Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 1988-1998 | ISSN:2251-6727

Development of Sustained Release Naproxen Emulsomes: A Box-Behnken Design Approach

Parina Gill[1], Md. Shamsuzzaman[2*], Rajni Bala[3], Ramandeep Kaur[4], Neha Jain[5]

1,2,5: School of Pharmacy, Raffles University, Japanese zone, Neemrana, Alwar Rajasthan

1,3,4: University School of Pharmaceutical Sciences, Rayat-Bahra University, Kharar, Mohali

(Received: 16 June 2025 Revised: 20 July 2025 Accepted: 04 August 2025)

KEYWORDS

Emulsomes, Box Behnken, Naproxen, optimization, ANOVA, nanoformulation

ABSTRACT:

Introduction: Emulsomes, vesicular carriers combining features of emulsions and liposomes, offer enhanced loading and sustained release for lipophilic drugs. Naproxen, a poorly water-soluble anti-inflammatory agent, can benefit from such nanocarrier systems.

Objective: To develop and optimize an emulsome-based nanoformulation of Naproxen using BBD for improved entrapment efficiency, zeta potential, and reduced particle size.

Methods: Emulsomes were prepared via modified thin-film hydration using lecithin, tristearin, and stearylamine. A 3-factor, 3-level BBD was employed with independent variables: drug-to-phospholipid ratio (A), tristearin-to-phospholipid ratio (B), and stearylamine-to-phospholipid ratio (C). Seventeen experimental runs were performed. The responses evaluated were particle size (R1), zeta potential (R2), and entrapment efficiency (R3). Data were analyzed using Design-Expert® software with quadratic modeling and ANOVA.

Results: Particle size ranged from 240–968 nm, zeta potential from 24.3–81.8 mV, and entrapment efficiency from 61.6–80.7%. Statistical modeling showed significant influence of formulation factors on all responses. The optimized formulation (3 mg drug, 5 mg tristearin, 10 mg stearylamine per 100 mg phospholipid) achieved a desirability of 0.9, with predicted values: particle size 523.5 nm, zeta potential 55.47 mV, and entrapment efficiency 75.23%.

Conclusion: BBD proved to be an efficient tool for optimizing Naproxen emulsome formulations. The developed nanoformulation demonstrated desirable physicochemical properties, indicating its potential for sustained drug delivery applications

1. Introduction

Vesicular drug delivery systems have drawn considerable attention from scholars over the past few decades due to their potential for controlled and selective medication delivery. A bilayer of phospholipids surrounding an aqueous core constitutes liposomes, which are among the most commonly utilized carriers in these systems. [1,2] Due to the hydrophilic nature of their interior core, however, liposomes possess a low ability to entrap lipophilic drugs. Owing to this limitation, advanced vesicular systems have emerged, e.g., Emulsomes, having a similar structure to liposomes but differing considerably in an effort to enhance lipophilic drug entrapment. [3]

A solid lipid core, generally triglycerides, is surrounded by one or more phospholipid bilayers to create emulsomes, a second-generation colloidal carrier system. [4,5] Greater loading of lipophilic drugs, which can be entrapped in the lipid core and phospholipid envelope alike, is facilitated by this two-part structure, which harnesses the beneficial properties of both emulsions and liposomes.[6] Emulsomes are also more effective than conventional liposomes in that they offer an improved sustained drug release profile, which can

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 1988-1998 | ISSN:2251-6727

last a maximum of 24 hours.[7] Phospholipids, triglycerides, drug molecules, and other excipients like charge inducers are some of the formulation factors that influence the physicochemical properties of emulsomes. Such critical properties as particle size, drug entrapment efficiency, and release kinetics might be significantly influenced by these factors. Thus, successful emulsome-based drug delivery system development relies on an understanding of the relationship between formulation factors and product attributes. [8]

A powerful statistical tool for systematically investigating such relationships is Design of Experiments (DoE). Recent experimental designs enable the simultaneous study of multiple factors and their interactions, as opposed to traditional methods focusing on a single variable at a time with other variables kept constant.[9] Pharmaceutical science extensively utilizes the following DoE methodologies: BBD, CCD, and DM. Specifically, BBD is suitable for evaluating three independent factors at three levels each, in which a quadratic model can be developed more easily to predict the influence of formulation and process variables on significant questions. In order to demonstrate how variations of two variables influence the response keeping other variables constant, graphical tools such as 2D contour plots and 3D response surface plots are employed. These plots assist in finding suitable conditions that meet pre-specified criteria of desirability.[10] To reduce the number of experimental trials and optimize knowledge, the current research is aimed to study the effects of various critical formulation parameters on emulsome characteristics to achieve a best formulation with improved performance using a methodical DoE approach.

2. Objectives

Optimization of naproxen-loaded emulsomes is crucial to address the drug's poor water solubility (BCS II), short biological half-life, and the gastrointestinal discomfort caused by frequent dosing. Emulsomes, composed of a solid lipid core and a surrounding phospholipid bilayer, improve the delivery of lipophilic drugs and enable sustained release. However, their effectiveness depends on key formulation factors like lipid type, phospholipid content, and surfactant concentration. Fine-tuning these variables enhances drug loading, release control, and stability. Utilizing a systematic approach such as Box-Behnken Design (BBD) helps to understand the interactions between variables, leading to the development of a more efficient and patient-friendly delivery system..

3. Methods

3.1. Materials

A gratuitous sample of naproxen was obtained from Dr. Reddy's Laboratories, Baddi, Himachal Pradesh, India. HiMedia Laboratories provided the soy lecithin and tristearin, whereas LOBA Chemie, Mumbai provided the cholesterol. Otto Chemie Pvt. Ltd., Mumbai provided the Stearylamine, whereas Sigma-Aldrich, USA provided the Sephadex G-50 used for column separation.

3.2. Emulsome Preparation

A thin-film hydration method modified, which was based on the conventional method, was employed to form emulsomes.[11] The lipid phase, consisting of lecithin, tristearin, and cholesterol, was dissolved first in chloroform after naproxen had been dissolved in methanol. A 500 mL capacity round-bottom flask was utilized to mix these two solutions. [12]

Solvents were steadily evaporated with a rotary evaporator under vacuum, establishing a uniform, thin lipid layer at the inner wall of the flask. [13-15] In order to form a lipid dispersion, the film was again hydrated with phosphate-buffered saline (PBS, pH 7.4). To reduce the vesicle size and form a stable nano-sized emulsomal solution, the resulting mixture was sonicated for 12 minutes at 40% amplitude by ultrasonic processor (Lark, India). The purity of the solution was achieved by column chromatography using Sephadex G-50 to remove free, unentrapped naproxen, resulting in a better formulation of naproxen-loaded emulsomes

3.3. Experimental Design

The Box-Behnken design (BBD), a response surface methodology (RSM), was employed for optimizing the emulsome formulations.[15-19] In analyzing the effect of three independent variables, each at three different levels, the use of this technique is highly beneficial. The independent variables employed for the current study were:

- A: Phospholipid to NAPROXEN ratio
- B: Phospholipid to Tristearin ratio

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 1988-1998 | ISSN:2251-6727

- C: Phospholipid to Stearylamine ratio

Design Expert Software, (Stat-Ease 360., Minneapolis, MN, USA) was utilized to design the experimental design matrix. Twelve edge points and five center points were part of the total of seventeen trial runs that were generated.[20,21] The center points confirmed the adequacy of the selected model and helped in measuring the experimental error. Each experiment was repeated three times, and additional statistical analysis was carried out based on the average data. To study how three independent variables (A, B, and C) impacted three significant dependent responses was the focus.

- R1: Particle Size
- R2: Zeta Potential
- R3: Entrapment Efficiency

The software was employed to prepare 3-dimensional response surface plots and 2-dimensional contour plots to evaluate these responses. The relationship among the variables was also explained by fitting the data into the quadratic polynomial equation. The generic form of the quadratic model is as follows:

$$Y=b0+b1X1+b2X2+b3X3+b12X1X2+b13X1X3+b23X2X3+b11X21+b22X22+b33X32$$

Here,

- Y represents the predicted response,
- b_0 is the intercept,
- b_1 to b_3 are the linear coefficients,
- b_{12} to b_{23} are interaction coefficients between variables,
- b_{11} to b_{33} are the quadratic coefficients, and
- X_1, X_2, X_3 represent the coded values of the independent variables.

Analysis of Variance (ANOVA), involving checking of F-values, adjusted R2, and estimated R2, was applied to identify the significance and validity of the model. These statistics helped in measuring the forecasting ability and robustness of the model. The following tables present the coded and actual independent variables (A, B, and C) and the respective answers (R1, R2, and R3).

Table 1: Variables and their levels in Box-behnken design

Variable code	Variable	Unit	Coded values/Actual values		
			-1	0	+1
A	PC:SA	%w/w of PC	5	10	15
B	PC:TRI	%w/w of PC	50	100	150
C	PC:DR	%w/w of PC	10	20	30

3.4. Characterization

I. Particle Size and Polydispersity Analysis

A Zetasizer 2000 instrument was employed to determine the particle size average and polydispersity index (PDI) via Dynamic Light Scattering (DLS) at a temperature of 25 ± 0.5 °C. A PDI ≤ 0.3 was confirmed by the research, demonstrating ultimate homogeneity and a limited particle size dispersion. For uniform drug release and bioavailability, this homogeneity is vital [22].

II. Determination of Zeta Potential

Using laser Doppler electrophoresis and a Zetasizer 2000, zeta potential was determined in order to evaluate the surface charge and colloidal stability of the emulsomes. Strong electrostatic repulsion between particles was indicated by a high absolute zeta potential value, which reduced aggregation and improved the formulation's shelf stability and biological compatibility [23].

III. Evaluation of Entrapment Efficiency

The efficiency of entrapment for naproxen was found by purifying the free drug using Sephadex G-50 gel filtration. The amount of encapsulated naproxen was quantified by UV-visible spectrophotometry following lysis of the retained emulsomes with 0.5% Triton X-100. Successful entrapment and drug compatibility of naproxen within the emulsomal lipid matrix were established by the findings, which were expressed as mean ± standard deviation (SD) for three replicates and had excellent drug loading efficiency [24]..

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 1988-1998 | ISSN:2251-6727

4. Results

A three-factor, three-level Box-Behnken design used to optimize a lipid-based nanoformulation is described in the Table 1. Drug to Lecithin ratio (Factor A), Tristearin to Lecithin ratio (Factor B), and Sterylamine to Lecithin ratio (Factor C) were the three main formulation factors that were examined at three different levels: -1, 0, and +1. Different combinations of medication, tristearin, and sterylamine concentrations were used in a total of 17 experimental runs. Particle size (nm), zeta potential (mV), and entrapment effectiveness (%) were the main responses assessed. The core formulation (20 mg medication, 100 mg tristearin, and 10 mg sterylamine) was repeated many times in order to evaluate model dependability. The findings demonstrated significant variation in entrapment effectiveness (61.6–80.7%), zeta potential (24.3–81.8 mV), and particle size (240–968 nm), underscoring the influence of formulation elements on the end result. By determining the best component combination for enhanced drug delivery efficacy, this design technique proved useful in formulation optimization.

Table 2: Experimental layout of 3 factors 3 levels Box-behnken design and values of response variables

RUNS	Coded Factors			Actual Factors			Responses		
	F1	F2	F3	F1	F2	F3	R1	R2	R3
	A:A (Drug: Lecithin) % w/w of Lecithin	B:B (Tristearin:Le... % w/w of Lecithin	C:C (Stearylamin... % w/w of Lecithin	A:A (Drug: Lecithin) % w/w of Lecithin	B:B (Tristearin:Le... % w/w of Lecithin	C:C (Stearylamin... % w/w of Lecithin	Particle size	Zeta potential	Entrapment efficiency
	Drug	Tristearin	Sterylamine	Drug (mg)	Tristearin	Sterylamine	(nm)	(mv)	(%)
1	+1	+1	0	30	150	10	968	56.1	80.7
2	0	0	0	20	100	10	603	59.8	69.3
3	0	0	0	20	100	10	495	48.6	73.1
4	0	-1	-1	20	50	5	311	26.3	61.6
5	0	0	0	20	100	10	443	61.8	66.4
6	+1	0	+1	30	100	15	624	74.6	72.6
7	+1	0	-1	30	100	5	770	31.1	76.1
8	-1	+1	0	10	150	10	816	47.1	64.6
9	-1	0	+1	10	100	15	846	81.8	68.1
10	0	+1	-1	20	150	5	783	24.3	71.2
11	0	0	0	20	100	10	568	62.2	75.6
12	-1	-1	0	10	50	10	240	54.3	63.7
13	0	-1	+1	20	50	15	361	78.1	66.1
14	0	+1	+1	20	150	15	813	81.6	77.8
15	0	0	0	20	100	10	622	59.1	74.4

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 1988-1998 | ISSN:2251-6727

16	+1	-1	0	30	50	10	568	63.6	68.6
17	-1	0	-1	10	100	5	641	326	66.8

Three crucial reaction parameters—particle size (R1), zeta potential (R2), and entrapment efficiency (R3)—are compared against actual and projected values for 17 experimental runs in the checkpoint analysis table. The accuracy of the optimization model is shown by the residual or percentage error, which shows the discrepancy between the experimental result and the model's forecast. Particle size data exhibit errors ranging from -192 to +192 nm, indicating both over- and under-predictions. Zeta potential forecasts vary a lot, particularly in runs (such runs 4, 7, and 10) when the actual values differed much from the model. Better predictability for entrapment efficiency was shown by comparatively small differences, often falling within ±7.%. Although the prediction of particle size and zeta potential shows significant variance, the model generally demonstrates a decent prediction capability, especially for entrapment efficiency. These findings support the model's partial dependability and point out that minor adjustments are necessary to improve forecast accuracy for all replies. Table 2.

Table 3: point prediction check point for optimizationactual value, experimental value and residual error (% error)

RUN	Particle size (R1)			Zeta potential (R2)			Entrapment efficiency (R3)		
	Actual value	Predicted value	Residual (% error)	Actual value	Predicted value	Residual (% error)	Actual value	Predicted value	Residual (% error)
1	968	777.5	192.5	56.1	55.47	0.62	80.7	73.46	7.24
2	603	692.8	-89.8	59.8	55.47	4.33	69.3	70.39	-1.09
3	495	367.5	127.5	48.6	55.47	-6.87	73.1	69.98	3.12
4	311	503.5	-192.5	26.3	55.47	-29.17	61.6	67.33	-5.73
5	443	552.5	-109.5	61.8	55.47	6.33	66.4	72.58	-6.18
6	624	692.8	-68.8	74.6	55.47	19.13	72.6	70.39	2.21
7	770	533.0	237	31.1	55.47	-24.37	76.1	67.74	8.36
8	816	706.5	109.5	47.1	55.47	-8.37	64.6	68.21	-3.61
9	846	711	-65	81.8	55.47	26.33	68.1	68.02	-0.52
10	783	692.8	90.2	24.3	55.47	-31.17	71.2	70.39	0.81
11	568	523.5	44.5	62.2	55.47	6.73	75.6	75.23	0.37
12	240	367.5	-127.5	54.3	55.47	-1.17	63.7	70.81	-7.11
13	361	405.5	-44.5	78.1	55.47	22.63	66.1	65.56	0.54
14	813	692.8	120.2	81.6	55.47	26.13	77.8	70.39	7.41
15	622	557	65	59.1	55.47	3.63	74.4	72.17	2.23
16	568	805	-237	63.6	55.47	8.13	68.6	73.04	-4.44

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 1988-1998 | ISSN:2251-6727

17	641	692	-51	32.6	55.47	-22.87	66.8	70.39	-3.59

Table 4: Summary of resuts of regression analysis for responses R1, R2, R3

Model	Sequential p-value	Lack of fit p-value	Adjusted R^2	Predicted R^2	Remarks
Response (Particle size, R1)					
Linear	0.5528	0.8195	-0.0535	-0.3565	**Suggested**
2FI	0.4739	0.8157	-0.0779	-0.6515	
Quadratic	0.6704	0.7107	-0.2508	-1.9628	
Response (Zeta potential, R2)					
Linear	0.0141	0.7731	0.4401	0.2798	**Suggested**
2FI	0.6499	0.7002	0.3777	-0.0333	
Quadratic	0.7201	0.5313	0.2569	-1.3461	
Response (Entrapment Efficiency, R3)					
Linear	0.2687	0.8503	0.0809	-0.2069	**Suggested**
2FI	0.7354	0.7696	-0.0580	-0.9163	
Quadratic	0.4952	0.7617	-0.4772	-1.3461	

The fit statistics for the three response variables entrapment efficiency (R3), zeta potential (R2), and particle size (R1) are shown in the Table 3 anmd 4. Particle size has the most variance (226.56), followed by zeta potential (15.57) and entrapment efficiency (5.63), according to the standard deviation values, which reveal data variability. In comparison to particle size (36.78%) and zeta potential (28.8%), the coefficient of variation (C.V.%) for entrapment efficiency (8%) is very low, indicating superior consistency. The zeta potential and entrapment efficiency have comparatively poor prediction, but the R2 values show a moderate match for particle size (0.4528). The present model may not adequately explain the data or forecast future events, as shown by negative adjusted and anticipated R2 values. Nonetheless, all three replies sufficient accuracy levels over 2 imply that the signal-to-noise ratio is appropriate. While the other two answers provide an adequate fit, the lack-of-fit p-values for particle size ($p = 0.49$) demonstrate statistical significance, suggesting a possible model mismatch. Table 5

Table 5: Fit statics for responces R1, R2, R3

	Particle size (R1)	Zeta potential (R2)	Entrapment efficiency (R3)
Std. Dev.	226.56	15.98	6.47
Mean	616	55.47	70.39
C.V. %	36.78	28.80	9.20
R^2	0.4528	0.6749	0.3654
Adjusted R^2	-0.2508	0.2569	-0.4506
Predicted R^2	1.9628	1.3461	-7.0332
Adeq Precision	3.061	4.463	2.4923
Lack of Fit (p-values)	0.49	0.86	0.84

In Table 6, the ANOVA summary for the models that forecast entrapment efficiency, zeta potential, and particle size in a lipid-based nanoformulation investigation is shown in the table. The models are not statistically significant for all three responses since the regression p-values (particle size: 0.6189; zeta potential: 0.8120; entrapment efficiency: 0.8700) are higher than 0.05. Additionally, the F-values are low, indicating that the independent factors contributed little to the replies. There may be a model mismatch for this reaction, as

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 1988-1998 | ISSN:2251-6727

shown by the significant particle size lack-of-fit test (p = 0.0302). The lack-of-fit for entrapment efficiency (p = 0.0923) and zeta potential (p = 0.7837), however, is not statistically significant, indicating that the model matches those responses. Overall, the residual error is still significant, particularly for particle size, which might be because of the present model's narrow scope or experimental variability. In order to increase forecast accuracy, this research highlights the need of either more influencing variables or model development.

Table 6: Analysis of variance (ANOVA) of calculated models for responses

Result of the ANOVA	Particle size (nm)	Zeta potential (mV)	Entrapment Efficiency (%)
Regression			
Sum of squares	3.089E+05	2239.53	168.91
Degree of freedom (df)	9	9	9
Mean square	34327.14	248.84	18.77
F-value	0.8189	0.5349	0.4478
p-value	0.6189	0.8120 or >0.1	0.8700
Interface	not significant	not significant	not significant
Lack of fit tests			
Sum of squares	2.554E+05	698.26	25.48
Degree of freedom (df)	3	3	3
Mean square	85118.00	232.75	75.16
F-value	8.94	0.3639	4.43
p-value	0.0302	0.7837	0.0923
Interface	significant	not significant	not significant
Residual			
Sum of squares	2.934E+05	3256.39	293.39
Degree of freedom (df)	7	7	7
Mean square	41916.97	465.20	41.91

Particle Size

The obtained *Model F-value* of 0.64 suggests that the model lacks statistical significance when compared to the variability caused by random noise. In fact, there is a 73.65% probability that such a high F-value could arise purely due to noise, rather than from meaningful model terms. Generally, a *p-value (Prob > F)* below 0.05 indicates statistically significant model terms. However, in this case, none of the model terms meet that threshold, indicating that they are not contributing significantly to the prediction. Moreover, model terms with *p-values* greater than 0.10 are typically considered non-significant. When multiple non-significant terms are present (excluding those necessary to maintain the hierarchical structure of the model), simplifying the model through reduction may help enhance its performance.

The *Lack of Fit F-value* is 0.49, indicating that the lack of fit is not significant compared to the pure error. With a 71.07% probability that this level of lack of fit is due to noise, this is actually favorable—it suggests the model fits the data reasonably well. However, the *predicted* R^2 of –5.8815 shows poor predictive capability, even though the *actual* R^2 is 0.5129. The *adjusted* R^2 of 0.0113 further highlights that the model explains very little variability in the response, in this case, particle size. Despite these shortcomings, the *adequate precision* value of 2.7862—being above the desired threshold of 2—indicates a reasonable signal-to-noise ratio, suggesting that the model still provides some degree of reliable measurement.

Journal of Chemical Health Risks

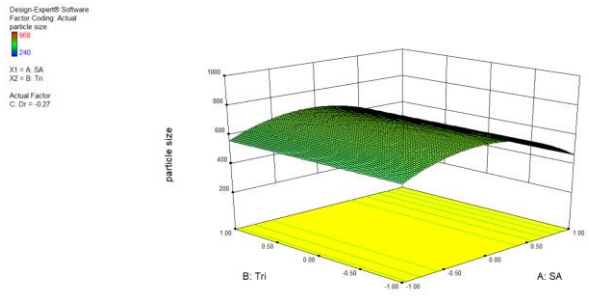
www.jchr.org

JCHR (2025) 15(4), 1988-1998 | ISSN:2251-6727

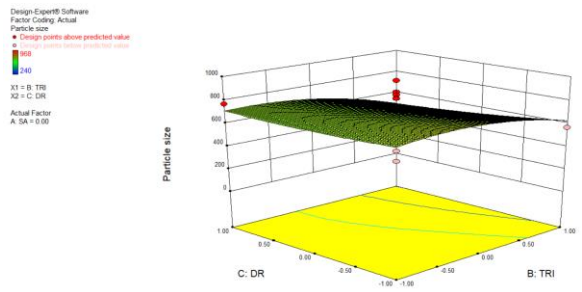
The second order polynomial equation relating the response of particle size (R1) is given below:

R1(particle size) = 697.20-63.37 A-79.87B - 37.75 C -167 A B - 35.25 A C - 28.25 B C -116.10 A2 -72.60 B2
+16.15 C2

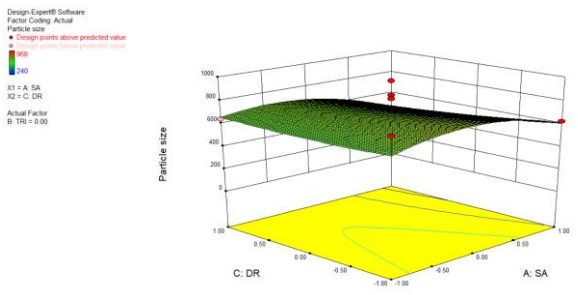
The negative values of variables indicates that it does not effect the particle size significantly.



(1a)



(1b)



(1c)

Fig.1: 3-Dimensional response surface plots showing interaction effect of variable A & B (1a), A & C (1b), B & C (1c) on particle size (R1).

Zeta potential

The *Model F-value* of 1.61 suggests that the overall model is not statistically significant when compared to the variability introduced by random noise. There is a 27.02% probability that such an F-value could occur purely by chance. Typically, model terms are considered significant if their *p-values (Prob > F)* are less than 0.05. In this analysis, factor **C** emerged as a significant term, indicating it has a meaningful effect on the response. Conversely, terms with *p-values* above 0.10 are deemed non-significant and may be candidates for removal, provided they are not required to maintain model hierarchy. Reducing these non-contributing terms could potentially enhance model performance and interpretability.

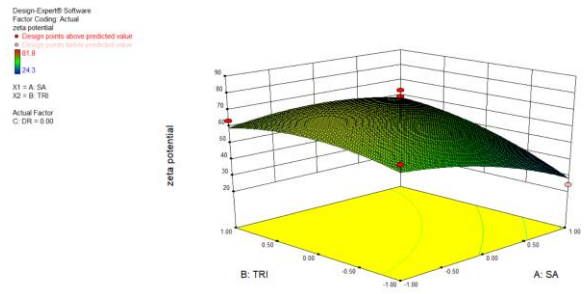
The *Lack of Fit F-value* of 0.86 indicates that the model's lack of fit is not statistically significant relative to the pure error, with a 53.13% chance that such a value could result from noise. This is a favorable outcome, as a non-significant lack of fit implies that the model sufficiently fits the experimental data.

Furthermore, the *predicted R^2* of 0.6749 is in reasonable alignment with the *actual R^2* of 0.6749, and the *adjusted R^2* of 0.2569 suggests the model captures a modest proportion of the variability in the zeta potential response. The *adequate precision* value of 4.4630—well above the desired threshold of 2—demonstrates a strong signal-to-noise ratio, confirming that the model is capable of making reliable predictions. The developed polynomial equation describing the relationship between the input variables and the zeta potential response is as follows:

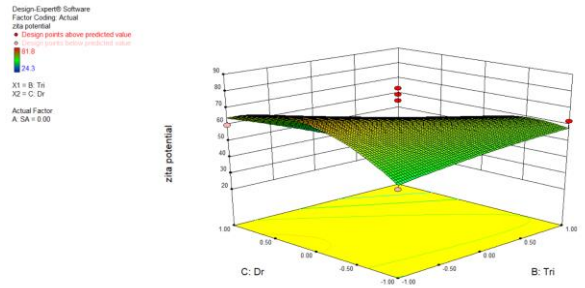
R2 = 62.34 - 7.51 A + 7.95B -15.96 C +7.05A B + 0.17AC + 6.40 BC - 5.31 A2 -6.03 B2 -3.26 C2

As shown in quadratic equation individual factor B and combined factor AB . BC and AC have synergistic effect, whereas individual factors A and C exhibited antagonastic effecton zeta potential. The positive sign of the factor B indicate that the addition of tristearin improve the stability of emulsomes formulation.

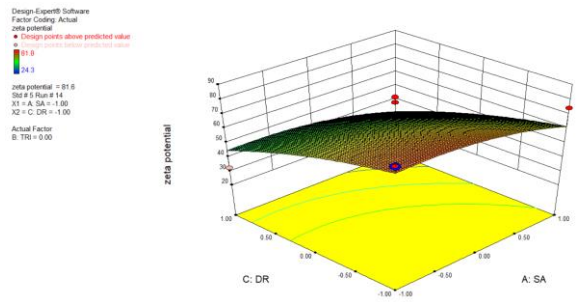
Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 1988-1998 | ISSN:2251-6727



(2a)



(2b)



(2c)

Fig.2: 3-Dimensional response surface plots showing interaction effect of variable A & B (2a), A & C (2b), B & C (2c) on zeta potential (Y2).)

Entrapment Efficiency

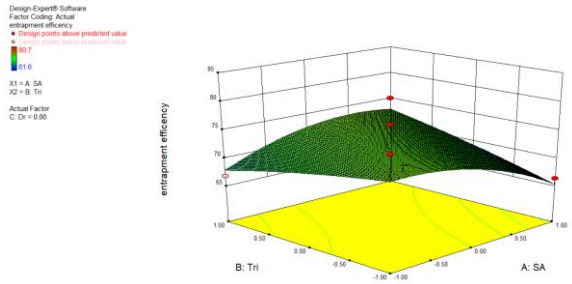
The *Model F-value* of 0.84 indicates that the model is not statistically significant when compared to the background noise. There is a 60.37% probability that an F-value of this magnitude could arise purely due to random variation, rather than from meaningful model effects. According to standard statistical criteria, model terms are considered significant when the *p-value (Prob > F)* is less than 0.0500. In this case, none of the model terms met that threshold, indicating that they do not significantly influence the response. Additionally, terms with *p-values* greater than 0.1000 are generally considered non-significant and may be candidates for removal—unless required to maintain model hierarchy. Reducing such terms could improve the model's clarity and performance.

The *Lack of Fit F-value* of 0.40 further suggests that the model's lack of fit is not significant when compared to the pure error. With a 76.17% chance that such a value could occur due to noise, this result indicates that the model fits the experimental data reasonably well—an outcome that is considered favorable.

The study also established a second-order polynomial equation to describe the relationship between the independent variables and the response of **entrapment efficiency**, which is presented as follows:

R3 (Entrapment Efficiency) = 71.54 - 2.21 + 2.63 B + 0.4375 C - 1.22 AB - 0.5500 AC + 2.83 BC + 0.7800 A^2 - 2.70 B^2 - 0.5200 C^2

According to the quadratic model, individual factors **B** and **C**, as well as their interaction (**BC**), demonstrated a synergistic effect on zeta potential. In contrast, individual factors **A** and **C** exhibited an antagonistic influence. The positive coefficients associated with **B** (tristearin) and **C** (drug concentration) suggest that increasing their levels leads to improved entrapment efficiency in the emulsome formulation. This enhancement can be attributed to the direct role of the drug concentration—higher levels of drug increase the availability of active compound in the system for encapsulation. Additionally, as tristearin serves as the core-forming lipid, an increased percentage provides more matrix volume for the drug to be entrapped. The combination of both higher tristearin and drug concentrations (BC interaction) further amplifies this effect, leading to greater entrapment efficiency.

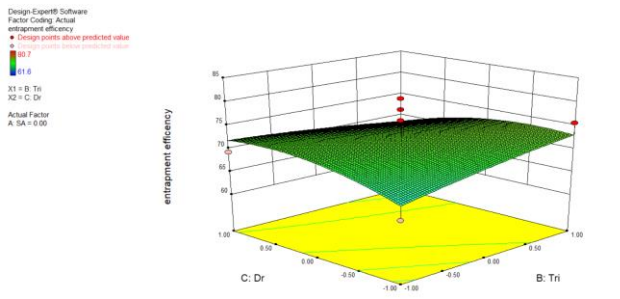


(3a)

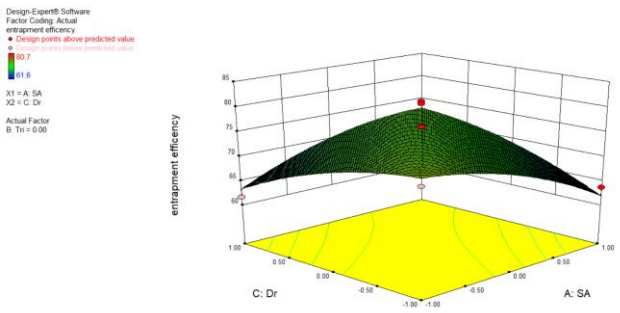
Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 1988-1998 | ISSN:2251-6727



(3b)



(3c)

Fig. 3: Three-dimensional response surface plots illustrating the interactive effects of formulation variables on entrapment efficiency (Y_3): (3a) Interaction between variable A and B, (3b) Interaction between variable A and C, and (3c) Interaction between variable B and C.

Optimization of data and validation of response surface methodology

The experimental results were analyzed using Design-Expert® software through a quadratic model involving ANOVA, as well as 2D and 3D response surface plots. The optimization of the formulation was guided by the goal of achieving maximum zeta potential and entrapment efficiency, while minimizing particle size. The software evaluated various combinations of the selected formulation variables within the defined design space to identify the most desirable outcome. Among the experimental runs, *Run 11* showed the highest desirability score. Desirability values range from 0 to 1, with values closer to 1 indicating better optimization. In this study, the optimal formulation achieved a high desirability value of 0.9. The selected combination included 3 mg of drug, 5 mg of tristearin, and 10% stearylamine relative to the total phospholipid content.

.

5. Discussion

Naproxen-loaded emulsomes were successfully developed using a modified lipid film hydration technique. A 3-factor, 3-level Box-Behnken design (BBD) was employed to optimize the formulation variables to achieve the desired characteristics—namely, maximum zeta potential and entrapment efficiency, along with the smallest possible particle size. Design-Expert® software facilitated the evaluation of individual and interactive effects of the three key formulation parameters: the drug-to-phospholipid ratio, tristearin-to-phospholipid ratio, and stearylamine-to-phospholipid ratio. A total of 17 experimental runs were conducted based on the BBD matrix, and the data were analyzed to determine the optimal formulation conditions using the desirability function. The final optimized formulation, corresponding to a desirability score of 0.9, was found to contain 3 mg of drug, 5 mg of tristearin, and 10% stearylamine with respect to the total phospholipid amount. This combination yielded a predicted particle size of 523.5 nm, a zeta potential of 55.47 mV, and an entrapment efficiency of 75.23%. Overall, this study confirms the utility of Box-Behnken design in effectively optimizing emulsome formulations by evaluating the influence of multiple formulation variables in a systematic and statistically valid manner..

Refrences

1. Zhang Y, Wang X, Liu J, Zhang Q. Advances in liposome-based drug delivery systems for cancer therapy. *J Control Release*. 2020;328:675–92.
2. Li M, Du C, Guo N, Teng Y, Meng X, Sun H, et al. Lipid-based nanocarriers for oral delivery of poorly soluble drugs: Recent advances and future perspectives. *Int J Pharm*. 2021;598:120374.
3. Kumar P, Sharma A, Chawla A, Goyal A. Emulsomes: A novel approach for sustained and targeted drug delivery. *Drug Deliv Transl Res*. 2022;12(5):1083–97.
4. Patel R, Patel M, Patel K. Design and optimization of emulsomes for enhanced topical drug delivery using a quality-by-design approach. *AAPS PharmSciTech*. 2023;24(2):56.
5. Chen L, Wang Y, Zhang X, Li J. Novel emulsome-based formulations for improved delivery of antiviral agents: A review. *Eur J Pharm Sci*. 2024;194:106704.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 1988-1998 | ISSN:2251-6727

6. Wang H, Zhang L, Liu Y, Chen S. Lipid-based nanoparticles for targeted drug delivery in cancer therapy. *Adv Drug Deliv Rev*. 2020;163–164:108–24.
7. Sharma S, Gupta N, Singh R. Emulsomes as a novel carrier for improved delivery of poorly soluble drugs. *J Drug Deliv Sci Technol*. 2021;65:102709.
8. Lee J, Kim S, Park H. Optimization of liposomal drug delivery systems using design of experiments: A comprehensive review. *Int J Nanomedicine*. 2022;17:2345–62.
9. Yang Q, Zhou X, Zhang T. Emulsomes for targeted delivery of biologics: Recent developments and challenges. *J Pharm Sci*. 2023;112(4):897–910.
10. Kim D, Lee H, Choi Y. Advances in emulsome technology for sustained release applications. *Drug Dev Ind Pharm*. 2024;50(3):215–28.
11. Xu W, Li Y, Zhao Q, Wang J. Lipid-based nanocarriers for enhanced oral bioavailability: Design and optimization strategies. *Pharmaceutics*. 2025;17(1):45.
12. Pal A, Gupta S, Jaiswal A, Dube A, Vyas SP. Development and evaluation of tripalmitin emulsomes for the treatment of experimental visceral leishmaniasis. J Liposome Res. 2012;22(1):62–71.
13. Wang F, Chen L, Jiang S, He J, Zhang X, Peng J, et al. Optimization of methazolamide-loaded solid lipid nanoparticles for ophthalmic delivery using Box–Behnken design. J Liposome Res. 2014;24:1–11.
14. Wehrle P, Stamm A. Statistical tools for process control and quality improvements in pharmaceutical industry. Drug Dev Ind Pharm. 1994;20:141–64.
15. Kaur P, Garg T, Rath G, Murthy RSR, Goyal AK. Development, optimization and evaluation of surfactant based pulmonary nanolipid carrier system of paclitaxel for the management of drug-resistant lung cancer using Box–Behnken design. Drug Deliv. 2016;23(6):1912–25.
16. Kraisit P, Sarisuta N. Development of triamcinolone acetonide loaded nanostructured lipid carriers (NLCs) for buccal drug delivery using Box–Behnken design. Molecules. 2018;23:982.
17. Moghddam SMM, Ahad A, Aquil M, Imam S, Sultana Y. Optimization of nanostructured lipid carriers for topical delivery of nimesulide using Box–Behnken design approach. Artif Cells Nanomed Biotechnol. 2017;45(3):617–24
18. Rane S, Prabhakar B. Optimization of paclitaxel containing pH-sensitive liposomes by 3-factor, 3-level Box–Behnken design. Indian J Pharm Sci. 2013;75(4):420–6.
19. Yasir M, Sara UVS. Preparation and optimization of haloperidol loaded solid lipid nanoparticles by Box–Behnken design. J Pharm Res. 2013;7:551–8.
20. Yasir M, Sara UVS, Chauhan I, Gaur PK, Singh AP, Puri D. Solid lipid nanoparticles for nose to brain delivery of donepezil: Formulation, optimization by Box–Behnken design, in vitro and in vivo evaluation. Artif Cells Nanomed Biotechnol. 2018;46(8):1838–51.
21. Soni K, Rizwanullah M, Kohli K. Development and optimization of sulforaphane loaded nanostructured lipid carriers by the Box–Behnken design for improved oral efficacy against cancer: In vitro, ex vivo and in vivo assessments. Artif Cells Nanomed Biotechnol. 2018;46(1):15–31.
22. Rathee S, Kamboj A. Optimization and development of antidiabetic phytosomes by Box–Behnken design. J Liposome Res. 2017;28(2):1–26.
23. Jores K, Mehnert W, Drechsler M, Bunjes H, Johann C, Mäder K. Investigations on the structure of solid lipid nanoparticles by photon correlation spectroscopy, field-flow fractionation and transmission electron microscopy. J Control Release. 2004;95(2):217–27.
24. Gill V, Gill B, Sharma V, Kumar SLH. Optimization of etodolac loaded emulsomes by Box–Behnken design of experiment. Int J Pharm Res. 2020;12(4):2444–52.