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Research Article

Quality-by-Design-Based Development and Optimization of Emtricitabine-Loaded Solid Lipid Nanoparticles

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Abstract

Background: Emtricitabine (FTC) is a nucleoside reverse transcriptase inhibitor widely used in combination antiretroviral therapy for HIV infection. Its pharmacokinetic characteristics and need for regular dosing may affect patient adherence. Solid lipid nanoparticles (SLNs) have been considered beneficial in terms of drug entrapment and drug delivery. The study was conducted using a Quality-by-Design (QbD) strategy along with Box–Behnken Design (BBD) in order to optimize FTC-loaded SLNs systematically.

Methods: A three-variable, three-level Box-Behnken Design consisting of seventeen runs has been employed to investigate the effects of stearic acid concentration, Tween 80 concentration, and homogenization speed on the entrapment efficiency of FTC.

Results: The optimized quadratic model exhibited exceptional predictive ability (R^2 =0.9989, adjusted R^2 = 0.9976, predicted R^2 = 0.9867), along with a non-significant lack of fit (p = 0.1047). It was found that stearic acid had the highest positive effect on EE%, while an increase in Tween 80 concentration and homogenization speed reduced the drug entrapment significantly. Results from the numerical optimization indicated that the optimized formulation had 164.68 mg of stearic acid, 2.09% of Tween 80 concentration, and a homogenization speed of 12,074.8 rpm, which yielded an EE% of 85.32%. Experimental validation indicated an EE% of 85.28%, which matched quite closely with the predicted value, yielding a prediction bias of 0.047%.

Conclusion: The QbD-driven BBD method developed a reliable and statistically valid optimization method for SLNs loaded with FTC. The optimization study provided an effective formulation with high entrapment efficiency and corroborated the reliability of the approach employed, leading to promising possibilities for the physical and chemical characterization, controlled release assessment, and future reproduction of emtricitabine-loaded lipid formulations.

Keywords – Emtricitabine[FTC]; Solid lipid Nanoparticles[SLNs]; Quality by Design[QbD]; Entrapment efficiency; Box-Behnken design [BBD]; Nanocarrier optimization.

1. INTRODUCTION

HIV infection remains one of the most significant global health issues, and successful management requires adequate and sustained systemic exposure to antiretroviral drugs[1].Emtricitabine (FTC) is an important part of first-line HIV treatments due to its strong action against HIV-1 reverse transcriptase, and proven ability to be used with other drugs but it has a short elimination half-life, requires a daily dosing regimen, and exhibits differences in absorption between various people making it hard for patients to comply with the treatment[2,3]. Problems of the method of delivery of emtricitabine have attracted attention of researchers to nanoparticle delivery systems able to alter the pharmacokinetics of the drug, improve its delivery to damaged cells, and probably enhance pharmacokinetics of emtricitabine[4].

Solid lipid nanoparticles (SLNs) have become a widely practised colloidal system for the transportation of both hydrophilic and lipophilic drugs[5]. The emulsions are manufactured using solid and biological materials by integrating the different advantages of their components including polymeric nanoparticles, emulsions, and liposomes. The SLN technology includes several advantages such as convenient of preparation method and good biocompatibility. The SLNs do not require any harmful solvents for their preparation and might be produced in larger scale by hot homogenisation, which allows to protect the drugs from degradation. The high water solubility of emtricitabine makes its encapsulating rather complicated and requires advanced technology [6,7].

The conventional one-factor-at-a-time (OFAT) optimization methods are not effective in dealing with the complex interactive and non-linear interactions between the process and formulation variables involved,

and they limit the understanding of the design space. The Quality by Design (QbD) principle, which is presented in ICH Q8(R2), Q9, and Q10 principles, provides a methodical, scientific, and risk-oriented approach whereby the quality is designed into the product, not tested after manufacturing[8]. In QbD, the target quality profile (QTPP) is established first; the critical quality attributes (CQAs) are determined based on their impact on the efficacy and safety of the product, the risk assessment method identifies the Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs) to be studied using design of experiments (DoE) approach in the result generation stage[9–11].

Box–Behnken design (BBD) is one of the response surface methods commonly employed for optimizing pharmaceutical formulations and particularly suitable for three-factor, three-level experiments [12]. Its characteristics include lower number of experiments in comparison with the full-factorial or central-composite design, absence of extreme (corner) combinations that may not be experimentally feasible or lead to unstabilized formulations, the rotatable nature of the design, and the capacity of BBD design to efficiently estimate linear, interaction and quadratic effects using second-order polynomial modelling[13]. In the current article, the Box–Behnken design with three factors and three levels has been applied within the framework of quality-by-design (QbD) concept to develop and optimize emtricitabine-loaded solid lipid nanoparticles. Concentration of stearic acid (A), concentration of Tween 80 (B) and homogenization speed (C) were determined as independent variables based on preliminary risk assessment, while drug entrapment efficiency (EE%) served as the critical response in the experiments. The relationships between the variables and EE% response were modeled using ANOVA method and allowed identifying the optimum formulation of the lipid formulation that is capable of providing maximum drug entrapment.

2. MATERIALS AND METHODS

2.1 Materials

Emtricitabine (FTC) was obtained as a gift sample. Stearic acid and Tween 80-were purchased from **Sigma-Aldrich, Bengaluru, India**. Double-distilled water was prepared in-house and used throughout the study. All chemicals and reagents used in this investigation were of analytical reagent (AR) grade.

2.2 Quality-by-Design (QbD) Approach

The approach used to develop emtricitabine-loaded solid lipid nanoparticles was based on the principles of Quality-by-Design set out in ICH Q8(R2)[8]. The QbD procedure used in this study comprised the following major stages: (i) formulation of the Quality Target Product Profile (QTPP); (ii) determination of Critical Quality Attributes (CQAs) which should be controlled to achieve the QTPP; (iii) conducting quality risk assessment (in accordance with ICH Q9) to identify and rank potential Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs) capable of influencing CQAs; (iv) development of a design of experiments (Box–Behnken) to establish quantitative cause-and-effect relationship between the selected high-risk factors and the CQA; (v) use of the developed statistical model to formulate the optimized composition in the established design space[14]. This structured approach ensured that formulation development was driven by sound scientific rationale and prior process/product knowledge rather than by empirical trial and error[15].

2.3 Quality Target Product Profile (QTPP)

The QTPP was defined prospectively to summarise the quality characteristics that the emtricitabine-loaded SLN formulation should possess in order to ensure the desired safety, efficacy, and performance [16,17]. The elements considered are summarised in Table 1.

Table 1. Quality Target Product Profile (QTPP)

QTPP Element	Target
Dosage form	Solid lipid nanoparticulate dispersion for oral administration
Route of administration	Oral
Nanoparticle type	Stearic-acid-based solid lipid nanoparticles (SLNs)
Particle size	Nanometric range (< 300 nm), suitable for enhanced permeability and cellular/lymphatic uptake
Zeta potential	Sufficient surface charge (positive or negative) to ensure colloidal stability
Polydispersity index (PDI)	Narrow, homogeneous size distribution (PDI < 0.3)
Drug entrapment efficiency (EE%)	Maximised entrapment of emtricitabine within the lipid matrix
Drug release profile	Controlled/sustained release to reduce dosing frequency
Stability	Physical and chemical stability on storage

2.4 Identification of Critical Quality Attributes (CQAs)

The critical quality attributes derived from the QTPP that are likely to impact the safety and efficacy of emtricitabine SLNs were identified. The critical quality attributes that were identified include particle size, zeta potential, PDI, percentage entrapment efficiency (EE%) and *in vitro* drug release. Since it can determine the effective dose of emtricitabine present in the drug delivery system, as well as having a direct impact on the drug-loading and release profile of the formulation, the percentage entrapment efficiency was selected as the critical quality attributes of the current study. As a result, the percent entrapment efficiency (%), as the dependent response (Y1) was used as the response of the Box–Behnken design-based optimization studies[18].

2.5 Risk Assessment

An initial risk assessment was carried out through the application of the Ishikawa (fishbone) cause-and-effect model followed by a risk estimation matrix. This risk assessment was intended to evaluate the physical characteristics and operating parameters of the CQA (EE%). The variables considered in this risk assessment included the kind and concentration of solid lipid, the type and concentration of surfactant, the drug-to-lipid ratio, the power and time of homogenization, the duration of sonication, and the cooling rate of the process. Low-risk factors and factors established from previous studies were maintained constant. Three variables of high risk affecting the efficiency of entrapment were then identified and selected as independent variables for optimization: amount of stearic acid (lipid matrix component, A), amount of Tween 80 used (surfactant/stabilizer, B), and the homogenization speed (process parameter, C). Using such risk assessment methodology, the complexity of the experiments was minimized, while ensuring that the experiment design concentrated on the most important process parameters responsible for drug entrapment[19,20].

2.6 Experimental Design (Box–Behnken Design)

In this study, a three-factor, three-level Box-Behnken design (BBD) was utilized through Design-Expert software to investigate the cumulative influence of the independent variables on percentage entrapment efficiency and to discover an optimal formulation. The amounts of stearic acid (A, mg), Tween 80 (B, % w/v), and homogenization speed (C, rpm) were varied at three coded levels; low (−1), medium (0) and high (+1); as tabulated in Table 2. There were a total of 17 experiments performed using the design, including 12 factorial (edge-midpoint) combinations, along with 5 replications of centre points, which enabled the computation of pure error and assessment of curvature (lack of fit) of the fitted model [21–23]. The experimental runs were performed in a randomized order to minimise the effect of uncontrolled variability, and the percentage entrapment efficiency obtained for each run was fitted to a second-order (quadratic) polynomial model of the general form:

$$Y = \beta 0 + \beta 1A + \beta 2B + \beta 3C + \beta 12AB + \beta 13AC + \beta 23BC + \beta 11A^2 + \beta 22B^2 + \beta 33C^2 + \varepsilon$$

where Y is the predicted response (EE%), β0 is the intercept, β1–β3 are linear coefficients, β12, β13 and β23 are interaction coefficients, β11, β22 and β33 are quadratic coefficients, and ε is the residual error term. The statistical significance and adequacy of the fitted model were evaluated by analysis of variance (ANOVA)[24].

Table 2. Independent Variables and Their Levels Used in Box–Behnken Design

Factor	Symbol	Low (−1)	Medium (0)	High (+1)
Stearic acid (mg)	A	100	150	200
Tween 80 (%)	B	1	2	3
Homogenization speed (rpm)	C	10,000	12,000	14,000

2.7 Preparation of Emtricitabine-Loaded Solid Lipid Nanoparticles

Emtricitabine loaded SLNs were prepared by hot homogenization-ultrasonication (melt-emulsification) technique. The lipid phase was prepared by melting the required amount of stearic acid (100–200 mg as per the design matrix) at a temperature approximately 5–10 °C above its melting point using a thermostatically controlled water bath, and a fixed amount of emtricitabine was dispersed/dissolved in the molten lipid to get a clear or homogeneous melt. The required concentration of Tween 80 (1-3% w/v) was dissolved in double distilled water to prepare the aqueous phase. The aqueous phase was separately heated to the same temperature as that of the lipid phase to prevent the premature solidification of the lipid on mixing. The hot aqueous surfactant solution was added to the drug-containing molten lipid phase and homogenised with a high-speed homogeniser at the pre-set speed dictated by the design matrix (10,000–14,000 rpm) for a fixed duration to yield a hot oil-in-water (o/w) nanoemulsion. The resulting pre-emulsion was then sonicated for a fixed time to reduce the particle size and to narrow the size distribution. The hot nanoemulsion was then rapidly cooled by dispersing it into cold water (2–4 °C) under continuous mechanical stirring, which allowed the lipid droplets to recrystallise and yield an aqueous dispersion of solid lipid nanoparticles. All seventeen batches prescribed by the Box–Behnken design matrix were prepared under the same drug loading and processing time conditions, changing only the three independent

variables under investigation and stored at 2–8 °C protected from light until further characterisation [25–27].

2.8 Characterization of Solid Lipid Nanocarriers

2.8.1 Preparation of Calibration Curve of Emtricitabine

A standard stock solution of Emtricitabine was prepared and serially diluted to obtain working standard solutions of suitable concentrations (2–20 µg/mL). The absorbance of each solution was measured at the predetermined λmax using a UV–Visible spectrophotometer. A calibration curve was constructed by plotting absorbance against drug concentration, and the regression equation and correlation coefficient (R^2) were determined using linear regression analysis[28,29]. The calibration curve was subsequently employed for the quantitative estimation of drug content, entrapment efficiency, and *in vitro* drug release studies.

2.8.2 Drug Entrapment Efficiency (EE%)

The percentage of drug entrapment efficiencies of different Box–Behnken batches was calculated through an indirect method which denotes the measurement of the drug that is not entrapped instead of measuring the entrapped drug. The precise amount of SLN dispersion was centrifuged or ultracentrifuged or ultrafiltered at a specific speed and temperature to segregate the nanoparticles from the aqueous phase. The transparent supernatant obtained after this operation which contained the free emtricitabine was appropriately diluted and analysed by spectrophotometer at the specified λmax as mentioned in Section 2.8.1[30,31]. The percentage entrapment efficiency was then calculated using the following equation:

EE (%) = [(Total amount of drug added – Amount of free drug in supernatant) / Total amount of drug added] × 100

Every determination was conducted for all the 17 batches of Box-Behnken design and the percentage entrapment efficiency values were used in the Response Surface Analysis as responses Y1.

3. RESULTS AND DISCUSSION

3.1 Box–Behnken Design Matrix and Response

The Box-Behnken design produced 17 experiments: 12 factorial points and 5 centre points for 3 variables: A (stearic acid, 100 - 200 mg), B (Tween 80, 1-3% w/v), and C (homogenization speed of 10,000 - 14,000 rpm). The EE% is in the range of 64.5 (Run 6; A = 100 mg, B = 3%, C = 12,000 rpm) to 84.8% (Run 14, the centre point; A = 150 mg, B = 2%, C = 12,000 rpm) with an average value of 78.56% and standard deviation of 6.67 (Table 3). This considerable spread of nearly 20 percentage points across the design space confirms that entrapment efficiency is markedly sensitive to the three selected variables and justifies their prior selection as high-risk parameters during the QbD risk assessment. Batches formulated with a low lipid content combined with a high surfactant concentration consistently gave the lowest EE%, whereas batches with higher stearic acid content and lower surfactant concentration or lower homogenization speed gave higher entrapment, an observation that is examined quantitatively in the sections that follow.

Table 3. Box–Behnken design matrix with experimental percentage entrapment efficiency (EE%) of emtricitabine-loaded SLNs

Std	Run	A: Stearic acid (mg)	B: Tween 80 (%)	C: Homogenization speed (rpm)	EE (%)
2	1	200	1	12000	82.9
6	2	200	2	10000	84.6
16	3	150	2	12000	84.7
7	4	100	2	14000	67.4
11	5	150	1	14000	79.7
3	6	100	3	12000	64.5
10	7	150	3	10000	77.3
15	8	150	2	12000	84.4
1	9	100	1	12000	70.8
17	10	150	2	12000	84.4
13	11	150	2	12000	84.3
5	12	100	2	10000	72.9
8	13	200	2	14000	79.8
14	14	150	2	12000	84.8
9	15	150	1	10000	83.5
12	16	150	3	14000	72.8
4	17	200	3	12000	76.8

3.2 Model Fitting and Selection

The experimental data were sequentially fitted to linear, two-factor interaction (2FI), quadratic and cubic models, and the fit summary statistics were compared to select the most appropriate polynomial for describing the EE% response (Table 4). The linear model gave a statistically significant sequential p-value ($p = 0.0077$) but showed a highly significant lack of fit ($p < 0.0001$), indicating that a first-order model was inadequate to describe the curvature present in the response surface. The 2FI model did not improve the fit appreciably (sequential $p = 0.9998$, non-significant) and likewise exhibited significant lack of fit. In contrast, the quadratic model showed a highly significant sequential p-value ($p < 0.0001$), a non-significant lack of fit ($p = 0.1047$), and markedly higher adjusted R^2 (0.9976) and predicted R^2 (0.9867) than the lower-order models. Although the cubic model showed a nominally higher adjusted R^2 (0.9989), it was flagged as aliased by the software because the Box–Behnken design does not contain sufficient distinct factor-level combinations to independently estimate all cubic terms, and it was therefore not considered a valid candidate. On the basis of the highest significant order with non-aliased, non-significant lack of fit and the best combination of adjusted and predicted R^2, the quadratic model was selected as the most appropriate model for percentage entrapment efficiency, consistent with the recommended model-selection strategy of maximizing the adjusted and predicted R^2 while avoiding over-fitting.

Table 4. Fit summary for percentage entrapment efficiency (EE%)

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	Remark
Linear	0.0077	< 0.0001	0.4920	0.3763	—
2FI	0.9998	< 0.0001	0.3402	−0.1201	—
Quadratic	< 0.0001	0.1047	0.9976	0.9867	Suggested
Cubic	0.1047	—	0.9989	—	Aliased

3.3 Analysis of Variance (ANOVA) for the Quadratic Model

The analysis of variance for the selected quadratic model is presented in Table 5. The model F-value of 728.11 ($p < 0.0001$) indicates that the model is highly significant, with less than a 0.01% probability that an F-value of this magnitude could arise from random noise. Among the individual model terms, the linear terms A (stearic acid), B (Tween 80) and C (homogenization speed), together with all three quadratic terms A^2, B^2 and C^2, were significant model terms ($p < 0.0001$ in every case). The two-factor interaction terms AB ($p = 0.7704$), AC ($p = 0.3236$) and BC ($p = 0.3236$) were not statistically significant, indicating that, within the ranges studied, the three variables act largely independently on EE% rather than through strong synergistic or antagonistic combinations; these interaction terms were nonetheless retained in the model to preserve hierarchy. The lack-of-fit F-value of 4.06 ($p = 0.1047$) was not significant relative to the pure error, which is a desirable outcome confirming that the quadratic model adequately represents the true relationship between the independent variables and the response, with no systematic unexplained variation.

Table 5. ANOVA for the quadratic model fitted to percentage entrapment efficiency

Source	Sum of Squares	df	Mean Square	F-value	p-value	Remark
Model	711.94	9	79.10	728.11	< 0.0001	significant
A-Stearic acid	294.03	1	294.03	2706.40	< 0.0001	
B-Tween 80	81.28	1	81.28	748.15	< 0.0001	
C-Homogenization speed	43.25	1	43.25	398.05	< 0.0001	
AB	0.0100	1	0.0100	0.0920	0.7704	
AC	0.1225	1	0.1225	1.13	0.3236	
BC	0.1225	1	0.1225	1.13	0.3236	
A^2	175.71	1	175.71	1617.34	< 0.0001	
B^2	78.22	1	78.22	719.93	< 0.0001	
C^2	14.96	1	14.96	137.71	< 0.0001	
Residual	0.7605	7	0.1086			
Lack of Fit	0.5725	3	0.1908	4.06	0.1047	not significant
Pure Error	0.1880	4	0.0470			
Cor Total	712.70	16				

3.4 Fit Statistics and Model Adequacy

The overall goodness-of-fit statistics for the quadratic model are summarised in Table 6. The model showed an excellent coefficient of determination (R^2 = 0.9989), indicating that 99.89% of the total variability in EE% is explained by the fitted model. The adjusted R^2 (0.9976), which accounts for the number of terms in the model, and the predicted R^2 (0.9867), which reflects the model's ability to predict new observations by a leave-one-out approach, were in reasonable agreement with each other (difference < 0.2), confirming that the model was neither under-fitted nor over-fitted. The coefficient of variation (C.V. = 0.42%) was very low, indicating a high degree of precision and reliability of the experimental runs. Most importantly, the Adequate Precision ratio, which measures the signal-to-noise ratio, was 79.39, far exceeding the minimum desirable value of 4; a ratio of this magnitude indicates an excellent signal and confirms that the model can be used with confidence to navigate the design space. Furthermore, the variance inflation factors (VIF ≈ 1.0–1.01) for all model terms and the low condition number of the coefficient matrix (1.18, well below the threshold of 100 associated with multicollinearity) confirmed the orthogonality of the Box–Behnken design and the absence of any troublesome collinearity among the model terms.

Table 6. Fit statistics for the quadratic model

Parameter	**Value**	Parameter	**Value**
Std. Dev.	0.3296	R^2	0.9989
Mean	78.56	Adjusted R^2	0.9976
C.V. %	0.4195	Predicted R^2	0.9867
		Adeq. Precision	79.3910

3.5 Regression Equation and Effect of Formulation Variables

The final polynomial equation relating percentage entrapment efficiency to the coded levels of the three independent variables was:

$$EE\ (\%) = +84.52 + 6.06A - 3.19B - 2.33C + 0.0500AB + 0.1750AC - 0.1750BC - 6.46A^2 - 4.31B^2 - 1.88C^2$$

In the coded equation, the intercept (84.52) represents the average response across all 17 runs, and the sign and magnitude of each coefficient reflect the relative direction and strength of the corresponding factor's effect within the studied range. Stearic acid (A) exhibited the largest positive linear coefficient (+6.06) and, by extension, the largest F-value (2706.40) among the linear terms, identifying it as the most influential variable governing entrapment efficiency: increasing the lipid content provides a greater matrix volume for accommodating the drug and reduces the relative surface area available for drug partitioning into the aqueous phase, thereby increasing EE%. However, the substantial negative quadratic coefficient for A (−6.46) indicates pronounced curvature, whereby entrapment efficiency increases with lipid content only up to an optimum level, beyond which excessive lipid promotes the formation of an imperfect crystal lattice with fewer accommodating imperfections, increases particle size and viscosity, and can lead to drug expulsion during lipid recrystallization, ultimately limiting further gains in EE%. Tween 80 concentration (B) showed a significant negative linear effect (−3.19) coupled with a significant negative quadratic term (−4.31): increasing surfactant concentration beyond the level required for adequate droplet stabilization promotes solubilization/micellization of the freely water-soluble emtricitabine into the aqueous continuous phase, thereby reducing the fraction of drug retained within the lipid core, while insufficient surfactant compromises emulsification and particle stability. Homogenization speed (C) also had a significant negative linear (−2.33) and quadratic (−1.88) effect, the smallest in magnitude of the three variables (consistent with its lowest F-value of 398.05): higher shear input during homogenization produces smaller lipid droplets with a proportionately larger surface-area-to-volume ratio, favouring drug diffusion/leaching into the aqueous phase before lipid solidification, whereas insufficient homogenization energy can yield coarser, less uniform particles. The non-significant interaction terms (AB, AC, BC) indicate that these three effects operate largely independently of one another over the ranges studied, so that the influence of one variable on EE% is not strongly modulated by the level of another.

The corresponding equation in terms of actual (uncoded) factor levels, useful for direct prediction of EE% at any combination of factor settings within the design space (though not for comparing the relative importance of the factors, owing to differing units and scale), was:

$$EE\ (\%) = -55.23250 + 0.873450\cdot(\text{Stearic acid}) + 14.95250\cdot(\text{Tween 80}) + 0.010060\cdot(\text{Homogenization speed}) + 0.001000\cdot(\text{Stearic acid} \times \text{Tween 80}) + 1.75000\times10^{-6}\cdot(\text{Stearic acid} \times \text{Homogenization speed}) - 0.000088\cdot(\text{Tween 80} \times \text{Homogenization speed}) - 0.002584\cdot(\text{Stearic acid})^2 - 4.31000\cdot(\text{Tween 80})^2 - 4.71250\times10^{-7}\cdot(\text{Homogenization speed})^2$$

3.6 Diagnostic (Residual) Analysis

Prior to interpreting the response surfaces, the underlying statistical assumptions of the ANOVA (normality, constant variance and independence of residuals) were verified by standard regression diagnostic plots (Figure 1). The normal probability distribution of the internal/external studentized

residuals indicated their proximity to a straight line (Fig. 1a) and the absence of significant deviations at both ends. This result validated the assumption of normality of the residuals and thus justified the use of ANOVA in the subsequent hypothesis testing. The predicted-against-actual plot (Fig. 1b) demonstrated a very tight grouping of actual EE% data points along the 45° line, confirming the high R^2 value and indicating a good predictive ability of the quadratic model. The residuals vs. run number plot (Fig. 1c) also showed random points scattered in the acceptable range and thus suggested that there is no time or other confounding factor correlation with residuals and the assumption for independence of errors is valid. Overall, all three tests indicated that the quadratic model is sound for statistical analysis and the prediction of entrapment efficiency of SLNs loaded with Emtricitabine.

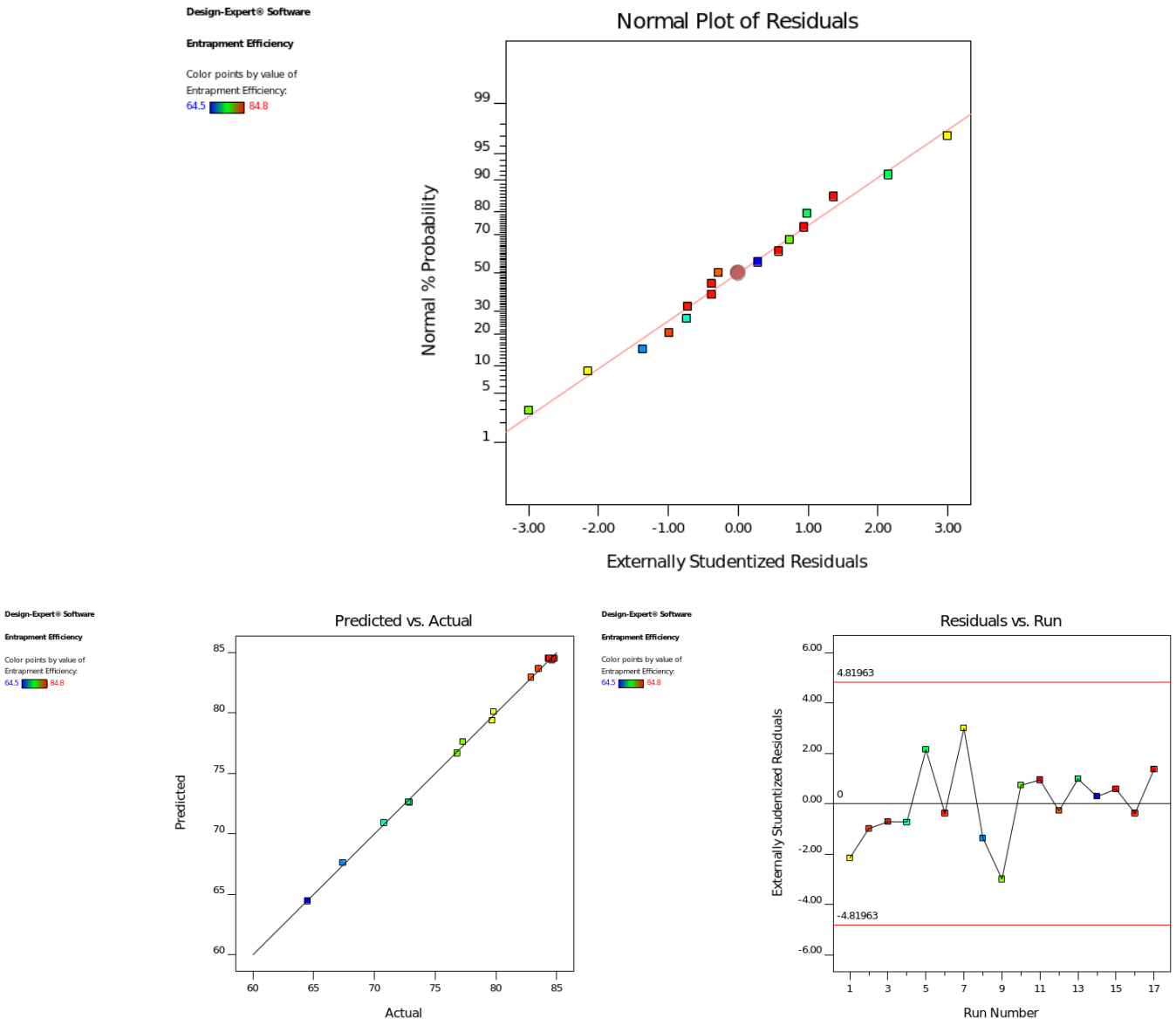

Figure 1. Diagnostic plots for the quadratic model: (a) normal probability plot of residuals, (b) predicted vs. actual plot, and (c) residuals vs. run number, for percentage entrapment efficiency (EE%).

3.7 Response Surface and Contour Plot Analysis

Three-dimensional response surface plot and corresponding two-dimensional contour plot have been constructed to illustrate different pair combinations of independent variables impacting on EE%, with the third variable kept constant at its average level, in order to assist in the interpretation of the interaction terms mentioned earlier.

Effect of stearic acid and Tween 80 (homogenization speed is established to be 12,000 rpm): In this case, the response surface for A and B (Figure 2) shows curved dome-like surface where EE% rapidly increases as stearic acid content moves from 100 towards about 150–165 mg and later decreases again with further rise in the amount of stearic acid approaching 200 mg, which fact confirms significance of negative A^2 factor. The study confirms that across the range of lipids tested the amount

of EE% is gradually decreased when the amount of Tween 80 rises from 1% to 3% indicating medication solubilization by surfactant. The elliptical and very close contours observed in the area of moderate-to-high stearic acid and low Tween 80 are strong indicators of the environment most capable of providing maximum entrapment whereas rather parallel position of contour lines across most of the plot confirms the absence of meaningful AB interaction.

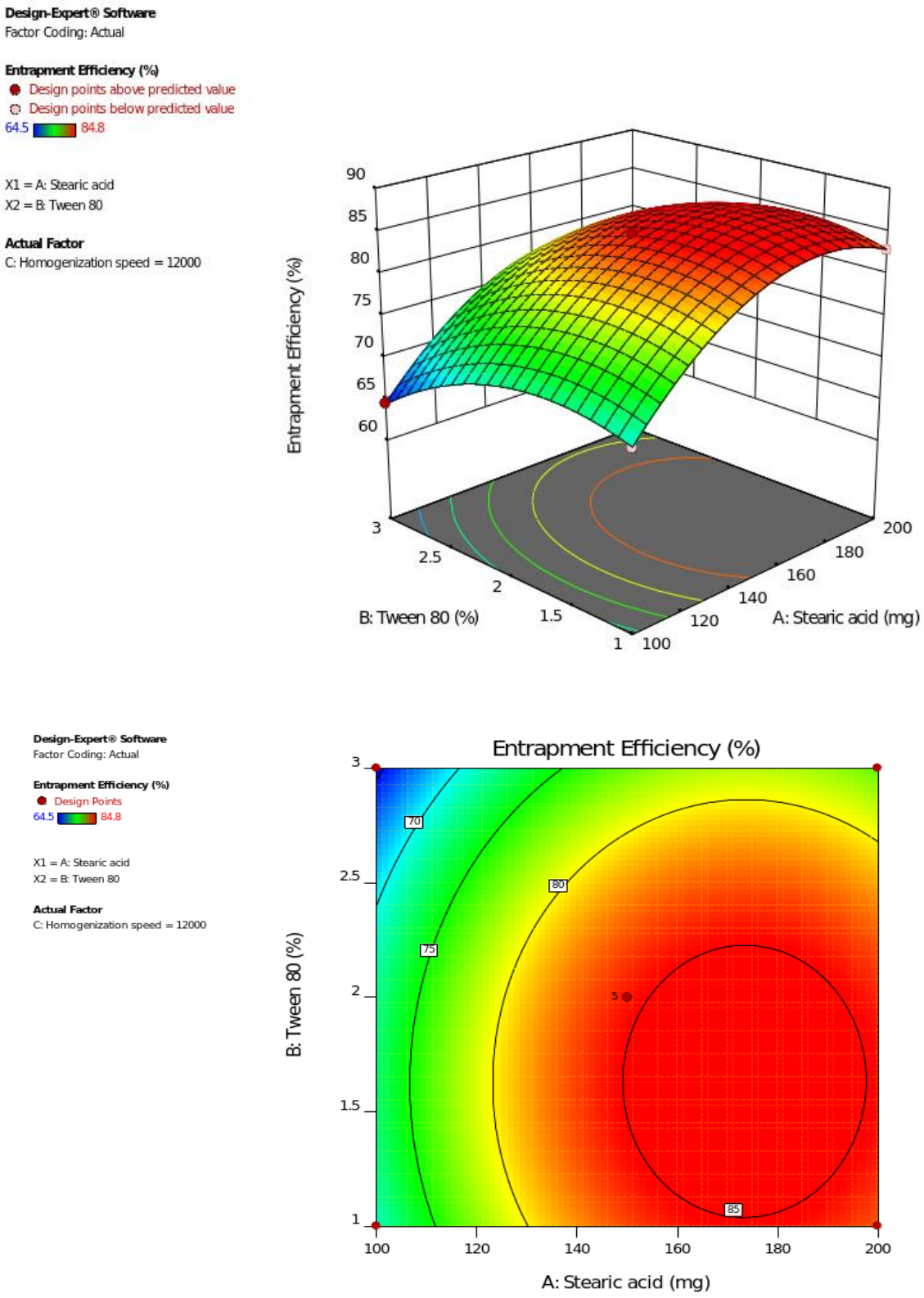

Figure 2. (a) 3D response surface and (b) contour plot showing the combined effect of stearic acid (A) and Tween 80 (B) on percentage entrapment efficiency (EE%) at a fixed homogenization speed of 12,000 rpm.

Effect of stearic acid and homogenization speed (Tween 80 fixed at 2%): The response surface for A and C (Figure 3) again displays the characteristic curvature along the stearic acid axis, with a maximum in the intermediate-to-high lipid region, while EE% declines at a much lesser pace and essentially in a linear manner with increase in homogenization speed from 10,000 to 14,000 rpm. Moreover, the fairly symmetrical contours further confirm the assumption of non-significant AC interaction term since the positive effect of increasing the lipid content on entrapment efficiency can be harvested to the same extent along all homogenization speeds that were studied.

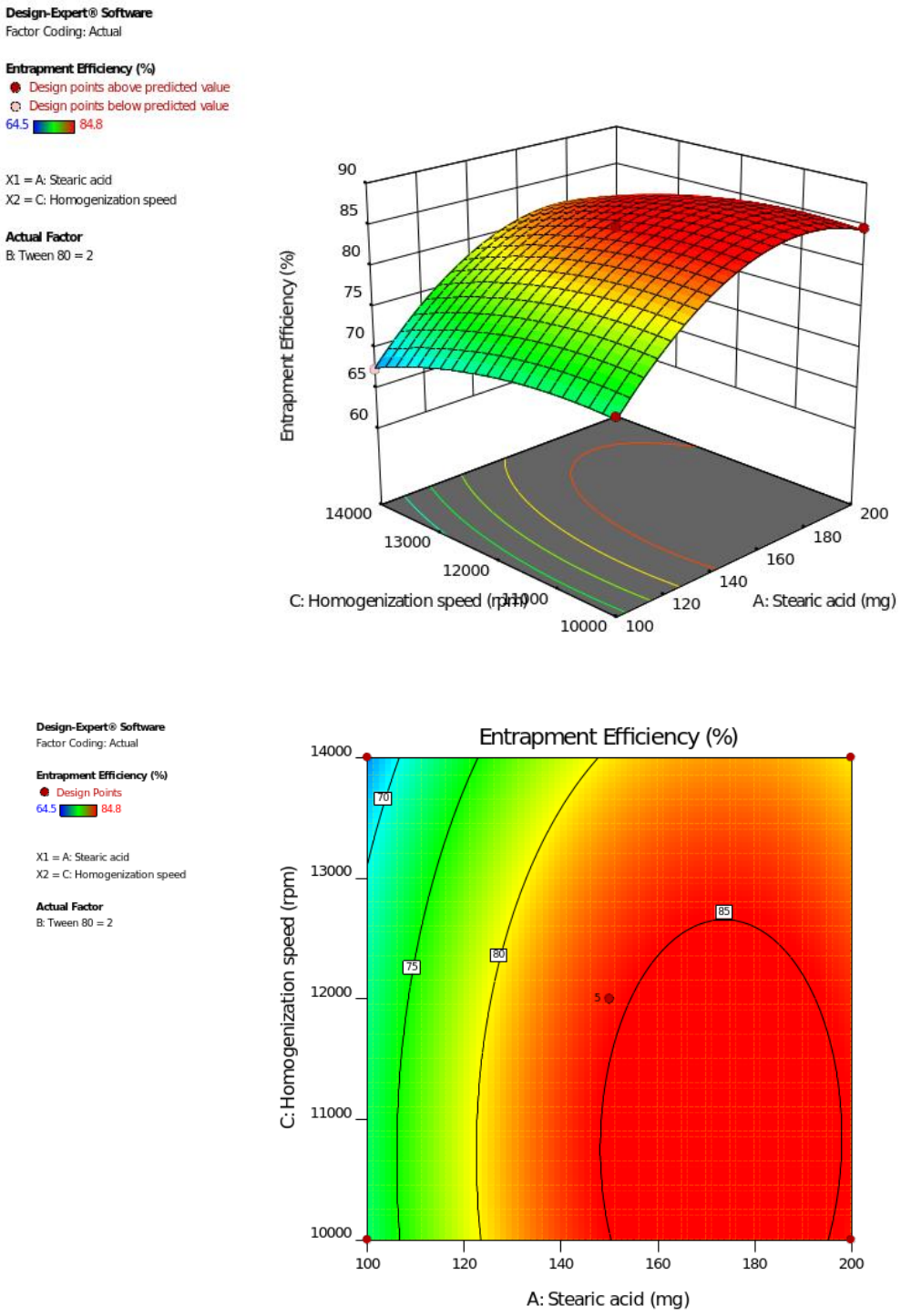

Figure 3. (a) 3D response surface and (b) contour plot showing the combined effect of stearic acid (A) and homogenization speed (C) on percentage entrapment efficiency (EE%) at a fixed Tween 80 concentration of 2%.

Effect of Tween 80 and homogenization speed (stearic acid fixed at 150 mg): The response surface for B and C (Figure 4) shows the highest EE% at the combination of low Tween 80 concentration and low-to-moderate homogenization speed, with the response falling progressively as either variable was increased, and most steeply as a function of Tween 80 concentration, in agreement with its larger F-value relative to homogenization speed. The gently curved, non-parallel but evenly spaced contour lines again reflect the statistically non-significant BC interaction while confirming that both surfactant concentration and shear input must be kept within moderate limits to preserve high entrapment efficiency.

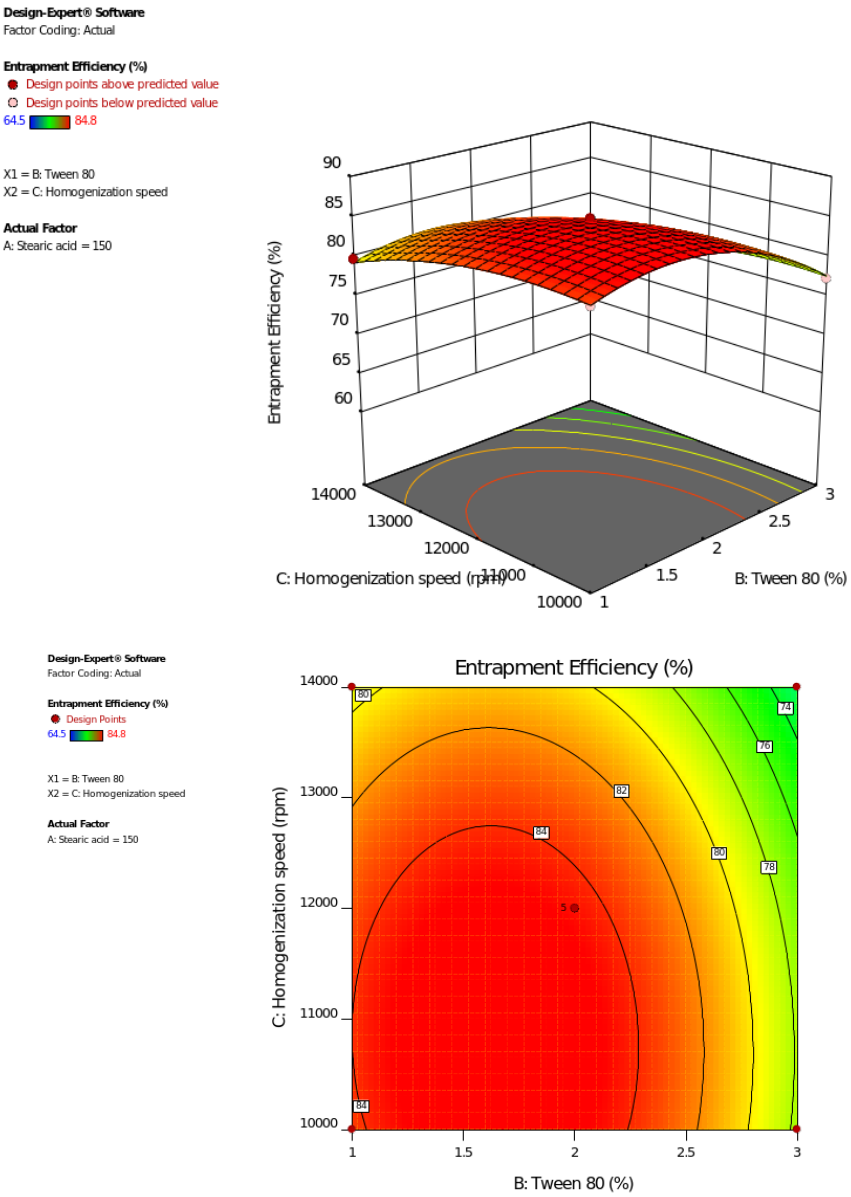

Figure 4. (a) 3D response surface and (b) contour plot showing the combined effect of Tween 80 (B) and homogenization speed (C) on percentage entrapment efficiency (EE%) at a fixed stearic acid amount of 150 mg.

3.8 Perturbation Analysis

The relative sensitivity of EE% to each of the three independent variables, at the reference (centre) point of the design space, is illustrated by the perturbation plot (Figure 5), in which all three factors are superimposed on a common coded scale (−1 to +1). The steepest curve corresponds to stearic acid (A), confirming it as the most influential variable, with EE% rising sharply from the low to the mid-level before curving downward toward the high level, mirroring the large linear and quadratic coefficients discussed in Section 3.5. Tween 80 (B) produced the second steepest response, decreasing monotonically across the coded range, while homogenization speed (C) produced the flattest curve of the three, indicating comparatively the smallest, though still statistically significant, influence on entrapment efficiency. This ranking (A > B > C) is fully consistent with the relative magnitude of the ANOVA F-values obtained for the three linear terms (2706.40, 748.15 and 398.05, respectively).

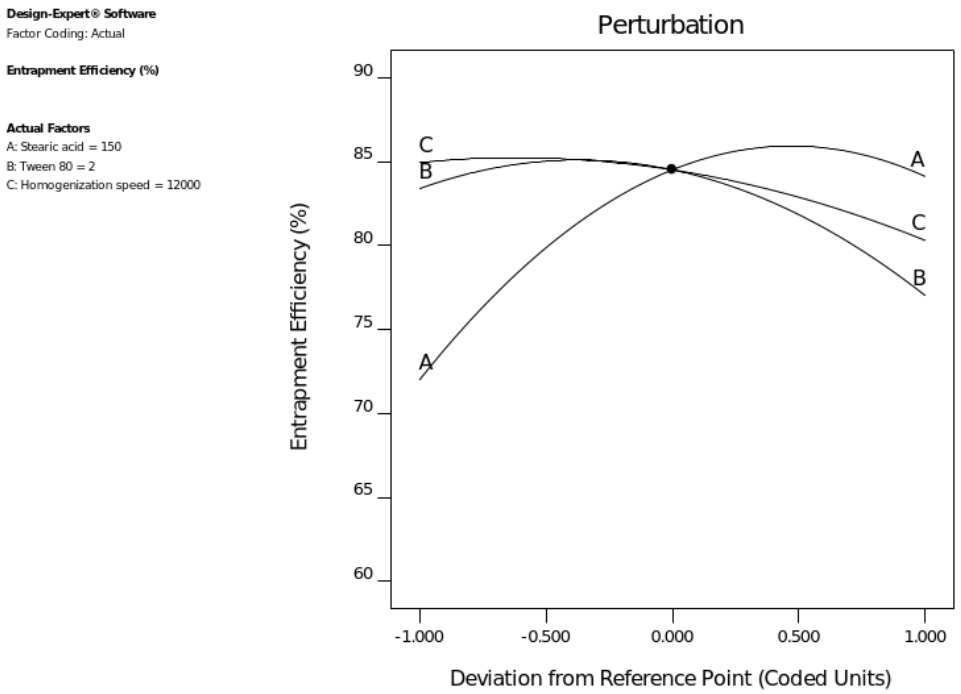

Figure 5. Perturbation plot showing the relative effect of stearic acid (A), Tween 80 (B) and homogenization speed (C) on percentage entrapment efficiency (EE%), at the reference point of the design space.

3.9 Numerical Optimization and Validation of the Optimized Formulation

Numerical optimization was performed using the desirability function approach in Design-Expert® software to maximize the entrapment efficiency (%) by optimizing the levels of the three independent variables. The optimization algorithm gave a stearic acid dose of 164.68 mg, a concentration of Tween 80 of 2.09% (w/v) and a homogenization rate of 12,074.8 rpm (as shown in Figure 6), and the model predicted a possible entrapment efficiency of 85.32 % (mean 95% Confidence Interval: 84.97-85.66%; prediction 95% interval: 84.47-86.17%). The computed optimum falls slightly above the maximum experimentally determined EE% (84.8% in Run 14), and this makes it reassuring in the sense that the numerically optimized position falls into, and not too far away from, the explored area of the design space.

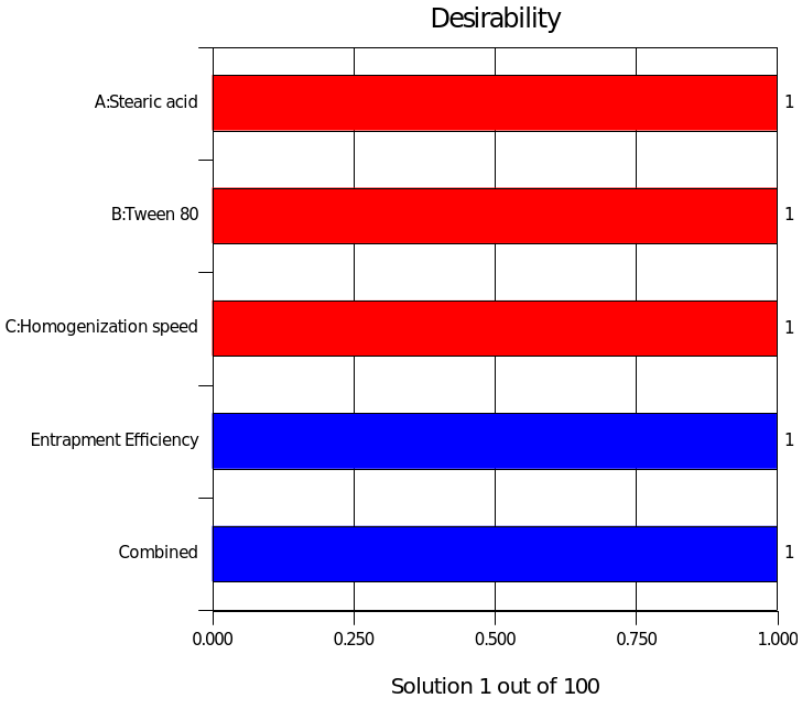

Figure 6. Desirability ramp for the numerical optimization of stearic acid, Tween 80 and homogenization speed to maximize percentage entrapment efficiency (EE%).

This optimized combination of variables represents the design space region predicted to yield the maximum attainable entrapment efficiency for the emtricitabine-loaded SLN system and was therefore selected for preparation of the final, optimized batch.

3.10 Preparation and Evaluation of the Optimized Formulation

To experimentally verify the predictive accuracy of the quadratic model, the optimized formulation identified by the desirability-based numerical optimization, namely stearic acid 164.68 mg, Tween 80 2.09% w/v and a homogenization speed of 12,074.8 rpm, was prepared in triplicate using the same hot homogenization-ultrasonication procedure described in Section 2.7, keeping the drug load and all other processing conditions identical to those used for the Box-Behnken batches. The resulting optimized SLN dispersion was evaluated for percentage drug entrapment efficiency by the indirect method described in Section 2.8.2. The optimized batch exhibited an experimentally determined entrapment efficiency of 85.28%, which is presented alongside the model-predicted value in Table 7 for comparison.

Table 7. Comparison of predicted and experimentally observed percentage entrapment efficiency (EE%) for the optimized emtricitabine-loaded SLN formulation

Optimized Formulation Variables	Predicted EE (%)	95% PI (Low-High)	Observed EE (%)	Residual	% Bias
Stearic acid: 164.68 mg; Tween 80: 2.09% w/v; Homogenization speed: 12,074.8 rpm	85.32	84.47 - 86.17	85.28	0.04	0.047%

The experimentally observed entrapment efficiency (85.28%) was found to lie well within the 95% prediction interval computed by the model (84.47-86.17%) and showed extremely close agreement with the model-predicted value of 85.32%, with an absolute residual of only 0.04 percentage points and a prediction bias of about 0.047%. The excellent predictive ability and practical validity of the quadratic model produced by the Box-Behnken design are confirmed by this low deviation between the observed and predicted responses. It also shows that the QbD-based optimisation strategy used in this study was able to consistently identify formulation and process conditions that maximise the entrapment efficiency of emtricitabine within the solid lipid nanoparticle matrix. This close correspondence between the predicted and experimentally verified EE% also validates, in retrospect, the adequacy of the risk assessment (Section 2.4) and the choice of stearic acid amount, Tween 80 concentration and homogenization speed as the critical material attributes/critical process parameters warranting formal optimization by response surface methodology. The optimized batch (stearic acid 164.68 mg, Tween 80 2.09% w/v, homogenization speed 12,074.8 rpm) was accordingly taken forward as the final optimized formulation for further characterization of the remaining CQAs (PDI, particle size, zeta potential and *in vitro* drug release) and subsequent stability evaluation.

4. CONCLUSION

The application of a Quality-by-Design-driven Box-Behnken design successfully identified stearic acid amount, Tween 80 concentration and homogenization speed as statistically significant determinants of the percentage entrapment efficiency of emtricitabine-loaded solid lipid nanoparticles, with a highly significant, non-aliased quadratic model (R^2 = 0.9989; adjusted R^2 = 0.9976; predicted R^2 = 0.9867; Adequate Precision = 79.39) providing an excellent fit to the experimental data. Stearic acid content exerted the dominant influence on EE%, with a positive linear effect and significant negative quadratic curvature, while increasing Tween 80 concentration and homogenization speed both reduced entrapment efficiency, and none of the pairwise interactions between the three variables were found to be statistically significant. Numerical optimization identified stearic acid 164.68 mg, Tween 80 2.09% w/v and homogenization speed 12,074.8 rpm as the optimized formulation, predicted to give 85.32% entrapment efficiency; the experimentally prepared optimized batch gave an observed entrapment efficiency of 85.28%, in excellent agreement with the model prediction (bias ~ 0.05%) and well within the 95% prediction interval, thereby validating the robustness and predictive accuracy of the quadratic model. These systematic, risk-based and statistically grounded, experimentally validated insights into the formulation and process variables governing entrapment efficiency provide a scientifically defensible basis for defining the design space and control strategy for further pharmaceutical development of emtricitabine-loaded SLNs.

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Author Contributions

S.M. conceived and designed the study, performed the experiments, analyzed the data, and drafted the manuscript. **A.S.** contributed to the experimental work, data collection, and validation. **A.V.** supervised the research, critically reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials: All data and materials related to this work are available in the manuscript.

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