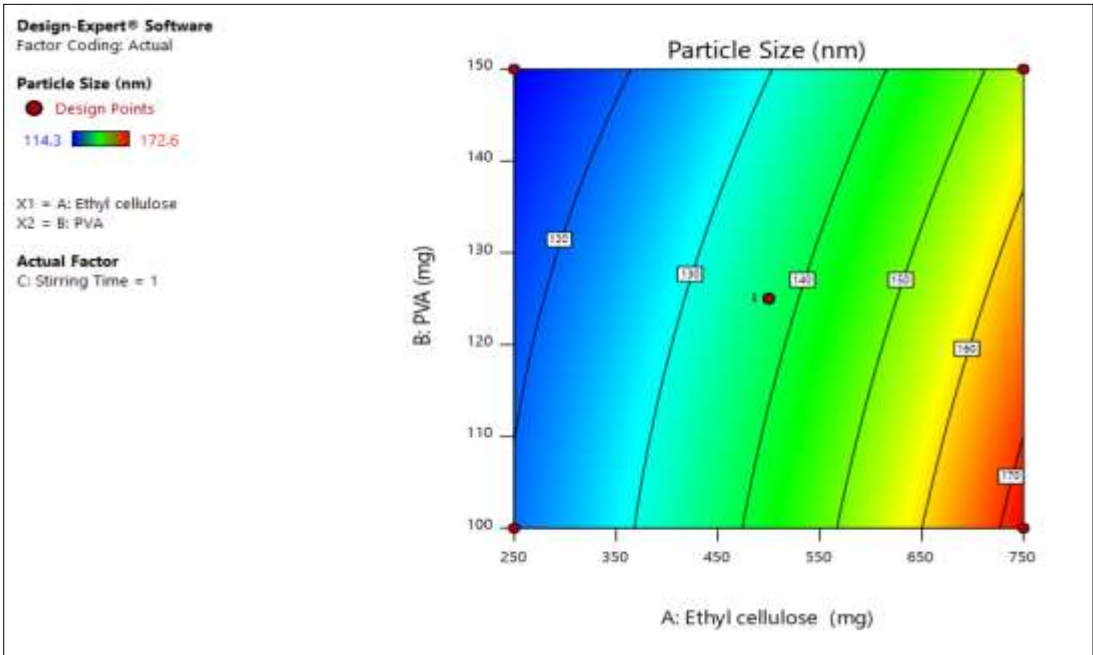


Figure 7: Contour plots for Particle size (between Ethyl cellulose vs. PVA)

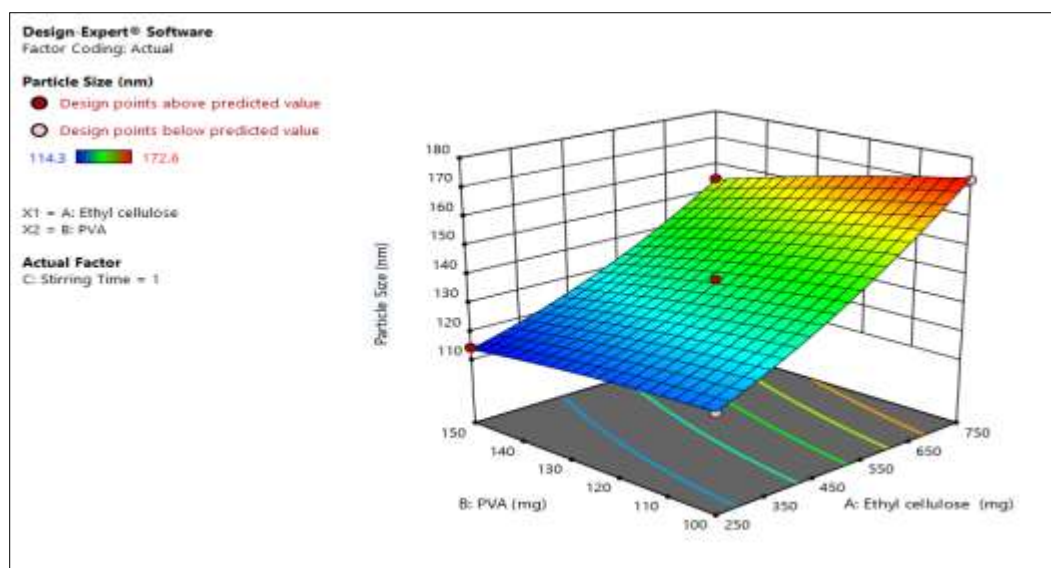


Figure 8: 3D surface plots for Particle size (between ethyl cellulose vs. PVA)

Table 4: Experimental data with predicted response

Run Order	Formulation Code				Parameters	Actual Value	Predicted Value
		Ethyl cellulose (mg)	PVA (mg)	Stirring Time (Hrs.)			
6	F13	250	125	1.5	Particle size	121.80	122.58
					Entrapment Efficiency (%)	90.25	87.75
					Zeta potential	-38.45	

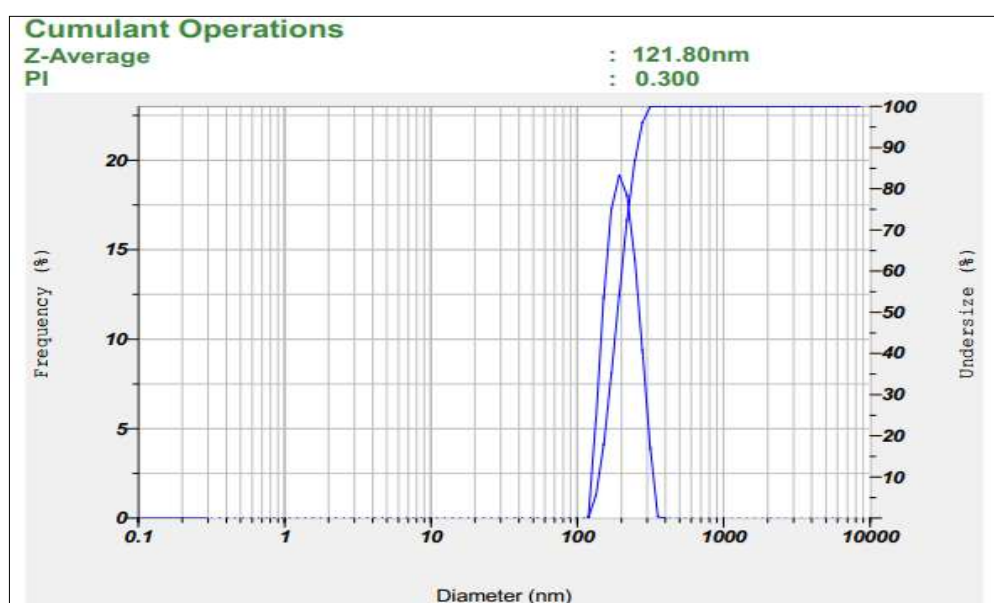


Figure 9: Graph of Particle size of optimized formulation F13

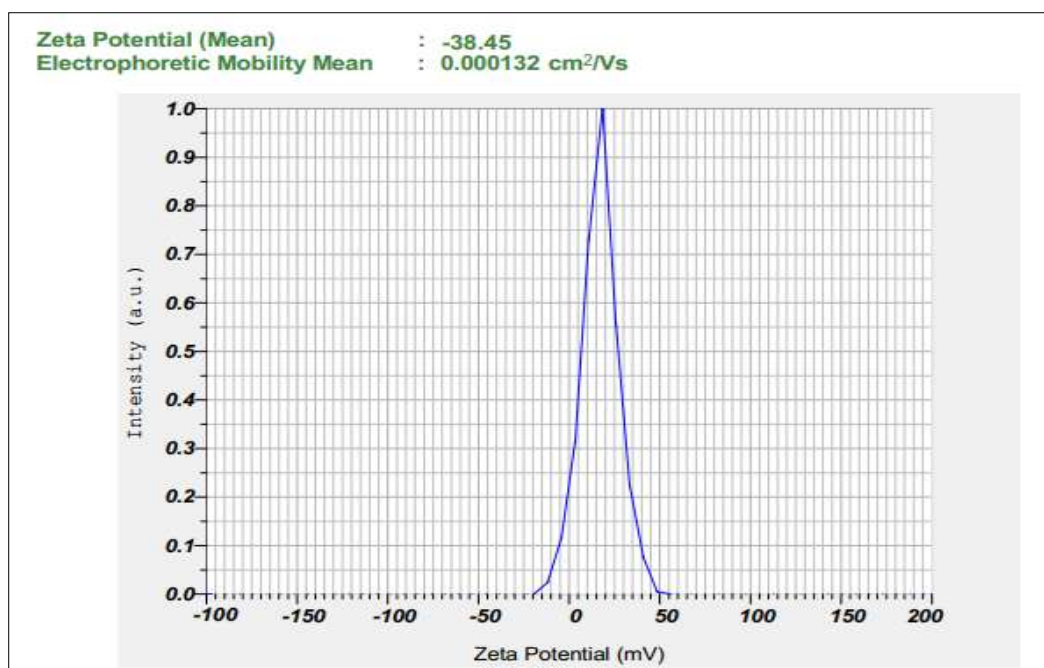


Figure 10: Graph of Zeta potential of optimized formulation F13

Table 5: *In-vitro* Drug Release Profile of Optimized 5-FU Nanosponges Gel (F13)

Time (h)	Cumulative % Drug Released (Mean $\pm$ SD)
0	0.00 $\pm$ 0.00
0.5	8.42 $\pm$ 0.51
1	15.76 $\pm$ 0.64
2	27.35 $\pm$ 0.82
3	38.64 $\pm$ 0.91
4	48.72 $\pm$ 1.03
5	57.85 $\pm$ 1.12
6	65.94 $\pm$ 1.18
8	74.86 $\pm$ 1.24
10	81.35 $\pm$ 1.31
12	86.72 $\pm$ 1.37
24	92.48 $\pm$ 1.42

CONCLUSION

The present study successfully developed 5-Fluorouracil (5-FU) loaded nanosponges using a Box–Behnken experimental design. The formulation variables were effectively optimized to obtain desirable nanosponge characteristics. The optimized F13 formulation showed an entrapment efficiency of 90.25% and particle size of 121.80 nm, with a zeta potential of -38.45 mV. The optimized nanosponges were successfully incorporated into a topical gel formulation with satisfactory physicochemical properties, homogeneity, spreadability, extrudability, and viscosity. The *in-vitro* drug release study demonstrated a gradual and sustained release of 5-FU over 24 h, with cumulative drug release of $92.48 \pm 1.42\%$. The developed 5-FU nanosponges gel demonstrated promising potential as a controlled topical drug-delivery system.

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