## Originales

## Artículo Especial

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# Optimization of Lovastatin Self-Nanoemulsifying Solid Dosage Form.

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**RESUMEN**

**Objetivo:** El objetivo del presente estudio fue desarrollar y optimizar la auto-nanoemulsión forma de dosificación sólida (SNESDF) de la Lovastatina para aumentar su solubilidad. Lovastatina (cuya solubilidad en agua es 0,4 x 10-3 mg / ml) se considera que es un fármaco razonable debido a su alto valor  de log P (4,3) y una buena solubilidad en aceites.

**Materiales y Métodos:** Las formulaciones fueron optimizadas por  el diseño estadístico Box-Behnken  en el cual  las variables independientes como relación de tensioactivo: tensioactivo co-(X1), aceite: tensioactivo co tensioactivo (X2), y% Aerosil (X3). Las formulaciones se caracterizan por sus variables dependientes, tales como tamaño de la gota (Y1), la transmitancia (Y3), el porcentaje de fármaco liberado en 5 minutos (Y3), y dentro de 15 minutos (Y4).

**Resultados y Conclusiones:** tamaño de la gota y el potencial zeta del lote optimizado resultó ser 21,89 nm y -6,4 mV, respectivamente. 44,32% y 90,78% del fármaco se encontró que se libera dentro de 5 min y 15 min, respectivamente. Por lo tanto, mediante la formulación en SNESDF de lovastatina ,  se encontró que la solubilidad mejoraba significativamente.

**PALABRAS CLAVE:** Diseño de Box-Behnken, lovastatina, Potencial Zeta, Diagrama de superficie de respuesta

**ABSTRACT**

**Aim:** The aim of present study was to develop and optimized self-nanoemulsifying solid dosage form (SNESDF) of Lovastatin for enhancing its solubility.  Lovastatin (whose water solubility is 0.4 x $10^{-3}$ mg/mL) is considered to be a reasonable drug because of its high log P value (4.3) and good solubility in oils.

**Materials and Methods:** The formulations were optimized by Box-Behnken statistical design in which the independent variables like Ratio of surfactant: co-surfactant ($X_1$), oil: surfactant co surfactant ($X_2$), and % Aerosil ($X_3$). The formulations were characterized for its dependent variables such as Droplet size ($Y_1$), transmittance ($Y_3$), percentage of drug released within 5 minutes ($Y_3$), and within 15 minutes ($Y_4$).

**Results and Conclusion:** Droplet size and zeta potential of the optimized batch was found to be 21.89 nm and -6.4 mV, respectively. 44.32 % and 90.78 % of the drug was found to be released within 5 min and 15 min, respectively. Hence, by formulating into SNESDF, the solubility of Lovastatin was found to be significantly improved.

**KEY WORDS:** Box-Behnken Design, Lovastatin, Zeta Potential, Surface Response Plot

# INTRODUCTION

Successful oral delivery of drug has always remained a challenge to the drug delivery field, since approximately 40% of new drugs have poor water solubility, and thus oral delivery is frequently associated with implications of low bioavailability[1]. To overcome these bioavailability problems, various formulation strategies have been reported including the use of surfactants, cyclodextrin inclusion complexes, solid dispersion, nanoparticles and absorption enhancers. However the most appropriate formulation and their metabolic products are still worth to be further investigated.

 Self-nanoemulsifying drug delivery systems (SNEDDS) have attracted considerable amount of interest as potential drug delivery vehicles, largely due to simplicity of preparation, clarity and ability to be filtered and incorporate wide range of drugs of varying solubility[2]. These SNEDDS is o/w type emulsion, most suitable formulation, which is expected to increase the solubility by dissolving compounds with low water solubility into an oil phase. They can also enhance oral bioavailability by reducing the droplet size (<100 nm), and hence increase the rate of absorption due to surfactant-induced permeability changes[3]. Lovastatin (LOV) lowers cholesterol levels through reversible and competitive inhibition of all HMG-CoA reducatse (3-hydroxy-3methylglutaryl-coenzyme A reductase), an enzyme involved in the biosynthesis of cholesterol. Lovastatin (water solubility is $0.4 \times 10^{-3}$ mg/mL) is considered to be a reasonable substrate because of its high log P valve (4.3) and good solubility in oils (55.10 and 83.54 mg/gm soybean oil and sunflower oil, respectively). Hence, the objective of this study was to enhance the solubility of Lovastatin by formulating self-nanoemulsifying solid dosage form (SNESDF).

Many statistical experimental designs have been recognized as useful techniques to optimize the process variables. Different types of screening designs, such as fractional factorial and Plackett- Burman screening designs have been used for preformulation evaluations[4]. Response surface methodology (RSM) is used when only a few significant factors are involved in optimization. Different types of RSM designs include 3-level factorial design, central composite design (CCD), Box Behnken design and D-optimal design[5]. A modified central composite experimental design, Box-Behnken design, is an independent, rotatable or nearly rotatable quadratic design (contains no embedded factorial or fractional factorial design), in which the treatment combinations are at the midpoints of the edges of the process space and at the center[6]. Among all the RSM designs, Box-Behnken design requires fewer runs (15 runs) in a 3-factor experimental design. A 3-factor, 3-level design would require a total of 27 unique runs without any repetitions and a total of 30 runs with 3 repetitions. Hence, the Box-Behnken design was applied in present investigation to optimize the Lovastatin self-nanoemulsifying solid dosage forms (SNESDFs) with constraints on the release of drug within 15 min.

# MATERIALS AND METHODS

## Materials
Lovastatin was received as a gift sample from the Torrent Pharmaceuticals Ltd. (Ahmedabad, India). Acrysol K 140 and Aerosil 200 were received as gift samples from Corel Chemical Ltd (Ahmedabad, India). Capmul MCM and Capmul MCM C8 were kindly gifted by Abitech Corporation (USA). Tween and Span were purchased from S.D. Fine Chemicals (Mumbai, India). All other chemicals and solvents were of analytical regent grades.

## Ternary phase diagram
Ternary phase diagram were constructed to obtain the appropriate components, and their concentration ranges that resulted in a large existence area of nanoemulsion were chosen. In order to optimize the concentration of oil phase, surfactant and co-surfactant, different batches of varied concentration were prepared and titrated with distilled water till transparency persisted. Ternary phase diagram was prepared by using a constant ratio of surfactant to co-surfactant. Three ratio of surfactant (Acrysol K 140) and co-surfactant (Capmul MCM C8) were selected. (1:1, 2:1, 3:1).

## Preparation of Lovastatin Self-nanoemulsifying Solid Dosage Form (SNESDF)

Box-Behnken statistical screening design was used to optimize and evaluate main effects, interaction effects, and quadratic effects of the formulation ingredients on the *in-vitro* performance of SNESDF. A 3-factor, 3-level design is suitable for exploring quadratic response surfaces and constructing second-order polynomial models. The nonlinear computer-generated Design Expert (Trial Version 8.0.4.1 STAT-EASE, Bangalore), quadratic model is given as

$$Y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{11} x_1^2 + b_{22} x_2^2 + b_{33} x_3^2 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3 \ldots \ldots$$

Where Y is the measured response associated with each factor level combination; $b_0$ is an intercept; $b_1$ to $b_{33}$ are the regression coefficients; and $X_1, X_2,$ and $X_3$ are the independent variables[7]. The selected dependent and independent variables are shown in Table 1. The Ratio of surfactant: co-surfactant $(X_1)$, oil: surfactant co-surfactant $(X_2)$, and % Aerosil $(X_3)$ used to prepare each of the 15 formulations are given in Table 2. Lovastatin SNESDFs were prepared by varying the concentrations of Sunflower oil, Acrysol K140, Capmul MCM C8 and Aerosil. Predetermined amounts of the Lovastatin (10 mg) were dissolved in the required quantity of Sunflower oil in screw-capped glass vial and were warmed in a water bath at 37°C. Acrysol K140 and Capmul MCM C8 were added to the above mixture as a fixed ratio and stirred for 1 hr. All the formulations with different concentrations of surfactant, co-surfactant, oil and solid adsorbent were filled into capsules size 3 and stored at room temperature until used in subsequent studies[8].

### Characterization of Self-nanoemulsifying Solid Dosage Form

**Droplet size and Zeta potential measurements**

The mean droplet size and zeta potential of the resultant nanoemulsion was determined by dynamic light scattering, using a zetasizer HSA 3000 (Malvern Instruments Ltd., UK)[9].

**Transmittance study**

Stability of the optimized SNESDF formulation with respect to dilution was checked by measuring transmittance at 650 nm with a UV Spectrophotometer (UV-1800, Shimadzu)[10].

### *In-vitro* Dissolution Testing

Dissolution rates from different SNESDF were determined in 900 mL of 0.1 N HCl at 37±0.5°C with stirrer rotation speed of 50 rpm using the USP dissolution test apparatus (TDT-08L, Electrolab, Mumbai, India) employing a basket stirrer (method 1). A 10 ml aliquot of dissolution medium was withdrawn at 5, 10 and 15 min with a pipette. The samples were suitably diluted and assay spectrophotomaterically (UV-1800, Shimadzu) at 238 nm[11].

### RESULTS AND DISCUSSION

**Preparation of Self-nanoemulsifying Solid Dosage Form**

The maximum amount drug was found to dissolve in sunflower oil (83.54 ±1.98 mg/gm). Therefore, this oil was selected for nanoemulsion formulation. The required HLB value to form o/w nanoemulsion should be between 12-18 and the selection of surfactant was mainly based on this. Co-surfactant was selected based on their capability to form a stable nanoemulsion with the relevant surfactant at a minimum concentration. Capmul MCM C8 was selected



Figure 1. Ternary phase diagram of nanoemulsion (a) Acrysol K 140/ Capmul MCM C8 (2:1) and (b) Acrysol K 140/ Capmul MCM C8 (3:1).
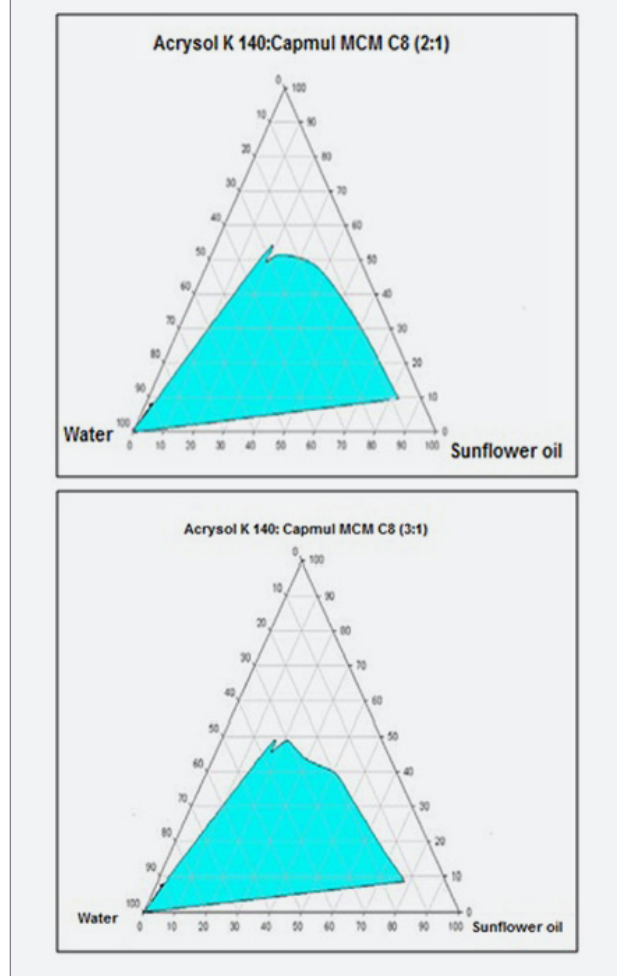
for Acrysol K140 containing nanoemulsion. Ternary phase diagram were constructed to obtain the appropriate components and their concentration range that can result in a large nanoemulsion existence area. From the ternary phase diagrams shown in Figure 1a and 1b, it was concluded that highest nanoemulsion zone was achieved for nanoemulsion containing Acrysol K140/Capmul MCM C8 at a ratio 3:1.

### Characterization of SNESDF

**Droplet size and Zeta potential measurements**

Droplet size for all the formulations was found < 90 nm regardless of the content used in formulation (Table 1). Lower value of the correlation coefficient (Eq. 1) clearly indicates that the response is independent of the factors studied. Once can be concluded that all these formulations resulted in acceptable droplet size range (< 100 nm) for nanoemulsions and no particular pattern was found (Table 1). As illustrated in Table 2, a p values of <0.05 for any factor in analysis of variance (ANOVA) indicated significant effect of the corresponding factor on the response. From

**Table 1.** Box-Behnken Design: Independent (X) and Dependent Variables (Y)*

| Batch | $X_1$ Acrysol K-140: Capmul MCM C8 (A*) | $X_2$ Sunflower oil: A* | $X_3$ % Aerosil 200 | $Y_1$ Droplet size (nm) | $Y_2$ Transmittance (%) | $Y_3$ % Dissolved in 5 min | $Y_4$ % Dissolved in 15 min | $Y_5$ Zeta potential (mV) |
|---|---|---|---|---|---|---|---|---|
| F1 | 1 | 1 | 0 | 40 | 91±0.98 | 44.5±1.34 | 89.56±2.12 | -6.7 |
| F2 | 1 | -1 | 0 | 32 | 69.8±1.02 | 23.3±0.87 | 56.23±1.67 | -9.5 |
| F3 | -1 | 1 | 0 | 30 | 85±2.67 | 37.8±2.09 | 82.56±0.95 | -4.3 |
| F4 | -1 | -1 | 0 | 45 | 69.45±3.21 | 34.23±1.23 | 74.78±0.56 | -5.8 |
| F5 | 1 | 0 | 1 | 31 | 74.23±2.56 | 34.55±1.89 | 69.89±1.09 | -3.2 |
| F6 | 1 | 0 | -1 | 65 | 89.35±1.98 | 22.67±2.3 | 43.78±2.45 | -7.8 |
| F7 | -1 | 0 | 1 | 29 | 78.23±2.16 | 39.34±2.76 | 75.23±1.23 | -6.7 |
| F8 | -1 | 0 | -1 | 32 | 78.56±0.87 | 41.76±1.56 | 81.23±1.45 | -5.7 |
| F9 | 0 | 1 | 1 | 26 | 84.56±2.35 | 35.23±2.32 | 64.34±1.67 | -4.9 |
| F10 | 0 | 1 | -1 | 22 | 94.34±2.31 | 36.23±1.27 | 70.98±2.12 | -6.3 |
| F11 | 0 | -1 | 1 | 34 | 76.34±2.56 | 29.34±2.32 | 53.23±1.67 | -7.1 |
| F12 | 0 | -1 | -1 | 89 | 78.89±1.45 | 38.34±1.27 | 65.34±1.34 | -6.9 |
| F13 | 0 | 0 | 0 | 20 | 93.23±2.13 | 47.34±1.56 | 86.23±1.54 | -5.8 |
| F14 | 0 | 0 | 0 | 21 | 89.99±2.24 | 46.31±1.67 | 86.23±1.54 | -6.3 |
| F15 | 0 | 0 | 0 | 19 | 94.21±1.23 | 44.67±1.23 | 84.29±2.34 | -5.7 |

| Variables | Levels used | | |
|---|---|---|---|
|  | Low | Medium | High |
| **Independent Variables** | | | |
| $X_1$ = Surfactant: Co surfactant | 1:1 | 2:1 | 3:1 |
| $X_2$ = Oil: Surfactant Co-surfactant | 7:3 | 8:2 | 9:1 |
| $X_3$ = Percentage Aerosil | 15% | 20% | 25% |
| **Dependent Variables** | | | |
| $Y_1$ = Droplet Size (nm) | | | < 100 |
| $Y_2$ = Transmittance | | | near to 100 |
| $Y_3$ = Dissolution after 5 min | 22.67 | 47.34 | Maximum |
| $Y_4$ = Dissolution after 15 min | 53.23 | 89.56 | > 85 |

A* is Acrysol K-140: Capmul MCM C8

the results of multiple regression analysis, it was found that the dependent variables, droplet size are strongly dependent on the independent variables (P> 0.05).

Droplet Size $(Y_1)$ = 20.00 + 4.00$X_1$ - 10.25$X_2$ - 11.00$X_3$ + 6.63$X_1X_1$ + 10.13$X_2X_2$ + 12.62$X_3X_3$ + 5.75$X_1X_2$ - 7.75$X_1X_3$ + 14.75$X_2X_3$ ($R^2$ = 0.8416)     (1)

Zeta potential results of all the batches were found to be -3.2 mV to -9.5 mV. Aggregation is not expected to take place, due to the slightly negative charge of the droplets.

**Transmittance Study**

The results of transmittance showed wide variation (Table 1). It can be inferred that these 3 factors have a profound effect on the transmittance. Formulations numbered 1, 3, 6, 9, 10, 13, 14 and 15 showed percentage transmittance of >80 %. From the results of multiple regression analysis, it was found that transmittance are strongly dependent on the independent variables (P<0.05, Table 2). The correlation coefficients indicate a good fit. Polynomial equation (Eq. 2) can be used to draw a conclusion after considering the magnitude of the coefficient and the mathematical sign it carries (positive or negative). The positive sign of variables $X_1$ and $X_2$ indicated positive effect of surfactant: co-surfactant and oil: surfactant co-surfactant ratio on transmittance.

### Table 2. Results of two way ANOVA for measured response.

| Droplet Size | | | | |
|---|---|---|---|---|
| Source | SS | DF | MS | F Ratio | P Value |
| Regression | 4172.33 | 9 | 463.59 | 2.95 | 0.1229 |
| Residual | 785.00 | 5 | 157.00 | | |
| Total | 4957.33 | 14 | | | |
| **Transmittance** | | | | |
| Source | SS | DF | MS | F value | P Value |
| Regression | 1024.30 | 9 | 113.81 | 17.57 | 0.0028 |
| Residual | 32.38 | 5 | 6.48 | | |
| Total | 1056.68 | 14 | | | |
| **% Dissolution after 5 min** | | | | |
| Source | SS | DF | MS | F value | P Value |
| Regression | 654.03 | 9 | 72.67 | 2.35 | 0.0793 |
| Residual | 154.30 | 5 | 30.86 | | |
| Total | 808.33 | 14 | | | |
| **% Dissolution after 15 min** | | | | |
| Source | SS | DF | MS | F value | P Value |
| Regression | 3498.75 | 9 | 388.75 | 24.14 | 0.0013 |
| Residual | 80.54 | 5 | 16.11 | | |
| Total | 3579.28 | 14 | | | |

DF is degree of freedom, SS is sum of square, MS is mean sum of square and F is Fischer's ratio.

Transmittance $(Y_2) = 92.47 + 1.64X_1 + 7.55X_2 - 3.47X_3 - 8.55X_1X_1 - 5.11X_2X_2 - 3.83X_3X_3 + 1.41X_1X_2 - 3.69X_1X_3 - 1.80X_2X_3$ $(R^2 = 0.9694)$ (2)

It is clear that the ratio of oil: surfactant co-surfactant $(X_2)$ has a more positive effect then the surfactant: co-surfactant $(X_1)$ ratio on transmittance, this may be due to the concentration of surfactant co-surfactant was decrease the interfacial tension between oil and water interface and give transparent nanoemulsion. Same finding was true for surfactant: co-surfactant is on the transmittance.

### *In-vitro* Dissolution Testing

From Table 1 it can be inferred that these three factors have a profound effect on the drug release profiles. Formulations numbered 1, 8, 13, 14 and 15 was >40% and only formulation 3 showed drug release of <40% drug release after 5 min. Lower value of the correlation coefficient (Eq. 3) clearly indicates that the response is independent of the factors studied. From the results of multiple regression analysis, it was found that the dependent variables, percentage dissolution after 5 min are less dependent on

the independent variables (P <0.05, Table 2). For finding best optimized batch study was further extended for drug release after 15 min.

$D_{5\ min}(Y_3) = 46.11 - 3.5X_1 + 3.57X_2 - 0.068X_3 - 5.68X_1X_1 - 5.47X_2X_2 - 5.85 X_3X_3 + 4.41 X_1X_2 + 3.57 X_1X_3 + 2.00 X_2X_3$ $(R^2= 0.8091)$ (3)

Formulations numbered 1, 13, 14, and 15 showed higher drug release of >85% after 15 minutes of dissolution. However, the percentage of drug released after 15 min from formulations 3 and 8 was <85%. In order to obtain a formulation having rapid drug release of >85% within 15 minutes, RSM optimization was used to determine the levels of these factors. The value of the correlation coefficient (Eq. 4) clearly indicates that the response is dependent of the factors studied. The positive value of the $X_2$ variable represents positive effects of oil: Surfactant co-surfactant, where as variable $X_1$ and $X_3$ have negative effects on drug released after 5 min. From the results of multiple regression analysis, it was found that the dependent variables, drug released after 15 min are dependent on the independent variables (P <0.05, Table 2). The correlation coefficients indicate a good fit.

$D_{15\ min}(Y_4) = 85.58 - 2.29X_1 + 7.23X_2 - 4.33X_3 - 7.37X_1X_1 - 2.43X_2X_2 - 19.68 X_3X_3 + 6.39X_1X_2 + 17.03X_1X_3 + 1.37X_2X_3$ $(R^2=0.8501)$ (4)

From the 3D plots, it is clear that the ratio of oil: surfactant co-surfactant $(X_2)$ has a major effect on determining drug release within 15 min from formulations (Figures 2a, b, and c). The Figure 2a shows that at a lower ratio of Acrysol K-140: Capmul MCM C8 (A*), the percentage drug released with an increase in the ratio of sunflower oil: A* (from 54.83 % to 82.73%). However, at a higher ratio of sunflower oil: A*, the percentage drug released from formulations decreased with an increase ratio of Acrysol K-140: Capmul MCM C8 (from 81.41% to 54.83%). This finding can be explained by the fact that co-surfactant by itself does not emulsify the oil, rather the co-surfactant acts by enhancing the emulsifying capability of surfactants. Hence, optimum ratio of surfactant and co-surfactant is a key factor in achieving an emulsion. The Figure 2b shows that at a low percentage of aerosil the percentage drug released from formulations increase with a decrease in the ratio of Acrysol K-140: Capmul MCM C8 (from 57.55% to 81.10%). Figure 2a and 2b indicated that for obtaining the higher percentage drug released after 15 min the ratio of Acrysol K-140: Capmul MCM C8 $(X_1)$ should be kept high whereas ratio of sunflower oil: A* $(X_2)$ should be kept medium to high. Figure 2b and 2c suggested that the % aerosil $(X_3)$ was effective at low level so should be kept medium for further study.

**Figure 2. Surface response plots for the effect of (a) independent variable $X_1$ and $X_2$ on % Dissolution after 15 min (b) independent variables $X_1$ and $X_3$ on % Dissolution after 15 min (c) independent variable $X_2$ and $X_3$ % Dissolution after 15 min**
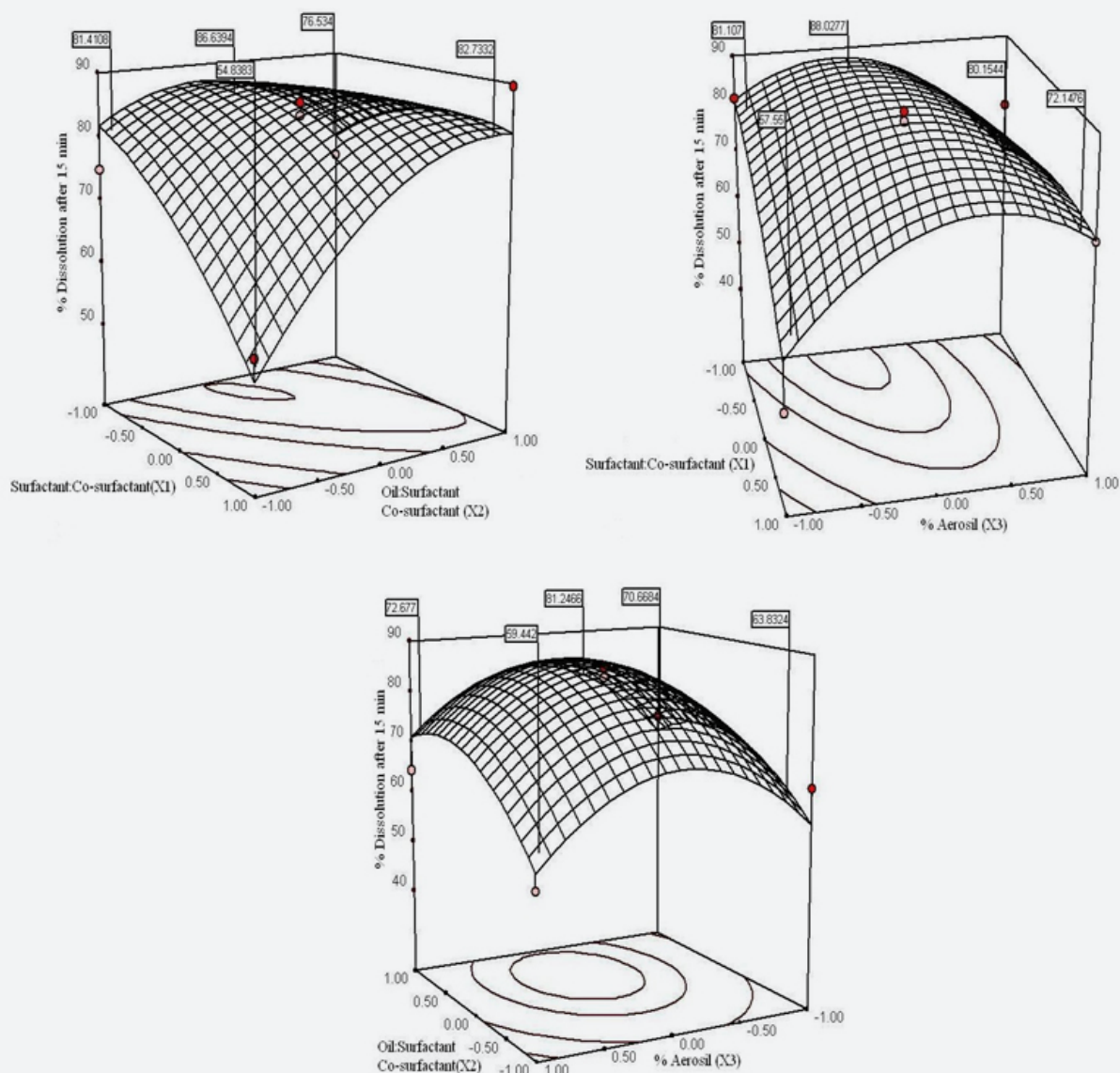


Figure 4 is a representative contour plot, which further elucidates the effects of varying ratio of Acrysol K-140: Capmul MCM C8 (A*) and Sunflower oil: A* with a fixed amount of aerosil on all responses. Figure 3 illustrates that the emulsification of Acrysol K-140: Capmul MCM C8 (A*) increases as the ratio of sunflower oil: A* is increased. Maximum transmittance and *in-vitro* drug released in 15 min were found at Acrysol K-140: Capmul MCM C8 (A*) from 0 to 1 with lower levels of Sunflower oil: A* from -0.25 to 1 as indicated by the yellow portion of the plot. Batch F1 falls in this region and therefore was selected as the best of the batches prepared according to the Box-Behnken design. Droplet size and percentage transmittance of batch F1 were found 40 nm and 91 ± 0.98, respectively. The in-vitro dissolution study indicated that more than 85% of the drug released in 15 min, indicating the non interference

**Figure 3. Drug release pattern of Lovastatin SNESDF formulations**
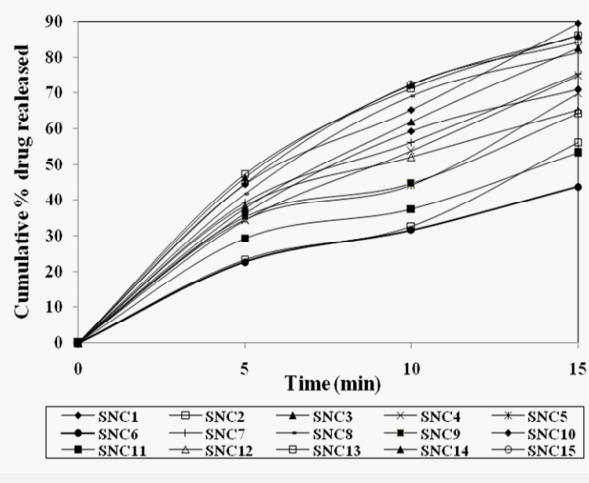
**Figure 4: Overlay plot in which the yellow region is the region where the constraints set on Particle size (< 19), Transmittance (> 90), % Dissolution in 5 min (>45) and in 15 min (> 80).**
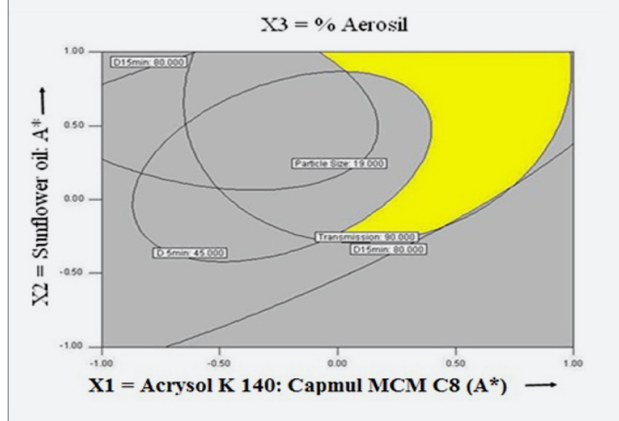


of the surfactant, co-surfactant, oil and aerosil with drug release. Batch F1 was selected as best optimized batch for further study.

**Validation of the evolved mathematical models**

To validate the evolved mathematical models, two check points were selected. Two batches CH1 and CH2 were prepared and evaluated (Table 3). As a confirmation process, a fresh formulation of Lovastatin SNCDF was prepared with Lovastatin (10 mg), Acrysol K-140: Capmul MCM C8 (A*) (2151 mg), Sunflower oil: A* (239 mg) and Aerosil (600 mg). The optimized levels of factors yielded a formulation (batch OPT) with droplet size was <30%, transmittance was >90%, rapid drug release of >40% within 5 min and of >90% within 15 min. The observed and predicted values were in very close agreement, thus strengthening the predictability of the mathematical model. Further the optimized formulation had zeta potential of -6.4 mV.

## CONCLUSION

Optimization of Lovastatin SNESDF using RSM, Box-Behnken design, was performed. The ratio of independent variables, Sunflower oil, Acrysol K 140, Capmul MCM C8 and Aerosil 200 showed a significant effect on the transmittance and drug release characteristics of the formulation. The optimum ratio of these factors at 3 levels was chosen based on the quantitative effect and the polynomial equations generated by RSM. The optimized formulation prepared by using these predicted levels of factors provided desired observed responses forming nanoemulsions with more than 94% transmittance and greater than 90% drug release within 15 min.

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## Table 3. Validation of the evolved mathematical models

| Response | Batch CH1 A= 0.4 & B= 0.6 | | Batch CH2 A= 0.6 & B= 0.4 | | Batch OPT A= 0.2 & B= 0.8 | |
|---|---|---|---|---|---|---|
| | Observed | Predicted | Observed | Predicted | Observed | Predicted |
| $Y_1$ | 20.08 | 21.54 | 24.78 | 23.69 | 21.89 | 20.26 |
| $Y_2$ | 93.89 | 94.79 | 90.89 | 92.92 | 94.67 | 95.45 |
| $Y_3$ | 43.45 | 45.03 | 46.23 | 43.58 | 44.32 | 45.24 |
| $Y_4$ | 89.75 | 88.48 | 84.67 | 85.59 | 90.78 | 90.07 |

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