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Optimization of Advance Novel Oral Self-Emulsifying Drug Delivery System (SEDDs) Involving Box-Behnken Design as Statistical Analysis Approach of Nateglinide Drug

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ABSTRACT

Background*:* Self-emulsifying drug delivery systems (SEDDs) have emerged as a promising approach to improve the solubility and bioavailability of poorly water-soluble drugs like Nateglinide (NTG). Optimization of formulation variables is essential for enhancing performance attributes such as dissolution rate and particle size.

Objective*:* To systematically investigate the primary, interaction, and quadratic effects of formulation variables on the performance of NTG-loaded SEDDs using a Box-Behnken Design (BBD).

Methodology*:* A 15-run BBD with three factors and three levels (3^3), including three center point replicates, was employed to analyze the impact of oil (Capryol 90), surfactant (Tween 80), and co-surfactant (PEG 400) as independent variables. Dependent variables were dissolution at 5 and 10 minutes and micelle particle size. Regression analysis, ANOVA, and desirability functions were used for model validation and optimization.

Results*:* Significant main and interaction effects of formulation variables were observed. The optimized formulation comprised 50.82 mg (10.15% w/w) oil, 250 mg (49.92% w/w) surfactant, and 200 mg (39.93% w/w) co-surfactant, achieving 91.0% dissolution after 5 minutes, approximately 100.0% dissolution after 10 minutes, and a micelle size of 49.23 nm. Predicted and experimental values were closely aligned, confirming model reliability.

Conclusion*:* The Box-Behnken Design effectively elucidated the relationships among formulation variables and enabled efficient optimization of NTG-loaded SEDDs, reducing time and labor while maximizing performance parameters.

Keywords: *design of experiments; optimizations; hyperglycaemic; self-emulsifying drug delivery system; box-behnken.*

1. INTRODUCTION

Oral administration remains the preferred method of drug delivery for both patients and manufacturers in treating various diseases. However, a significant percentage of newly discovered chemical entities in the pharmaceutical industry are poorly water-soluble or lipophilic, hindering their consistent oral absorption and therapeutic effectiveness. This issue not only impacts the delivery of new drugs but also affects the absorption of existing medications. Poorly water-soluble compounds face challenges related to solubility and dissolution, leading to bioavailability (BA) issues. The absorption of such compounds is often limited by dissolution rate, which is directly linked to solubility. These compounds are typically classified as Biopharmaceutical Classification System (BCS) class II or class IV, with the absorption of class II compounds heavily reliant on the performance of the formulated product. With the right formulation design, these drugs can be effectively prepared for oral administration to ensure reliable BA [1].

The various techniques have been reported to enhance the absorption of BCS class II compounds, such as solid lipid nanoparticles, nano-crystals, nano-suspensions, solid dispersions, emulsions, micro-emulsions, nano-emulsions, self-emulsifying system, and liposomes [2]. Among these, self-emulsifying drug delivery systems (SEDDs) stand out as a newer lipid-based innovation with great potential to improve the absorption of poorly water-soluble drugs [20-22]. SEDDs are liquid mixtures consisting of lipids, surfactants, drugs, and co-surfactants, which form stable and transparent micro emulsions upon dilution with water and gentle agitation [1-3].

The self-emulsifying properties of these formulas are attributed to their low requirement for free energy in the formation of micro-emulsions. The spontaneous formation of micro emulsions is advantageous as it allows the drug to be presented in a dissolved form, and the small size of the resulting globules provides a large surface area for drug release and absorption. Some examples of marketed products in this category include Sandimmune Neoral (Cyclosporine A), Norvir (Ritonavir), Fortovase (Saquinavir), Aptivus (Tipranavir), and Kaletra (lopinavir and ritonavir). However, SEDDS in the form of liquids or encapsulated in hard/soft gelatin capsules offer a more stable and robust dosage form with lower manufacturing costs [4]. NTG is a pharmaceutical drug used primarily to manage high blood sugar levels in individuals with type 2 diabetes. It belongs to a class of medications known as meglitinides, which work by stimulating the release of insulin from the pancreas. This action helps control blood glucose levels, especially after meals [5-6].

This conducted research objective is to optimize the SEDDs novel oral drug carriers for the treatment of several types of diseases, and individual Type-II diabetes. The optimization conduced involving the design of experiments and box-behnken design for the statistical purposes carefully.

2. CHEMICALLY OVERVIEW OF NTG

2.1. Chemical Structure: Nateglinide has the chemical name (2R,3S,4S,5S)-2-(2-(3-methylphenyl)propanoylamino)-3-phenyl-4-(4-(trifluoromethyl)phenyl)-5-hydroxy-N-(propan-2yl) cyclohexanecarboxamide. Its chemical formula is $C_{19}H_{27}N_3O_3F_3$, and it has a molecular weight of approximately 389.43 g/mol. NTG is a derivative of D-phenylalanine, an amino acid. Its structure includes a cyclohexane ring, an amide bond, and an ester group [7]. The chemical structure of NTG as Fig. **1** below followings:

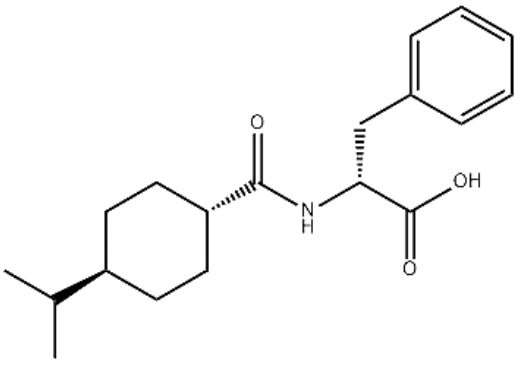


Fig. (1). Chemical structure of Nateglinide (NTG) drug; (2R)-2-({[trans-4-(1-methylethyl) cyclohexyl] carbonyl} amino)-3-phenylpropanoic acid

2.2. Functional Group: IUPAC, Where; (2R) this indicates the stereochemistry at the second carbon atom. The "R" refers to the configuration around this center. 2; the carbon atom bonded to the amino group is numbered 2. ({[trans-4-(1-methylethyl)cyclohexyl]carbonyl}amino) this describes the side chain attached to the second carbon. It includes a cyclohexane ring with an isopropyl group (1-methylethyl) at the 4th position in the trans configuration, a carbonyl group (C=O), and an amino group (NH_2). 3-phenyl a phenyl group (benzene ring) is attached to the third carbon atom and propanoic acid the molecule has a three-carbon chain (propanoic) ending in a carboxylic acid group (COOH) [6-7].

2.3. Mechanism of Action: NTG exerts its pharmacological effects by targeting the pancreatic β cells. It binds to the sulfonylurea receptor (SUR1) on the beta cell surface, specifically the Kir6.2 subunit of the ATP-sensitive potassium channel (KATP channel). This binding leads to the closure of the KATP channel, depolarization of the cell membrane, and subsequent opening of voltage-gated calcium channels. The influx of calcium ions triggers insulin secretion from the beta cells into the bloodstream, thereby lowering blood glucose levels [8].

2.4. Physicochemical Properties of Nateglinide: The physicochemical properties of NTG drug mentioned in the given Table **1** as below followings:

Table 1. The list of physicochemical properties of NTG drug with their description [8-10]

Property	Description
Chemical Formula	$C_{19}H_{27}N_3O_3F_3$
Molecular Weight	Approximately 389.43 g/mol
Appearance	White to off-white crystalline powder
Solubility	Sparingly soluble in water (approximately 1.5 mg/mL at 25°C)
Melting Point	141-145°C
pKa	4.25
Log P (Partition Coefficient)	3.67
Stability	Stable under normal storage conditions
Storage Conditions	Store in a tightly closed container, protected from light and moisture, at room temperature (20-25°C)

These physicochemical properties are important for understanding the behavior of NTG in terms of its solubility, stability, and storage requirements, which are crucial considerations in pharmaceutical formulation and use.

2.5. Application of Nateglinide: NTG is indicated for the management of type-II diabetes mellitus, particularly in patients who experience postprandial hyperglycemia (high blood sugar levels after meals). It is often prescribed in combination with other antidiabetic medications, such as metformin or thiazolidinediones, to achieve glycemic control [11].

3. MATERIAL AND METHODOLOGY

3.1. Materials: NTG was gift sample from Pharmaceutical Industry. PEG 400 was generously provided by Abitec Corporation in the USA. Capryol 90 and Tween 80 were acquired from ACS. Hard gelatin capsules were supplied by TCI Pvt. Ltd. All excipients and chemicals (analytical grade) were utilized as received.

3.2. Preparation of SEDDs formulations: SEDDs formulations were prepared using mixture of Capryol 90 as oil, Tween 80 as surfactant and PEG 400 as co-surfactant. NTG was dissolved into the mixture of oil, surfactant, and co solvent at 60°C in an isothermal water bath to facilitate solubilization. The resultant mixture was vortexed until a clear solution was obtained and stored at room temperature until further use [12].

3.3. Drug Solubility Determination: The solubility of NTG in different solvents was determined using the shake flask technique [13]. In summary, an excess amount of NTG was added to 1gm of each solvent, and the mixture was placed in sealed vials. The vials were then placed in a water bath shaker for 72 hr. to achieve equilibrium. After that, the sample was centrifuged at 5,000 RPM for 15 minutes using a centrifuge and the supernatant was filtered through a 0.45 µm membrane filter. The concentration of NTG was then measured using a UV-visible spectrophotometer at λ max 242 nm (UV-1700 Shimadzu).

3.5. Optimization of Self emulsifying drug delivery system by BBD: The BBD statistical screening design was utilized to optimize the formulation parameters and assess the main effects, interaction effects, and quadratic effects of the formulation ingredients on the dissolution and droplet size of SEDDs [14]. The study was conducted with a three-component system consisting of the oil phase X, surfactant Y, and co-surfactant Z, with a total concentration of 100%. The oil phase ranged from 10-20%, surfactant from 30-50%, and co-surfactant from 30-50%. The concentration ranges for each component were determined based on previous phase diagram results: X (50-75mg), Y (150–250mg), and Z (150–250mg). The responses measured were particle size, dissolution after 5 minutes, and dissolution after 10 minutes. Design-Expert® software (version 9.0.6.2; Stat-Ease Inc.) was used to analyze the responses of all model formulations. Linear, 2FI (two-factor interaction), and quadratic models were considered, with the best fitting model selected based on statistical parameters such as standard

deviation (SD), multiple correlation coefficients (R^2), adjusted multiple correlation coefficients (Adjusted R^2), and predicted residual sum of square (PRESS). The chosen model was validated by Design-Expert software, with emphasis on the PRESS value to ensure a good fit to the data [14-15].

The software has chosen a group of potential points to serve as the foundation for the design. These points encompassed factorial points, which represented both high and low levels within the constraints of each factor. Additionally, the software considered the centers of edges, constraint plane centroids, axial check points, and the overall centroid. The base design consisted of a total of 15 runs. From this study, the optimal formulation was determined to achieve a droplet size of less than 65 nm. Furthermore, the desired percentage of dissolution at 5 minutes ranged between 75% and 85%, while the percentage of dissolution at 10 minutes ranged between 90% and 100% [16]. The specific design layout can be found in Table **2** as below followings:

Table 2. The design layout of NTG formulation and optimization

Chemicals	Units	Min.	Max.	Coded	Value Range	Mean
X-Oil (Capryol 90)	mg	50	75	-1.000=50	1.000=75	65.5
Y-Surfactant (Tween 80)		100	225	-1.000=100	1.000=225	225
Z-Co surfactant (PEG 400)		125	300	-1.000=125	1.000=300	225

4. EVALUATION PARAMETERS

4.1. Droplet size analysis: The SEDDs globule size was measured utilizing a photon correlation spectrometer that relies on laser light scattering principles to examine light scattering fluctuations. A helium-neon gas laser with an intensity of 4 mw served as the light source. The light scattering measurements were conducted at a 90° angle and 25 °C. SEDDs samples were diluted 100 times with purified water for the globule size analysis [17-18].

4.2. Determination of self-emulsification time: The self-emulsification duration of SEDDs formulations was evaluated utilizing the USP dissolution apparatus. 250mL of purified water at 37 ± 0.5 °C served as the medium for each formulation, which was added drop wise. A standard stainless steel dissolution paddle rotating at 50 RPM provided gentle agitation. Emulsification time was determined visually. The ability to spontaneously create a transparent, clear, or slightly bluish emulsion was considered “good”, while poor or minimal emulsification with the presence of large oil droplets was deemed “bad”.

4.3. *In-vitro* drug release: Dissolution studies were carried out on NTG 2mg using both SEDDs and a marketed product. The experiments were conducted utilizing a USP dissolution type-II apparatus. The dissolution medium consisted of 900 ml of pH 5.0 buffer, which was maintained at a temperature of 37 ± 0.5°C. The paddle speed was set at 75 RPM. Samples were collected at specific time points (5, 10, 15, 30, 45, 60 min), with an aliquot of five ml each time, and replaced with fresh dissolution medium. The collected samples were then analyzed for NTG content using UV spectroscopy [19-20].

4.4. Thermodynamic stability studies SEDDs: The objective of thermodynamic stability is to evaluate the phase separation and effect of temperature variation on SEDDs formulations. NTG SEDDs were diluted with aqueous medium and centrifuged at 15000 RPM for 15 minutes and formulations were observed visually for phase separation. No phase separation was observed in any sample [21-22]. The formulations were subjected to freeze thaw cycles (-20°C for 2 days followed by +40°C for 2 days). No change in the visual description of samples after freeze-thaw cycles.

5. RESULTS AND DISCUSSION

5.1. Solubility studies: After evaluating different oils and surfactants, Capryol 90 was chosen as the oil, Tween 80 as the surfactant, and PEG 400 as the co-surfactant for additional research due to their superior solubility of NTG [23]. The solubility findings can be found in Table **3** and visualized in Fig. **2** as below followings:

Table 3. The Solubility of NTG in various oils and surfactant/co-surfactant

Name of the surfactant	Solubility of the drug(mg/ml)	Parts of the solvent (ml) required for 1part drug
Water	0	0.00
Isopropyl myristate (oil)	3.42	293.77
Soyabean oil (oil)	7.68	129.83

Captex355 (surfactant)	7.90	128.28
Tween 80 (surfactant)	32.09	31.24
PEG-400 (surfactant)	33.38	30.08
Span80 (surfactant)	41.47	25.72
Capmul mcm (oil)	43.88	25.47
Captex350 (surfactant)	66.76	16.23
Labrafil m1944cs (surfactant)	72.78	13.75
Maisine35-1 (oil)	75.71	14.38
Acconon c30 (surfactant)	101.93	9.91
Capryol 90 (surfactant)	151.14	3.69

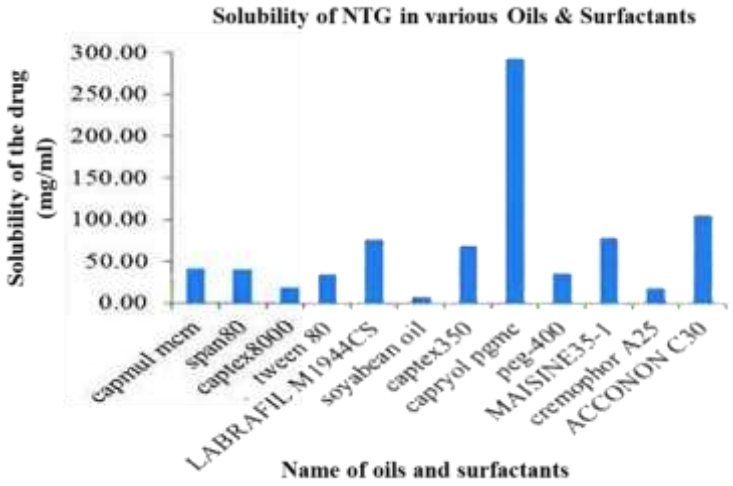


Fig. (2). Solubility expression of NTG in oils & surfactants

5.2. Droplet size analysis: The droplet size globule size of each of the 15 SEDDs formulations, which were prepared according to the experimental design, varied from 50-105nm. Notably, smaller globule sizes were observed when the oil levels were lower and the surfactant levels were higher [24]. The particle size graph of the optimized formulation is shown in Fig. **3** as followings:

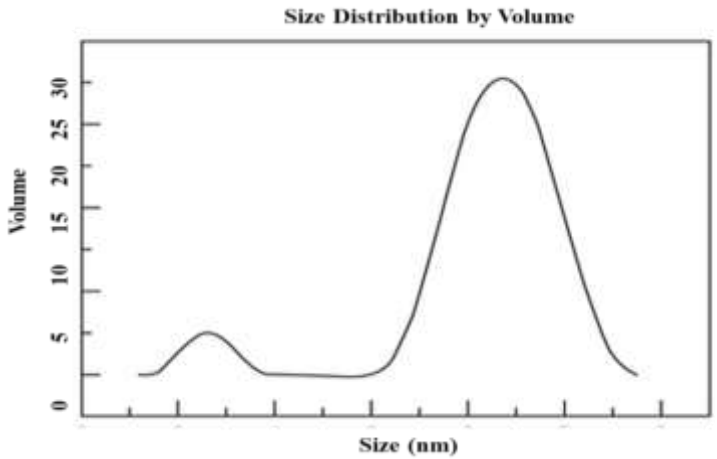


Fig. (3). Determination of size analysis distribution in term of Volume of NTG

5.3. Determination of self-emulsification time: The self-emulsification time for SEDDs formulations was determined to be under 1 minute. Each of the 15 SEDDs formulations exhibited the spontaneous formation of a transparent or slightly bluish emulsion, which was considered to be of high quality [25].

5.4. Box–Behnken Statistical analysis: The selection of system components was based on the ability of the preliminary prepared pseudo ternary system to create nano emulsion with the highest oil content. To efficiently achieve the optimal drug loaded SEDDs, BBD was utilized in this research. Capryol 90 was chosen as the oil, Tween 80 as the surfactant, and PEG 400 as the co-surfactant for the formulation variables, while particle size, dissolution after 5 minutes, and dissolution after 10 minutes were selected as response variables Table **2**. The results of these formulations are outlined in Table **4.** The relationship between independent and response variables was established using a polynomial equation with statistical analysis conducted through DoE software. The approximation of response values based on the quadratic model was deemed most appropriate due to its smallest PRESS value. A positive coefficient signifies a synergistic effect, whereas a negative term indicates an antagonistic effect on the response [25-27].

Table 4. The Observed Responses for different formulations of BBD

Details	**Factor 1**	**Factor 2**	**Factor 3**	**Response 1**	**Response 2**	**Response 3**
Run	**X: Oil (Capryol 90)**	**Y: Surfactant (Tween 80)**	**Z: Co-surfactant (PEG 400)**	**Dissolution at 5min.**	**Dissolution at 10min.**	**Particle size**
Units	mg	mg	mg	%	%	nm
1	55	160	225	75	81	77
5	80	160	225	62	74	102
12	55	275	225	94	101	53
4	80	275	225	75	83	84
3	55	225	160	82	91	67
2	80	225	160	65	74	102
10	55	225	275	86	95	54
11	80	225	275	76	83	90
13	65.5	160	160	61	72	104
14	65.5	275	160	85	93	61
15	65.5	160	275	76	84	74
8	65.5	275	275	86	95	55
7	65.5	225	225	73	86	66
6	65.5	225	225	74	86	64
9	65.5	225	225	74	84	64

The second order quadratic model was used to fit all the data, and the model's validation was conducted through the analysis of variance (ANOVA) test, lack of fit test, and correlation coefficient (R^2). The statistical evaluations of the models for each response can be found in Tables **5** and 5. ANOVA was employed to determine the statistical significance of the ratio of mean square variation due to regression and mean square residual error [28]. According to Table **5**, it was observed that for responses Y1, Y2, and Y3, the quadratic fitting was significant at a 5% significance level (p-value <0.05). The high value of F indicates that a significant portion of the variation in the response can be explained by the regression equation. The model was considered significant at a 5% significance level if the significance p-value was less than 0.5 and exhibited a significant lack of fit. The lack of fit measures the model's failure to represent data in the experimental domain at points that are not included in the regression. A significant lack of fit is a desirable statistical parameter to demonstrate the model's fitting on the responses [29]. The Table **5** shows that all models exhibit a significant lack of fit. When calculating the correlation coefficient (R^2) for the responses Y1, Y2, and Y3, the "Predictable R^2" is reasonably close to the "Adjustable R^2," with a difference of less than 0.2 Table **6**. The coefficients in Table **7** represent the quantitative effects of the independent variables

(X1, X2, and X) and their interactions on the responses. Coefficients with more than one term and those with higher-order terms indicate interactions and quadratic effects, respectively. A positive sign indicates a synergistic effect of the factor, while a negative sign represents an antagonist effect on the response [29-31].

Table 5. The ANOVA for Response Surface Quadratic model

Table 5.1. Response 1: Dissolution at 5 min.

Source	Sum of Squares	df	Mean Square	F Value	p-value Probability > F
Model	1295.53	9.5	133.73	104.43	< 0.0002
X-Oil (Capryol 90)	466.14	1	466.14	363.45	< 0.0002
Y-Surfactant (Tween 80)	579.00	1	579.02	451.41	< 0.0002
Z-Co-surfactant (PEG 400)	121.15	1	121.15	94.70	0.0003
XY	0.29	1	0.28	0.22	0.5775
XZ	1.12	1	1.00	0.90	0.4269
YZ	7.28	1	7.26	5.86	0.0685
A^2	16.34	1	16.34	14.62	0.0245
B^2	8.42	1	8.42	5.66	0.0715
C^2	2.65	1	3.69	2.29	0.3195
Residual	7.43	5.4	2.29	--	--
Lack of Fit	7.65	6	1.63	6.78	0.1616
Pure Error	0.96	4	0.44	--	--
Corrected Total	1180.93	15	--	--	--

Note: Values of "Prob. > F" less than 0.0500 indicate model terms are significant.

Table 5.2. Response 2: Dissolution at 10min.

Source	Sum of Squares	df	Mean Square	F Value	p-value Probability > F
Model	1220.04	6.7	204.16	67.80	< 0.0002
X-Oil (Capryol 90)	598.15	1	468.14	154.12	< 0.0002
Y-Surfactant (Tween 80)	572.33	1	562.14	174.49	< 0.0002
Z-Co-surfactant (PEG 400)	145.51	1	145.51	46.52	0.0005
XY	13.55	1	13.23	5.02	0.0698
XZ	1.02	1	1.05	0.35	0.5931
YZ	5.04	1	5.05	1.35	0.2954
Residual	26.34	9	2.06	--	--
Lack of Fit	23.36	7	4.73	3.75	0.2368
Pure Error	2.01	2.5	1.05	--	--
Corrected Total	1356.34	15	--	--	--

Table 5.3. Response 2: Particle Size range

Source	Sum of Squares	df	Mean Square	F Value	p-value Probability > F
Model	4642.83	9.5	505.67	52.66	0.0003
X-Oil (Capryol 90)	2246.14	1	2256.14	223.84	< 0.0004
Y-Surfactant (Tween 80)	1360.62	1	1502.52	135.70	< 0.0004
Z-Co-surfactant (PEG 400)	567.14	1	466.14	48.53	0.0007
XY	2.75	1	2.76	0.23	0.6289
XZ	0.040	1	0.030	0.000	1.0201
YZ	242.62	1	265.53	25.07	0.0542
A^2	312.37	1	312.28	32.37	0.0224
B^2	73.04	1	73.04	7.52	0.0508
C^2	50.62	1	50.65	5.18	0.0812
Residual	48.93	6	11.59	--	--
Lack of Fit	46.26	4	21.29	11.31	0.0925
Pure Error	3.68	2.5	2.34	--	--
Corrected Total	4378.73	15	--	--	--

Table 6: The Correlation coefficients for responses

Response: 1- Dissolution at 5min.			
Std. Dev.	1.15	R^2 Range	0.9846
Mean	78.08	Adjustable R^2 Value	0.9952
C.V. %	1.50	Predictable R^2 Value	0.9522
PRESS	94.80	Adeq Precision	35.9781
Response: 1- Dissolution at 10 min.			
Std. Dev.	1.75	R^2 Range	0.9705
Mean	85.86	Adjustable R^2 Value	0.9559
C.V. %	3.70	Predictable R^2 Value	0.9137
PRESS	121.65	Adeq. Precision	25.460
Response: 3- Particle Size			
Std. Dev.	3.12	R^2 Range	0.9647
Mean	75.89	Adjustable R^2 Value	0.9619
C.V. %	5.14	Predictable R^2 Value	0.8507
PRESS	720.12	Adeq. Precision	26.046

Table 7. The Final Equation in Terms of Coded Factors

Dissolution at 5min. = +79.34-7.79* A+ 9.60* B+4.89* C-0.28* B+0.52* AC-1.26* BC-3.18* A^2-2.43* B^2-1.68* C^2
Dissolution at 10min. = +85.68-7.98* A+9.38* B+5.26* C-2.79* AB+0.52* AC-2.01* BC
Particle size = +64.66+17.39* A-13.78* B-8.62* C+0.81* AB+5.545E-019* AC+8.65* BC+8.19* A^2 +5.43* B^2 +4.68* C^2

5.5. Response Surface Analysis: The graphical representations of the regression equation, including three-dimensional response surface plots and two-dimensional contour plots, illustrate the relationship between two independent variables and the response variable (Fig**. 4 to 5**). Through response surface analysis, the statistically significant relationship between the dependent and independent variables is further explained [32-33]. In the response surface and contour plots, the factors with the least significant values are held constant at low, center, and high levels. Fig. **4A to 4C** display the response surface and contour plots depicting the effects of Oil (Capryol 90) and surfactant (Tween 80) on dissolution at 5 minutes across all three levels of co-surfactant (PEG 400) [34].

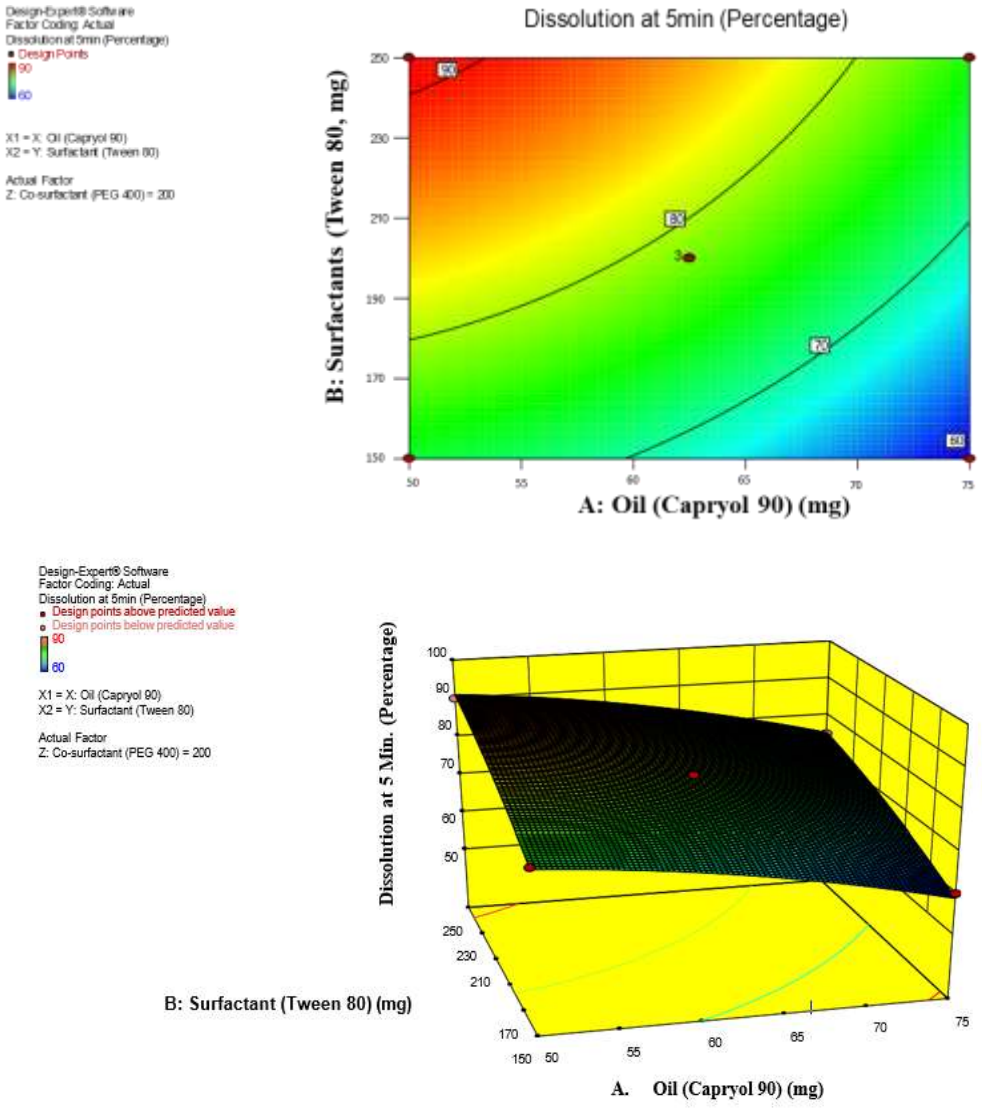


Fig. 4 (A). The response Surface and Contour Plots Showing the Effects of Oil and surfactant on Dissolution at 5min. (Co-surfactant is Constant at centre point i.e.200mg)

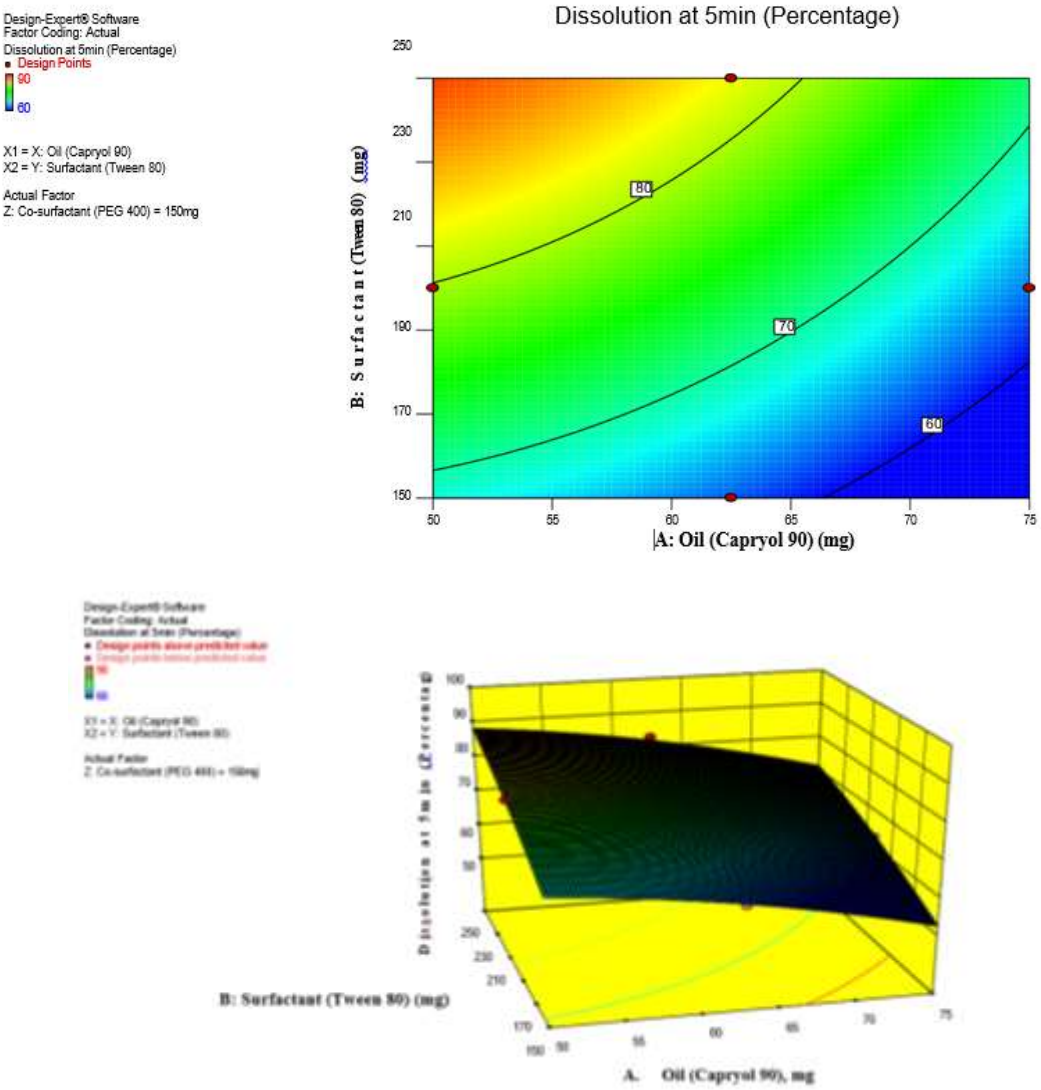
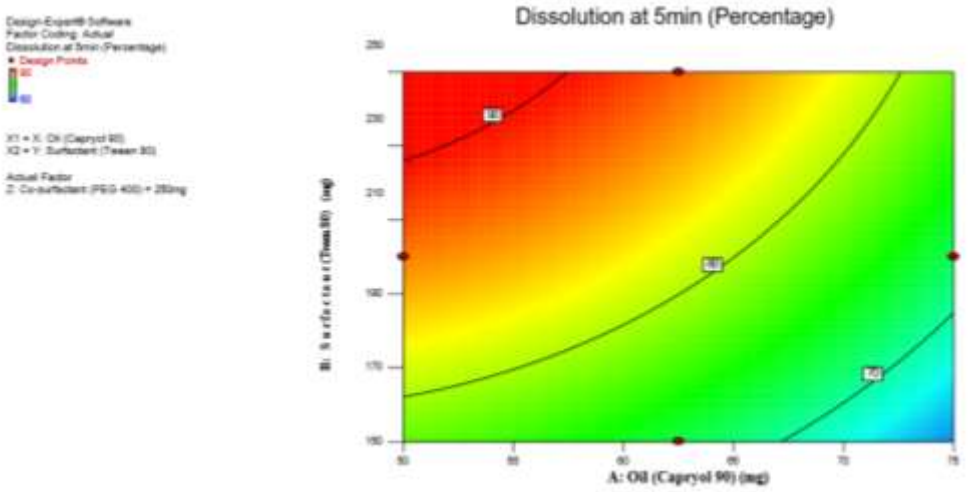


Fig. 4 (B). The response Surface and Contour Plots Showing the Effects of Oil and surfactant on Dissolution at 5 min. (Co-surfactant is Constant at centre point i.e.200mg)



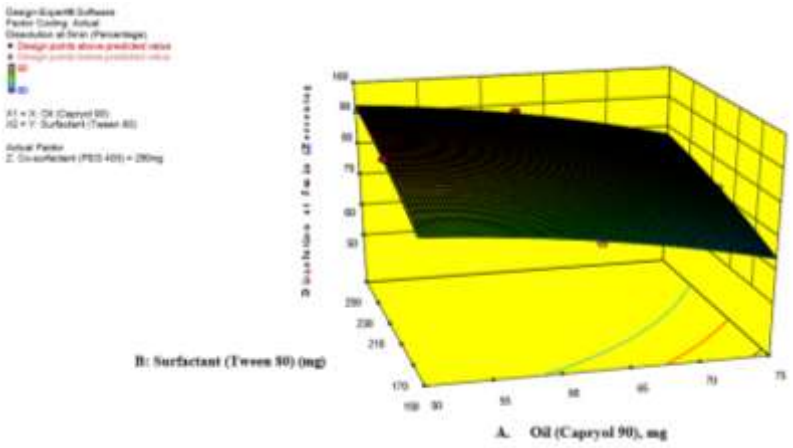
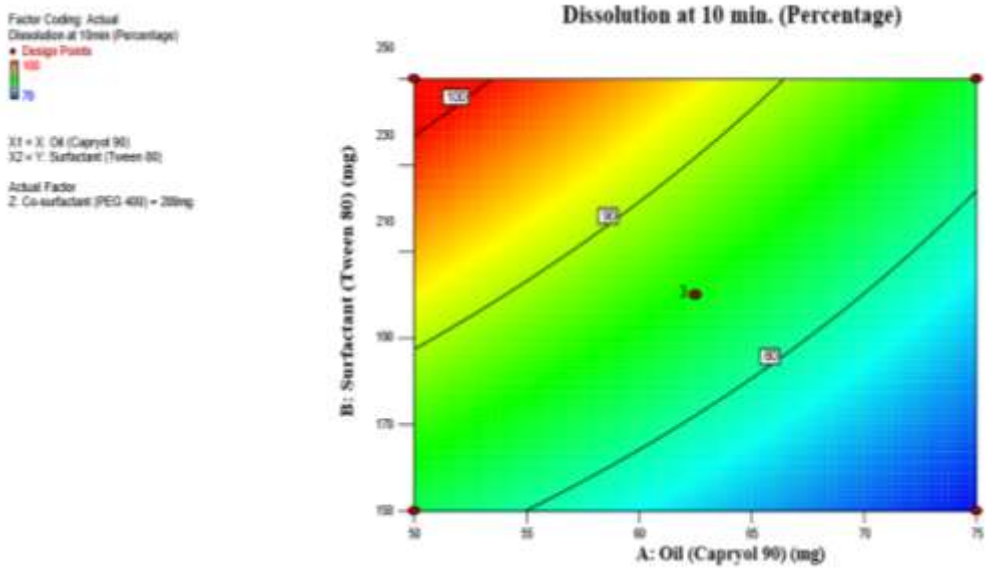


Fig. 4 (C). The response Surface and Contour Plots Showing the Effects of Oil and surfactant on Dissolution at 5 min. (Co-surfactant is Constant at highest point i.e.250mg)

The significant main effects were observed for all independent variables in Tables **7** and **8**, indicating their impact on dissolution at 5 minutes ($p<0.05$). Although the interaction effects were not very pronounced ($0.05<p<0.6$), the amounts of oil and surfactant demonstrated significant quadratic effects on dissolution at 5 minutes ($p<0.1$). As the Co-surfactant increased in the formulation (with a positive coefficient), the dissolution at 5 minutes also increased. This can be attributed to the availability of more surfactant, allowing for the formation of a more closely packed surfactant film with reduced curvature at the oil/water interface. Conversely, a decrease in the amount of co-surfactant resulted in a more marked decrease in dissolution at 5 minutes. Fig. **4A to 4C** display the response surface and contour plots, illustrating the effects of soil and surfactant on dissolution at 5 minutes across all levels of co-surfactant [35]. It is preferable to achieve a high percentage of drug release for the sake of reproducible bioavailability.

In Fig. **5A to 5C** depict the response surface and contour plots illustrating the impact of Oil (Capryol 90) and surfactant (Tween 80) on dissolution at 10 minutes across all three levels of co-surfactant (PEG 400). According to Tables **7** and **8**, all independent variables exhibited significant main effects ($p<0.05$) on dissolution at 10 minutes. The interaction effects were not particularly pronounced ($0.07<p<0.58$), although the quantities of oil and surfactant demonstrated significant quadratic effects on dissolution at 10 minutes ($p<0.1$) [36-37]. As the co-surfactant concentration increased in the formulation (with a positive coefficient), the dissolution at 10 minutes also increased. This can be attributed to the availability of more surfactant, which facilitates the formation of a more tightly packed surfactant film with reduced curvature at the oil/water interface. Conversely, a decrease in the amount of co-surfactant resulted in a more noticeable decrease in dissolution at 10 minutes. Fig. **5A to 5C** showcase the response surface and contour plots, highlighting the effects of Oil and surfactant on dissolution at 10 minutes across all levels of co-surfactant. It is preferable to achieve a high percentage of drug release in order to ensure reproducible bioavailability [38].



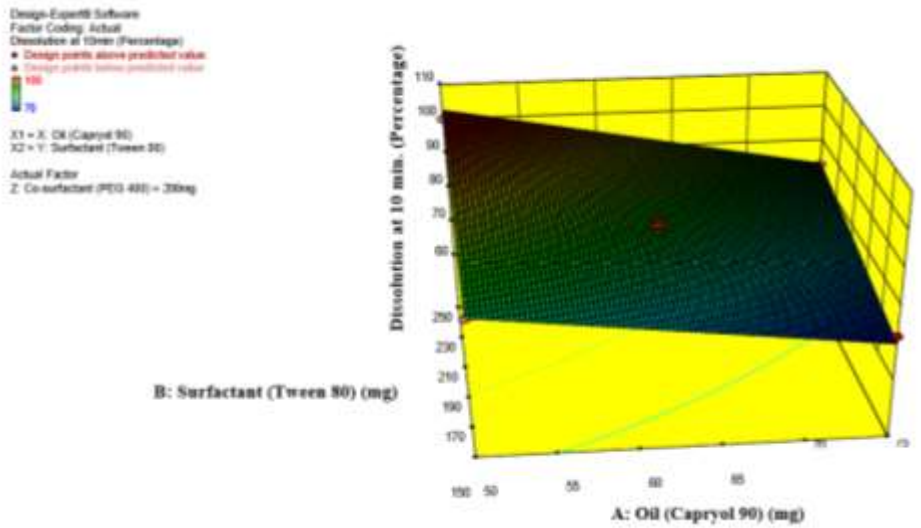


Fig. 5 (A). The response Surface and Contour Plots Showing the Effects of Oil and surfactant on Dissolution at 10 min. (Co-surfactant is Constant at centre point i.e.200mg)

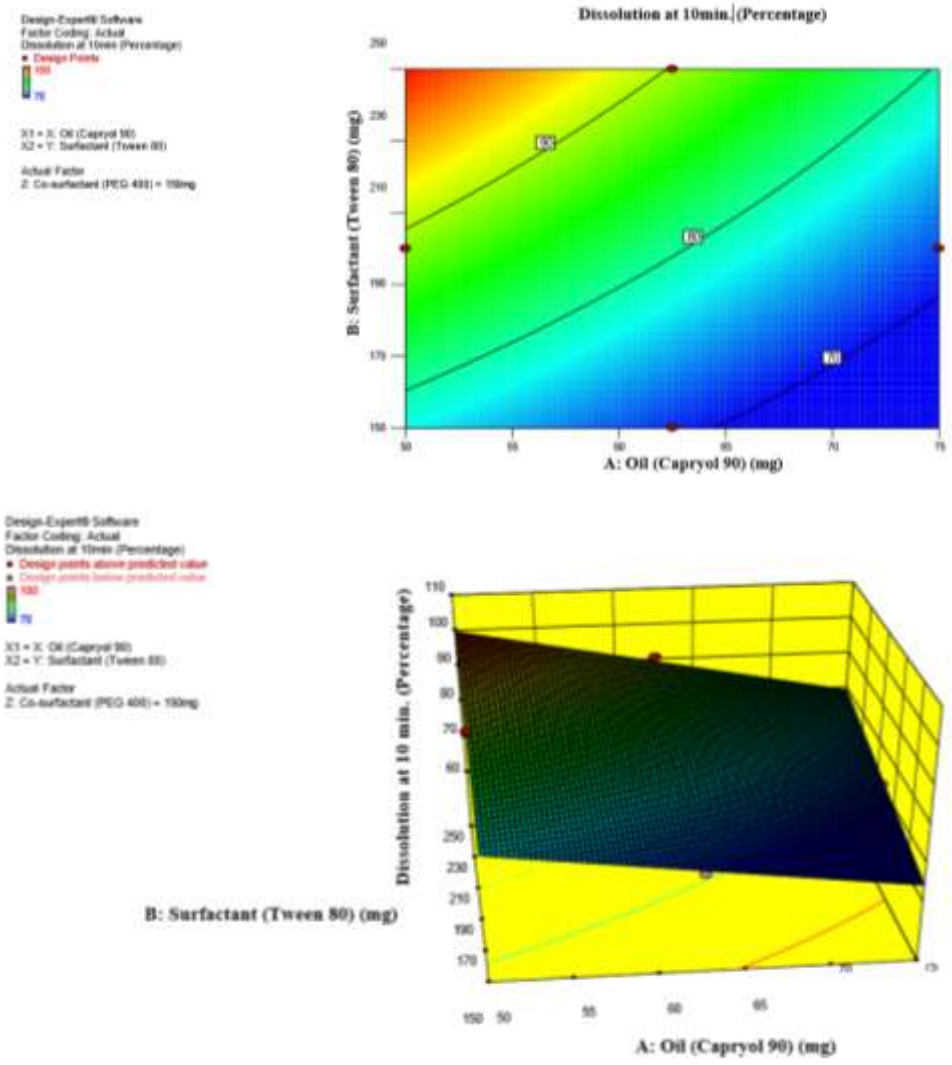


Fig. 5 (B). The response Surface and Contour Plots Showing the Effects of Oil and surfactant on Dissolution at 10 min. (Co-surfactant is Constant at lowest point i.e.150mg)

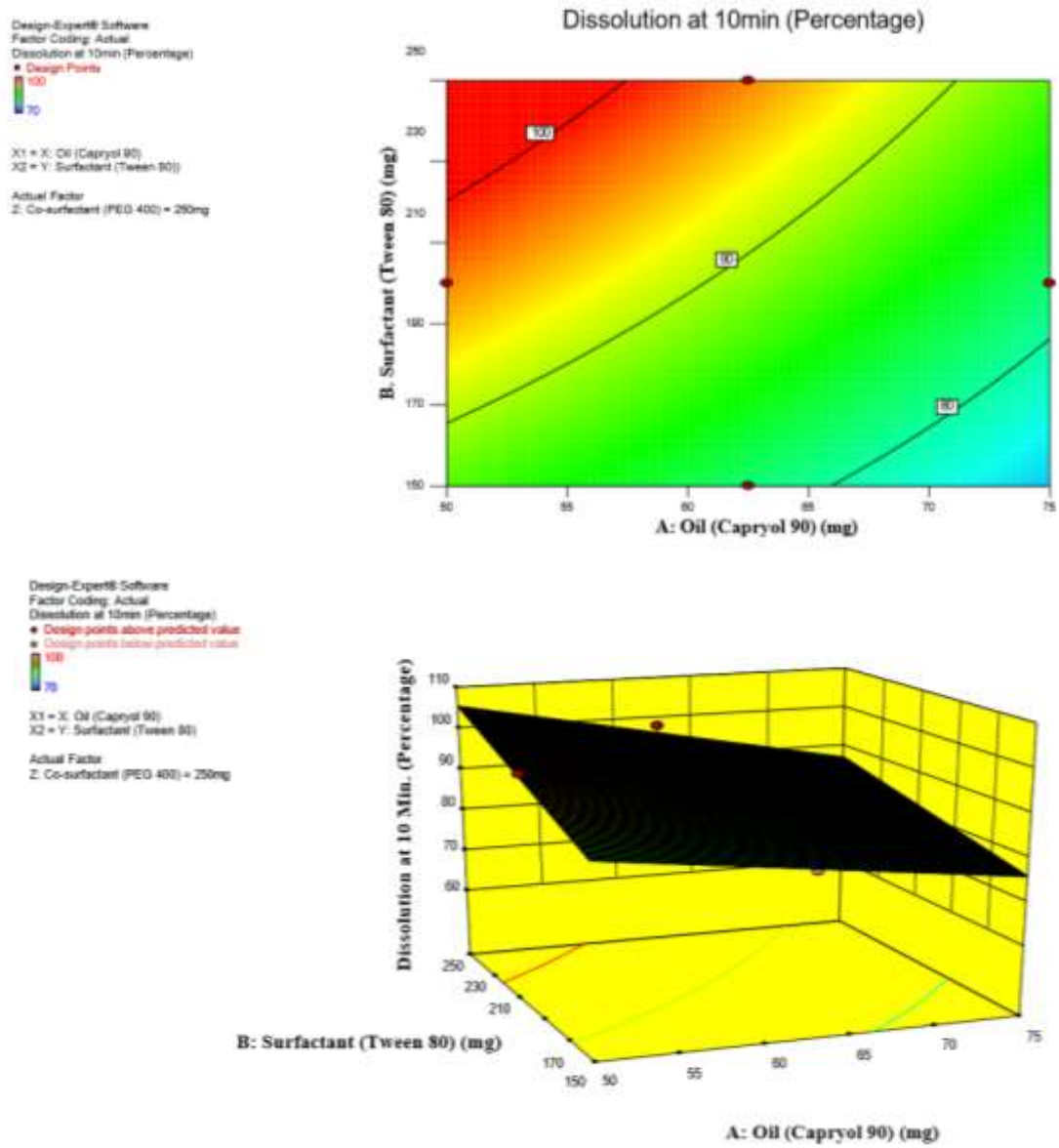


Fig. 5 (C). The response Surface and Contour Plots Showing the Effects of Oil and surfactant on Dissolution at 10 min (Co-surfactant is Constant at lowest point i.e.250mg)

Similarly, Fig. **6A to 6C** depict the response surface and contour plots illustrating the impact of Oil (Capryol 90) and surfactant (Tween 80) on particle size across all three levels of co-surfactant (PEG 400). Analysis of Tables **7** and **8** reveals that all independent variables exhibit significant main effects ($p<0.05$) on particle size. Although the interaction effects are not particularly pronounced ($0.05<p<1.0$), the quantities of oil and surfactant demonstrate significant quadratic effects on particle size ($p<0.1$). Notably, as the co-surfactant (with a -ve coefficient) increases in the formulation, the particle size decreases [39]. This phenomenon can be attributed to the availability of more surfactant for the formation of a more densely packed surfactant film with reduced curvature at the oil/water interface. Conversely, a decrease in the amount of co-surfactant leads to a more pronounced increase in particle size. The response surface and contour plots in Fig. **6A to 6C** provide a visual representation of the effects of Oil and surfactant on particle size at all levels of co-surfactant. It is important to achieve a high percentage of drug release for the purpose of ensuring reproducible bioavailability [40].

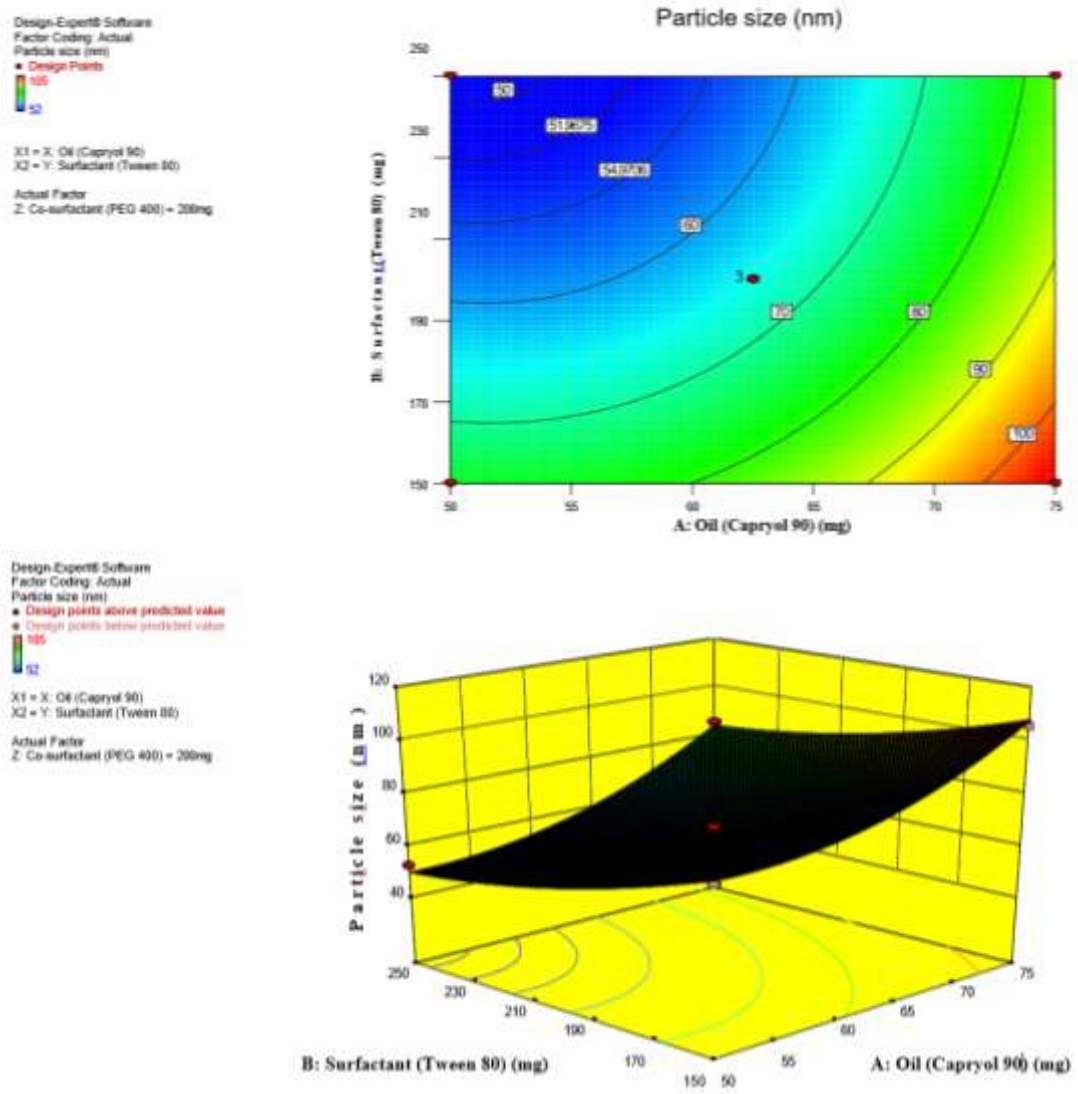
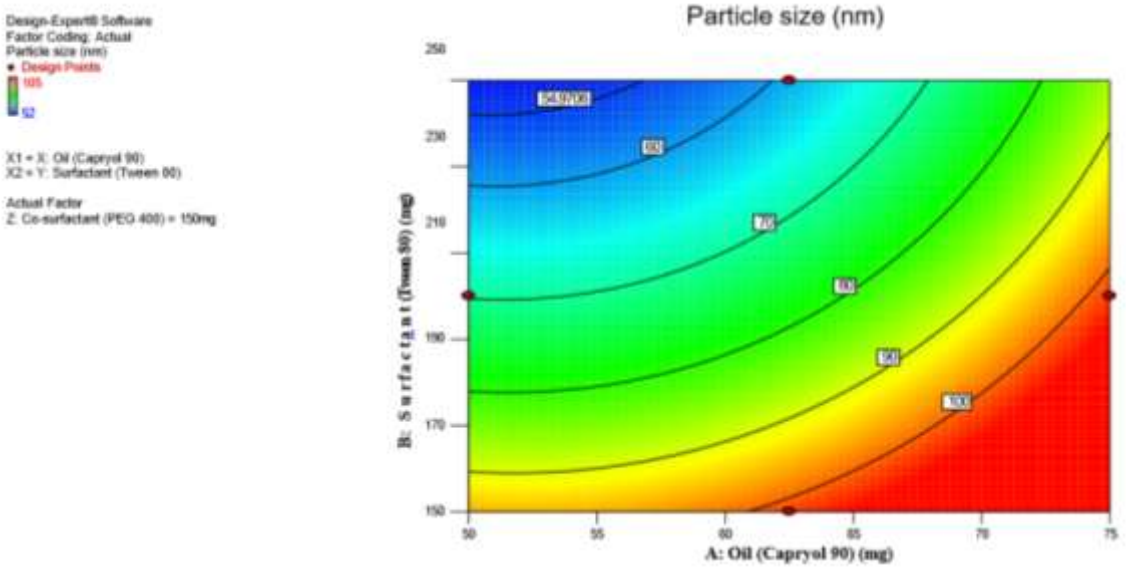


Fig. 6 (A). The response Surface and Contour Plots Showing the Effects of Oil and surfactant on particle size (Co-surfactant is Constant at centre point i.e.200mg)



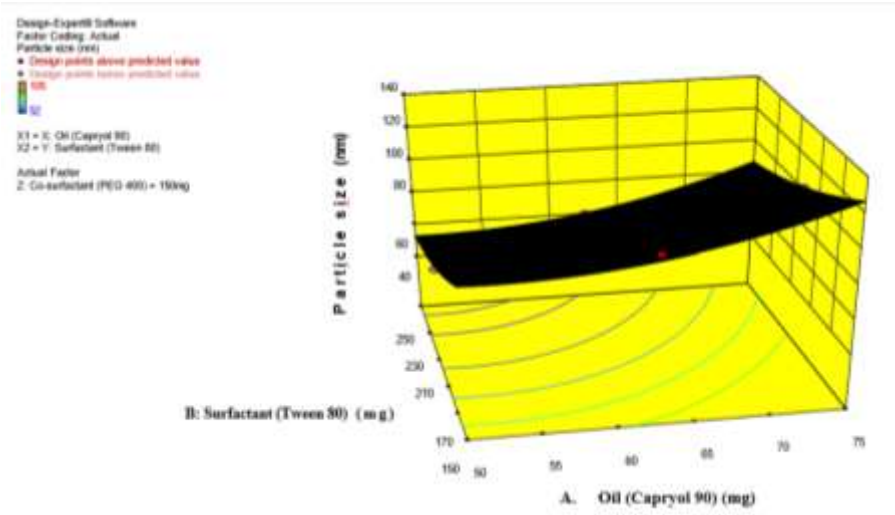
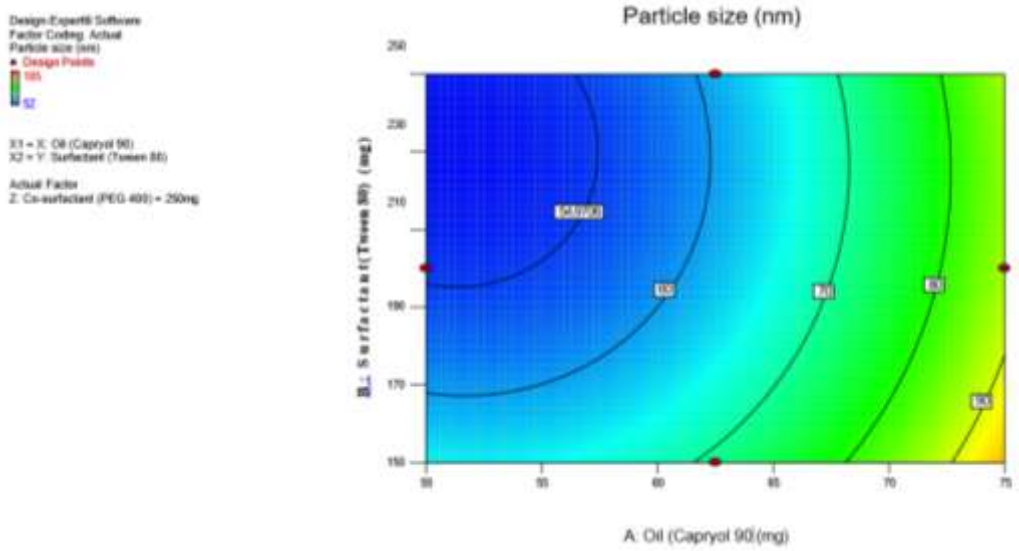


Fig. 6 (B). The response Surface and Contour Plots Showing the Effects of Oil and surfactant on particle size (Co-surfactant is Constant at centre point i.e.150mg)



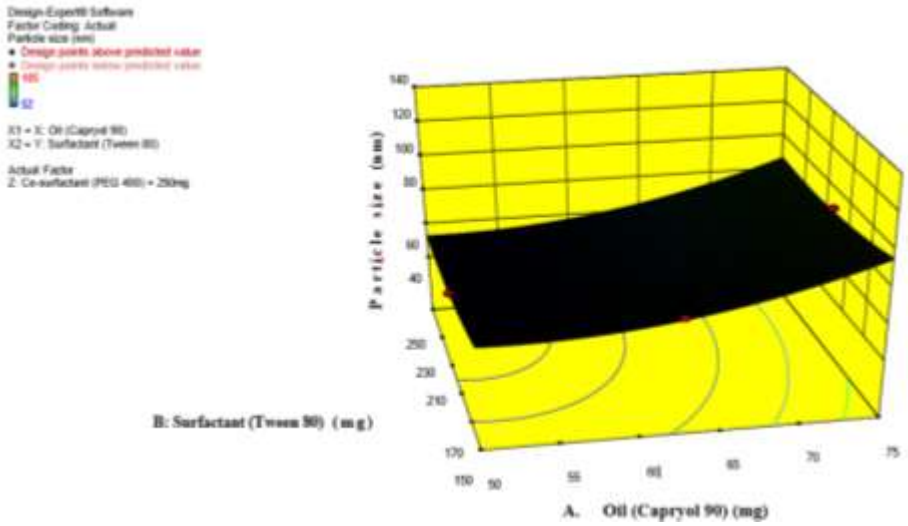


Fig. 6 (C). The response Surface and Contour Plots Showing the Effects of Oil and surfactant on particle size (Co-surfactant is Constant at centre point i.e.250mg)

5.6. Optimization by Using Desirability Function:

Upon generating polynomial equations to establish relationships between dependent and independent variables, the optimization process was carried out simultaneously for three responses using a desirability function. Y1, Y2, and Y3 responses were converted into a desirability scale, with factors set within a specified range and constraints applied to all responses. Y1 and Y2 were targeted for maximization, while Y3 was aimed to be minimized, with equal weight assigned to all responses [42]. The global desirability value was computed by combining individual desirability functions through the geometric mean, utilizing an extensive grid and feasibility search across the domain. The recommended optimized formulation comprised 50.82 mg of oil, 250 mg of surfactant, and 200 mg of co-surfactant, yielding a Desirability (D) value of 0.922. This factor level combination resulted in predicted responses of Y1=91.89%, Y2=103%, and Y3=52.34nm [43-45]. To validate the model's adequacy for prediction, two batches of the optimized formulations were prepared and all responses were assessed for each formulation Table **8** as followings:

Table 8. The predicted and Measured Values of Responses with confidence interval

Responses	Predicted Mean	Observed Mean	Std. Dev.	n	SE Pred.	95% PI low	Observed Mean	95% PI high
Dissolution at 5min	91.8943	94.233	2.13495	2	1.25	89.75	92.01	95.05
Dissolution at 10min	203.234	103.20	1.78805	2	1.78	98.94	103.01	104.07
Particle size	48.1653	48.25	3.0956	2	3.93	43.76	52.25	58.97

The optimized NTG-loaded SEDDs exhibited a particle size of 49.23 nm, dissolution rates of 91.0% after 5 minutes, and 100.0% after 10 minutes. It can be inferred that the experimental values closely matched the predicted values, demonstrating the success of the design in evaluating and optimizing the SEDDs formulation [44-46].

6. CONCLUSION

The present study aimed to investigate the effects of three formulation factors, namely Capryol 90 as oil, Tween 80 as surfactant, and PEG 400 as co-surfactant, on the main characteristics of NTG, SEDDs. The 3-level, 3-factor (3^3) BBD was employed for this purpose. The results indicated that all three factors had a significant impact on particle size, dissolution after 5 minutes, and dissolution after 10 minutes. The amount of oil and surfactant used played a major role in influencing these factors. Additionally, the formulation factors exhibited interaction and quadratic effects on the studied responses. Through the utilization of the desirability function, an optimized formulation was successfully developed, with the experimental values closely aligning with the predicted values. Notably, the in vitro dissolution study of the optimized formulation demonstrated a significant increase in release, reaching approximately 100% after 10 minutes. Consequently, it can be concluded that the implementation of BBD facilitated a better understanding of the inherent relationship between formulation variables and responses, ultimately leading to the optimization of NTG SEDDs in a cost-effective, time-efficient, and labor-saving manner.

LIST OF ABBREVIATIONS

SEDDs: self-emulsifying drug delivery systems**, BA:** bioavailability, **BBD:** Box-behnken design, **NTG:** nateglinide**, BCS:** biopharmaceutical classification of drug.

ETHICAL APPROVAL

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

HUMAN AND ANIMAL ETHICAL RIGHT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, and no funding was required to conduct these review data.

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AVAILABILITY OF DATA AND MATERIALS

The data supporting this study's findings will be available in the cited references.

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AUTHOR CONTRIBUTION

This study was the result of collaborative efforts from all co-authors contributing equally to the preparation of the final draft. All authors have reviewed and approved the final version of the manuscript for publication.

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