WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 16, 1458-1477. Research Article ISSN 2277– 7105

# FORMULATION AND EVALUATION OF SELF NANOEMULSIFYING DRUG DELIVERY SYSTEM CONTAINING POORLY WATER SOLUBLE ANTILIPIDEMIC DRUGS

**Milan D. Limbani*[1] and Dr. L. D. Patel[2]**

[1]Ph.D. Scholar, Sharda School of Pharmacy, Pethapur - 382610, Gandhinagar, Gujarat, India.

[2]Former Principal, Sharda School of Pharmacy, Pethapur - 382610, Gandhinagar, Gujarat, India.

Article Received on
11 July 2018,

Revised on 01 August 2018,
Accepted on 23 August 2018,

DOI: 10.20959/wjpr201816-13273

***Corresponding Author**
**Milan D. Limbani**
Ph.D. Scholar, Sharda School of Pharmacy, Pethapur - 382610, Gujarat Technological University, Ahmedabad.

## ABSTRACT

The present work was aimed for the formulation development of stable SNEDDS of Fenofibrate and Atorvastatin Calcium using concentration of oil and surfactant/cosurfactant on the basis of preliminary trials. The $3^2$ factorial design was employed using concentration of Capmul MCM oil and Cremophor RH 40: Transcutol-P (3:1) as independent variables. The Globule size (GS), Polydispersity index (PDI), Zeta potential (ZP) and drug release at 15 minutes for Fenofibrate and Atorvastatin calcium were selected as dependent variables. The optimized batch was selected on the basis of arbitary criteria using Design Expert software employing overlay plot with desirability approach. The SNEDDS formulations were evaluated for their physico-chemical parameters such as globule size, zeta potential, polydispersity index, drug release profile and physico-chemical stability. The composition of optimized formulation consisted of Capmul MCM Oil as oil (0.471ml), Cremophor RH 40 as surfactant (1.206ml) and Transcutol-P as cosurfactant (0.402ml), containing 10mg of Atorvastatin and 45mg of Fenofibrate showing drug release for liquid SNEDDS formulation (>95%), droplet size (78.3nm), Zeta potential (-23.13), and infinite dilution capability. *In-vitro* drug release of the optimized batch was highly significant ($p<0.05$) as compared to marketed conventional capsule (Fenostat) of Fenofibrate and Atorvastatin Calcium.

**KEYWORDS:** Capmul MCM oil, non-ionic surfactant/co-surfactant, Nanoemulsion, SNEDDS.

## INTRODUCTION

SNEDDS are isotropic mixtures made up of oil, surfactant and sometimes cosurfactant or cosolvent. In an aqueous environment a homogeneous, transparent (or at least translucent), isotropic and thermodynamically stable dispersion will result up on mild agitation.[1,2] SNEDDS is best suited for dosage for development of poorly soluble drugs. Lipophilic drug compounds which exhibit dissolution rate-limited absorption, these systems may offer an improvement in rate and extent of absorption.[1,2] SNEDDS formulations are known to reduce inter- and intra-individual variations in bioavailability, which is believed to be caused by a decreased sensitivity of formulation performance to pre-absorptive solubilisation and dietary status.

Atorvastatin Calcium inhibits HMG-CoA reductase and reduces serum concentrations of LDL-cholesterol, VLDL-cholesterol, Apo lipoprotein B and triglycerides.[3] Atorvastatin shows low aqueous solubility and rapidly absorbed after oral administration. Food decreases the rate and extent of drug absorption by approximately 25% and 9% respectively. Fenofibrate decrease LDL-cholesterol, triglyceride and Apo B concentrations and to increase concentrations of HDL-cholesterol in the management of mixed dyslipidemia and primary hypercholesterolemia, including heterozygous familial hypercholesterolemia.[4] Fenofibrate shows bioavailability problems due to poor water and physiological fluids solubility. Fenofibrate shows increase in absorption in fed condition of patient compare to fasting condition of patient. It exhibits additive antilipidemic effects when used concomitantly with other antilipidemic agents.[5]

The first step in oral absorption process is dissolution of the drug compound in gastrointestinal lumen contents, poor aqueous solubility is rapidly becoming the leading hurdle for formulation scientist working on oral delivery of such drug compounds. Hence, SNEDDS formulation were considered for enhance solubility, release rate and oral absorption of poorly soluble antilipidemic drugs.

To the best of our knowledge, no information is available in the literature on the improvement of Fenofibrate and Atorvastatin Calcium dissolution and bioavailability using mixture of Capmul MCM oil, Cremophor RH 40 and Transcutol-P by SNEDDS methodology. The present work described the formulation development of stable SNEDDS. The $3^2$ factorial design was employed using concentration of Capmul MCM oil and Cremophor RH 40: Transcutol-P (3:1) as independent variables. The Globule size (GS),

Polydispersity index (PDI), Zeta potential (ZP) and drug release at 15 minutes for Fenofibrate and Atorvastatin calcium were selected as dependent variables. The optimized batch was selected on the basis of arbitary criteria using Design Expert software employing overlay plot with desirability approach.

Two check point batches was prepared and performed to validation the evolved polynomial equations in the formulation development of Fenofibrate and Atorvastatin Calcium SENDDS.

Stability study for optimized SNEDDS was performed as per ICH guidelines by keeping in stability chamber at room temperature (25°C) & 60% RH and accelerated condition (40°C) & 75% RH for 6 months. The optimized formulation was subjected to in vitro dissolution to evaluate improvement in drug release as compared to marketed product.

## MATERIAL AND METHODS

### Materials

Labrasol and Transcutol-P were generous gift from Gattefose for research. Cremophor RH 40 was gifted from BASF. Capmul MCM oil was gifted from Abitech. Atorvastatin calcium and Fenofibrate were gifted from Cadila Healthcare Limited. Water used in the preparation of formulations was distilled water, whereas ultra-pure water, used in analyses, was obtained with a Milli-Q apparatus. All other chemicals and reagents used were of pharmaceutical grade or HPLC grade.

## METHODS

### Solubility Study

Screening of solubilizing excipients was done by determining the solubility of Fenofibrate and Atorvastatin Calcium in different solubilizing vehicle like oils, surfactants and co-surfactants. An excess quantity of Fenofibrate or/and Atorvastatin Calcium were added to the 2 ml of the solubilizing vehicle. Both components were mixed in a vial for 5 minutes using cyclomixer. The mixtures in vial was shaken at room temperature for 48 hour using controlled temperature rotary shaker. The mixtures were centrifuged at 5000 RPM for 20 minutes. The drug content was analysed using UV-Visible spectrophotometer at 287 and 246 nm for Fenofibrate or Atorvastatin Calcium, respectively.[6,7]

**Preliminary Trials**

The oil and surfactant/co-surfactant mixture was used to found suitable concentration in formulation development of SNEDDS. The efficiency of self-emulsifying systems was measured from the rate of emulsification upon hydration with mild agitation. The time taken for the formation of fine emulsion was noted as dispersion time. Preliminary batches were formulated and their results of dispersion status as clear, turbid or clear & translucent in SNEDDS and dispersion time were recorded.

**Preparation of SNEDDS[7]**

Accurately weighed Fenofibrate and Atorvastatin Calcium were placed in a glass vial, and required quantity of oil, surfactant, and co-surfactant were added. The mixture was mixed by gentle stirring and vortex mixing at 40ºC on a magnetic stirrer, until drugs were dissolved.[7]

The mixture was stored at room temperature in closed container for further use.

**$3^2$ factorial design for optimization of formulation parameters of Fenofibrate and Atorvastatin Calcium SNEDDS**

The preliminary trials were carried out using different concentration of Capmul MCM oil, Cremophor RH 40 and Transcutol-P (3:1). On the basis of results of preliminary trials for selection of lipid vehicle, the concentration of Capmul MCM oil ($X_1$) and Concentration of Cremophor RH 40: Transcutol-P (3:1) ($X_2$) were taken as independent variables at three levels. The GS ($Y_1$), PDI ($Y_2$), ZP ($Y_3$), drug release at 15 minutes of Fenofibrate ($Y_4$) and drug release at 15 minutes of Atorvastatin Calcium ($Y_5$) were considered to play significant role in formulation performance of SNEDDS and all the five were taken as dependent parameters in present study. Multiple regression analysis, contour plot and 3D response surface plot were used to study the main and interaction effects of the variables. The responses were measured for each trials and then either simple linear equation, or interactive equation or quadratic equation model was fitted by carrying out multiple regression analysis and F-statistics to identify statistically significant term.

Microsoft EXCEL was used to identify non-significant terms. A coefficient is significant if ti>tcrit(v), where v denotes the degrees of freedom of residual variance. The refined model may be used for calculating the residuals or for drawing the contour plot.

**Contour Plot**

Contour plot is a diagrammatic representation of values of the response and it is helpful in explaining visually the relationship between independent and dependent variables. The reduced model was used to plot two dimension contour plot using demo version of Design Expert 11 software.

**Response Surface Plot**

Response surface plot is helpful in understanding the main and the interaction effects of variables in the formulation development. The effect of level of independent variable on the response parameter can be understood from the respective response surface plot.

**Optimization of SNEDDS formulation overlay plot by Design Expert software**

The desirability function approach is a technique for the simultaneous determination of optimum settings of input variables that can determine optimum performance levels for one or more responses.[7] The optimization of SNEDDS formulation was performed using Design Expert software employing overlay plot with desirability approach.

**Checkpoint Analysis**

A check point analysis was performed to validation the evolved polynomial equations in the formulation development of SNEDDS. Difference of theoretically computed values of GS, PDI, Zeta potential and drug release at 15 minutes for Fenofibrate and Atorvastatin Calcium and then mean values of experimentally obtained GS, PDI, Zeta potential and drug release at 15 minutes for Fenofibrate and Atorvastatin Calcium were compared by using Student's t-test.

**Measurement of evaluation parameters of SNEDDS Formulations**

**Measurement of Globule Size, Polydispersity Index (PDI) and Zeta Potential[8]**

Globule size, Polydispersity index (PDI) and zeta potential of SNEDDS were determined using Zetasizer Nano ZS (Malvern Instruments, UK), which follows principle of LASER light diffraction. SNEDDS was added (after suitable dilution with purified water) to the sample cell and put into the sample holder unit and measurement was carried out with the help of software of same instrument.[8]

**Drug Content**[9]

Fenofibrate and Atorvastatin Calcium from pre-weighed SNEDDS was extracted by dissolving in 25ml methanol. Then methanolic extract was separated out and Fenofibrate and Atorvastatin Calcium content in methanolic extract were analysed HPLC Method at 248nm, against standard methanolic solution of Fenofibrate and Atorvastatin Calcium.

**In-Vitro Drug Release Study**[7]

In vitro drug release study was carried out for all formulations, marketed product and active drug substance using USP Type II dissolution test apparatus. The dissolution medium (900 ml water) maintained at 37 ± 0.5°C and rotated at 50rpm. Aliquots were collected periodically and replaced with fresh dissolution medium. Aliquots, after filtration through 0.45µ PVDF filter paper, were analysed by HPLC at 248nm for Fenofibrate and Atorvastatin Calcium content.[9,10]

**Stability Study of Fenofibrate and Atorvastatin Calcium SNEDDS**

Stability of optimized formulation was carried out at 40±2°C/75±5%RH and 25±3°C (room temperature) as per ICH guidelines. SNEDDS was stored in glass vial for 6 months. Samples was withdrawn at 0, 1, 3 and 6 months and analysed periodically.[7,10]

**Comparison of in vitro drug release between Optimized SNEDDS formulation, pure drug powder and marketed product**

In vitro drug release study was performed as method described above for optimized SNEEDS formulations, marketed product and active drug substance to compare the in vitro drug release profile.

## RESULT AND DISCUSSION

**Solubility Study**

Vehicles should have good solubilizing capacity for the drug substance, which is essential for formulating SNEDDS. Fenofibrate and Atorvastatin Calcium had highest solubility in Capmul MCM Oil, Cremophor RH 40 and Transcutol-P (Table 1). Capmul MCM Oil as oil, Cremophor RH 40 as surfactant and Transcutol-P as co-surfactant were selected for SNEDDS formulation resulting in improved drug loading capability.

**Table 1: Solubility of Fenofibrate and Atorvastatin Calcium.**

| Material | Average (mg/ml) ± SD | |
|---|---|---|
| | Fenofibrate | Atorvastatin Calcium |
| Castor Oil | 72.18 ± 0.15 | 11.60 ± 0.06 |
| Labrafac PG | 58.85 ± 0.14 | 28.14 ± 0.04 |
| Oleic Acid | 21.43 ± 0.11 | 19.40 ± 0.10 |
| Capmul MCM Oil | 178.93 ± 0.38 | 52.97 ± 0.07 |
| Light Liquid Paraffin | 25.70 ± 0.12 | 10.69 ± 0.09 |
| Tween-80 | 74.80 ± 0.20 | 40.13 ± 0.04 |
| Span-20 | 47.22 ± 0.24 | 26.06 ± 0.07 |
| Labrafac Lipophile WL 1349 | 63.89 ± 0.22 | 42.02 ± 0.03 |
| Cremophor EL | 61.48 ± 0.18 | 30.43 ± 0.05 |
| Labrasol | 119.93 ± 0.46 | 74.48 ± 0.08 |
| Capmul GMO-50 | 36.29 ± 0.14 | 26.74 ± 0.08 |
| Captex 355 | 25.19 ± 0.08 | 14.31 ± 0.08 |
| PEG-400 | 36.39 ± 0.11 | 38.67 ± 0.07 |
| Propylene Glycol | 34.17 ± 0.11 | 10.74 ± 0.09 |
| Transcutol-P | 177.11 ± 0.43 | 82.28 ± 0.08 |
| Cremophor RH 40 | 112.85 ± 0.31 | 71.32 ± 0.28 |

**Selection of Concentration of Oil, Surfactant and Cosurfactant**

Capmul MCM oil and Cremophor RH 40: Transcutol-P mixture (3:1) were used to found their suitable concentration in formulation development of SNEDDS of Fenofibrate and Atorvastatin Calcium.

**Table 2: Preliminary trials for development of Fenofibrate and Atorvastatin Calcium SNEDDS.**

| Preliminary Batches | Oil (ml) | S:Co-S (3:1) (ml) | Dispersion | Dispersion time (Seconds) |
|---|---|---|---|---|
| P1 | 0.5 | 0.5 | Turbid | 68 |
| P2 | 0.5 | 1.0 | Clear & Translucent | 60 |
| P3 | 0.5 | 1.5 | Clear & Transparent | 44 |
| P4 | 0.5 | 2.0 | Clear & Transparent | 41 |
| P5 | 0.4 | 0.5 | Clear & Translucent | 65 |
| P6 | 0.4 | 1.0 | Clear & Transparent | 64 |
| P7 | 0.4 | 1.5 | Clear & Translucent | 60 |
| P8 | 0.4 | 2.0 | Clear & Transparent | 58 |
| P9 | 0.6 | 0.5 | Clear & Translucent | 71 |
| P10 | 0.6 | 1.0 | Clear & Translucent | 63 |
| P11 | 0.6 | 1.5 | Clear & Transparent | 46 |
| P12 | 0.6 | 2.0 | Clear & Transparent | 45 |

The preliminary trials were carried out using different concentration of Capmul MCM oil (0.4mL – 0.6mL), and Cremophor RH 40 and Transcutol-P (3:1) (0.5mL – 2.0mL). The

results of dispersion status and dispersion time were presented in Table 2. The result of preliminary trial with 0.5mL of Capmul MCM oil (batch P3) was found satisfactory. Apart from oil concentration, Concentration of S/Cos mixture (3:1) was also important in formulation development of SNEDDS and 1.5mL of Cremophor RH 40: Transcutol-P mixture (3:1) was found appropriate in the preliminary study.

**Optimization of SNEDDS of Fenofibrate and Atorvastatin Calcium using factorial design**

The concentration of oil and surfactant/Cosurfactant play important role in stable formulation of SNEDDS; hence concentration of Capmul MCM oil (0.5mL) and concentration of Cremophore RH 40:Transcutol-P (3:1) (1.5mL) were selected as independent variables in factorial design on the basis of preliminary trials (Table 2). The $3^2$ factorial design was employed using concentration of oil and surfactant/Cosurfactant as independent variable $X_1$ and $X_2$ respectively. The GS ($Y_1$), PDI ($Y_2$), ZP ($Y_3$), drug release at 15 minutes of Fenofibrate ($Y_4$) and Atorvastatin Calcium ($Y_5$) were selected as dependent variables. The coded and actual value of independent variable were shown in Table 3. The runs and responses for factorial batches were presented in Table 4.

**Table 3: Factors and levels of independent variables in $3^2$ factorial design for formulation of Fenofibrate and Atorvastatin Calcium SNEDDS.**

| Independent variables | Level | | |
|---|---|---|---|
| | Low (-1) | Medium (0) | High (+1) |
| Capmul MCM oil conc. ($X_1$) | 0.4 | 0.5 | 0.6 |
| Cremophor RH 40: Transcutol-P (3:1) conc. ($X_2$) | 1.2 | 1.5 | 1.8 |

**Table 4: Experimental runs and measured responses of $3^2$ factorial design for SNEDDS.**

| Batch | $X_1$ | $X_2$ | Globule size (nm) ($Y_1$) | PDI ($Y_2$) | Zeta potential (mV) ($Y_3$) | Drug release at 15 min for Fenofibrate ($Y_4$) | Drug release at 15 min for Atorvastatin Calcium ($Y_5$) |
|---|---|---|---|---|---|---|---|
| T1 | -1 | -1 | 357.0 | 0.428 | -15.12 | 91.8 | 92.4 |
| T2 | 0 | -1 | 64.1 | 0.283 | -16.40 | 93.9 | 93.6 |
| T3 | 1 | -1 | 55.8 | 0.221 | -17.12 | 93.1 | 91.6 |
| T4 | -1 | 0 | 332.0 | 0.427 | -15.68 | 93.8 | 92.9 |
| T5 | 0 | 0 | 20.7 | 0.189 | -27.96 | 96.7 | 97.4 |
| T6 | 1 | 0 | 44.0 | 0.233 | -17.60 | 94.1 | 93.4 |
| T7 | -1 | 1 | 307.0 | 0.426 | -15.96 | 93.5 | 92.3 |
| T8 | 0 | 1 | 26.6 | 0.195 | -24.28 | 94.5 | 95.8 |
| T9 | 1 | 1 | 29.2 | 0.191 | -21.12 | 95.0 | 96.0 |

Multiple regression analysis was carried out for the responses using MS Excel. The reduced model was obtained by using significant terms ($p > 0.05$ was considered non-significant and such termed were neglected) for all the responses. The contour and response surface plot were constructed using Design Expert version 11 (Demo version).

**Globule size ($Y_1$)**

A full model equation of globule size ($Y_{FGS}$) was written as Equation 1.

$Y_{FGS} = 31.9889–144.5X_1–19.0167X_2+150.3667X_1^2+7.7167X_2^2+5.85X_1X_2$ …… (Equation 1)

The reduced model for globule size ($Y_{RGS}$) was presented as Equation 2.

$Y_{RGS} = 37.1333 – 144.5X_1 – 19.0167X_2 + 150.3667X_1^2$ ……. (Equation 2)

**Polydispersity index (PDI) ($Y_2$)**

A full model equation of polydispersivity index ($Y_{PDI}$) was written as Equation 3.

$Y_{FPDI} = 0.2172 – 0.106X_1 – 0.02X_2 + 0.0987X_1^2 + 0.0077X_2^2 – 0.007X_1X_2$ …… (Equation 3)

The reduced model for polydispersity index ($Y_{RPDI}$) was presented as Equation 4.

$Y_{RPDI} = 0.2223 – 0.1060X_1 – 0.0200X_2 + 0.0987X_1^2$ .....…….. (Equation 4)

**Zeta potential (ZP) ($Y_3$)**

A full model equation of zeta potential ($Y_{FZP}$) was written as Equation 5.

$Y_{FZP} = -24.2667 – 1.5133X_1 – 2.12X_2 + 5.78X_1^2 + 2.08X_2^2 – 0.79X_1X_2$ …… (Equation 5)

The reduced model for zeta potential ($Y_{RZP}$) was presented as Equation 6.

$Y_{RZP} = -19.0267 – 1.5133X_1 – 2.1200X_2$ ...…….....……. (Equation 6)

**Drug release at 15 minutes for Fenofibrate (DRF) ($Y_4$)**

A full model equation of drug release at 15 minutes for Fenofibrate ($Y_{FDRF}$) was written as Equation 7.

$Y_{FDRF} = 95.8556 + 0.5167X_1 + 0.7X_2 – 1.4833X_1^2 – 1.2333X_2^2 + 0.05X_1X_2$ … (Equation 7)

The reduced model for drug release at 15 minutes for Fenofibrate ($Y_{RDRF}$) was presented as Equation 8.

$Y_{RDRF} = 94.0444 + 0.5167X_1 + 0.7X_2$ ……...……. (Equation 8).

**Drug release at 15 minutes for Atorvastatin Calcium (DRA) ($Y_5$)**

A full model equation of drug release at 15 minutes for Atorvastatin Calcium ($Y_{FDRA}$) was written as Equation 9.

$Y_{FDRA} = 96.2333 + 0.5667X_1 + 1.0833X_2 – 2.5X_1^2 – 0.95X_2^2 + 1.125X_1X_2$ ….. (Equation 9)

The reduced model for drug release at 15 minutes for Atorvastatin Calcium ($Y_{RDRA}$) was presented as Equation 10.

$Y_{RDRA} = 95.6000 + 0.5667X_1 + 1.0833X_2 – 2.5X_1^2$ ……..... (Equation 10).

**Table 5: ANOVA of full model and reduced model.**

| **Responnse Y1** | **Model** | **DF** | **SS** | **MS** | **F** | **$R^2$** | **F cal** |
|---|---|---|---|---|---|---|---|
| Regression | FM | 5 | 172927.6 | 34585.51 | 360.64 | 0.9983 | 1.334 |
| | RM | 3 | 172671.6 | 57557.19 | 529.32 | 0.9968 | |
| Error | FM | 3 | 287.701 | 95.900 | | | |
| | RM | 5 | 543.685 | 108.737 | | | |
| **Responnse Y2** | Model | dF | SS | MS | F | $R^2$ | F cal |
| Regression | FM | 5 | 0.0896 | 0.01792 | 14.286 | 0.9597 | 1.541 |
| | RM | 3 | 0.0892 | 0.0297 | 36.502 | 0.9563 | |
| Error | FM | 3 | 0.0003 | 0.0012 | | | |
| | RM | 5 | 0.0040 | 0.0008 | | | |
| **Responnse Y3** | Model | dF | SS | MS | F | $R^2$ | F cal |
| Regression | FM | 5 | 118.673 | 23.734 | 1.713 | 0.7407 | 1.876 |
| | RM | 2 | 40.707 | 20.353 | 1.021 | 0.2541 | |
| Error | FM | 3 | 41.547 | 13.849 | | | |
| | RM | 6 | 119.513 | 19.918 | | | |
| **Responnse Y4** | Model | dF | SS | MS | F | $R^2$ | F cal |
| Regression | FM | 5 | 11.994 | 2.398 | 2.892 | 0.8282 | 2.996 |
| | RM | 2 | 4.541 | 2.270 | 1.370 | 0.3136 | |
| Error | FM | 3 | 2.487 | 0.829 | | | |
| | RM | 6 | 9.940 | 1.656 | | | |
| **Responnse Y5** | Model | dF | SS | MS | F | $R^2$ | F cal |
| Regression | FM | 5 | 28.335 | 5.667 | 5.053 | 0.8938 | 3.062 |
| | RM | 3 | 21.468 | 7.156 | 3.497 | 0.6772 | |
| Error | FM | 3 | 3.364 | 1.121 | | | |
| | RM | 5 | 10.231 | 2.046 | | | |

**Contour Plots and Response Surface Plots**

Two dimensional contour plots were constructed for all dependent variables i.e. GS, PDI, ZP and drug release at 15 minutes for Fenofibrate (DRF) and Atorvastatin Calcium (DRA) and shown in Figure 1, 2, 3, 4, and 5. Response surface plots are very helpful in learning about both the main and interaction effects of the independent variables.

**Globule size (GS)**

Figure 1 showed contour plot for globule size at prefixed values. The contour plot was found to be linear, thus the relationship between independent variables for GS could be linear.

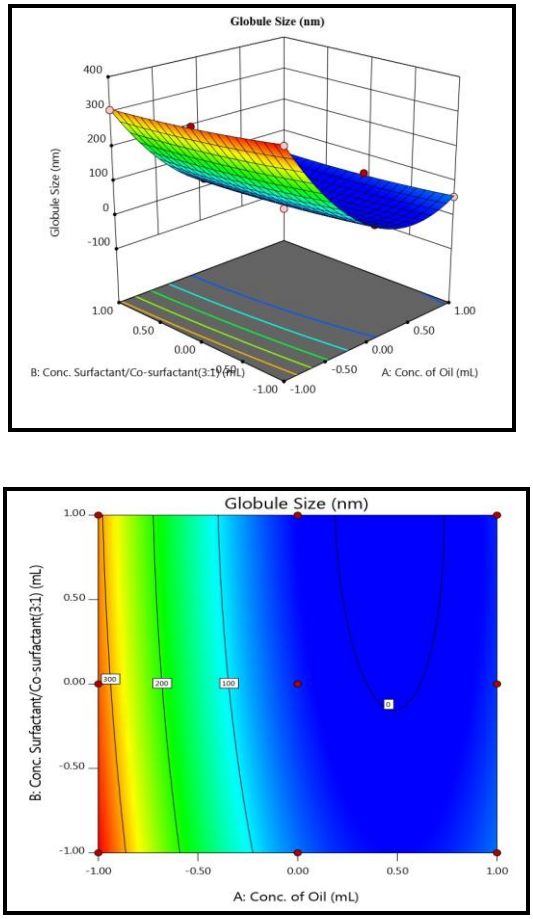
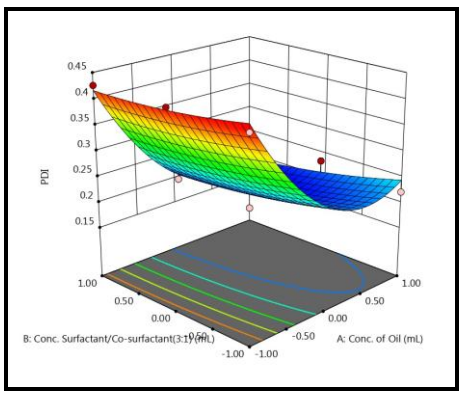



**Figure 1: Contour plot and 3D surface plot for the effect on globule size.**

The response surface plot showed decrease in globule size with increase in the conc. of Capmul MCM oil and conc. of Cremophor RH 40: Transcutol-P (3:1).

**Polydispersity index (PDI)**

Figure 2 showed contour plot for polydispersity index at prefixed values. The contour plot was found to be linear. Hence, the relationship between independent variables for polydispersity index could be linear.



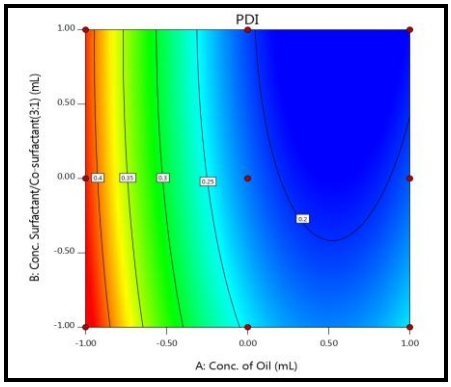
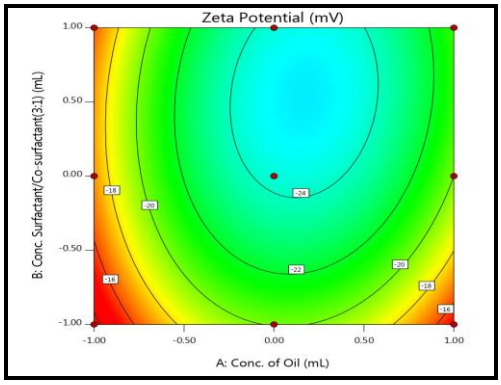



**Figure 2: Contour plot and 3D surface plot for the effect on PDI.**

The response surface plot showed decrease in polydispersity index with increase in the conc. of Capmul MCM oil and conc. of Cremophor RH 40: Transcutol-P (3:1).

**Zeta Potential (ZP)**

Figure 3 showed contour plot for zeta potential at prefixed values. The contour plot was found to be non-linear. Hence, the relationship between independent variables for zeta potential could be non-linear.



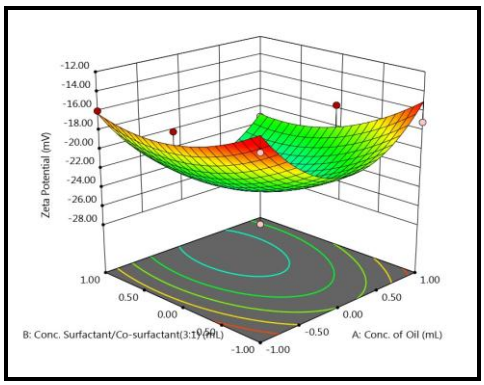




**Figure 3: Contour plot and 3D surface plot for the effect on Zeta Potential.**

The response surface plot showed decrease in zeta potential with increase in the conc. of Capmul MCM oil and conc. of Cremophor RH 40: Transcutol-P (3:1).

**Drug release at 15 minutes for Fenofibrate (DRF)**

Figure 4 showed contour plot for drug release at 15 minutes for Fenofibrate (DRF) at prefixed values. The contour plot was found to be non-linear. Hence, the relationship between independent variables for drug release at 15 minutes for Fenofibrate (DRF) could be non-linear.

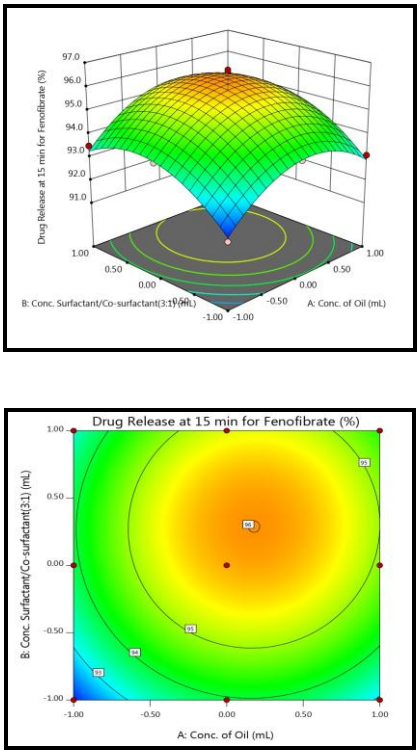



**Figure 4: Contour plot and 3D surface plot for the effect on Drug Release at 15 min for Fenofibrate.**

The response surface plot showed increase in drug release with increase in the conc. of Capmul MCM oil and conc. of Cremophor RH 40: Transcutol-P (3:1).

**Drug release at 15 minutes for Atorvastatin Calcium (DRA)**

Figure 5 showed contour plot for drug release at 15 minutes for Atorvastatin Calcium (DRA) at prefixed values of 92.75, 93.75, 94.75, and 95.5. The contour plot was found to be non-linear. Hence, the relationship between independent variables for drug release at 15 minutes for Atorvastatin Calcium (DRA) could be non-linear.

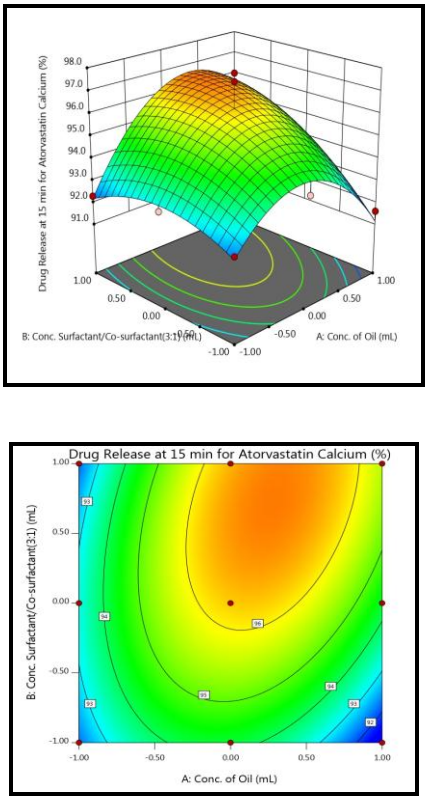






**Figure 5: Contour plot and 3D surface plot for the effect on Drug Release at 15 min for Atorvastatin Calcium.**

The response surface plot showed increase in drug release with increase in the conc. of Capmul MCM oil and conc. of Cremophor RH 40: Transcutol-P (3:1).

**Optimiztion of SNEDDS Formulation**

Optimized formulation was selected by arbitrarily fixing the criteria of 20.7 – 357nm of GS (minimize), 0.189 – 0.428 PDI (maximize), -30mV to -21mV ZP (is target = -27), more than 95% drug released at 15 minutes for Fenofibrate and Atorvastatin Calcium. The recommended concentrations of the independent variables were calculated by Design Expert software using overlay plot with desirability approach (Figure 6). The results gave one optmized solution with theoretical target profile characteristics which were shown in Table 6.

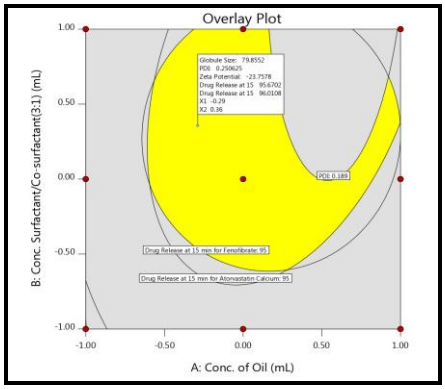



**Figure 6: Overlay plot for optimization of SNEDDS formulation.**

**Table 6: Solution proposed by Design Expert.**

| Sol. Run | Conc. of oil (mL) | Conc. of S:Co-s (3:1) (mL) | GS (nm) | PDI | ZP (mV) | Drug Release at 15 min for Fenofibrate | Drug Release at 15 min for Atorvastatin Calcium |
|---|---|---|---|---|---|---|---|
| 1 | 0.471 | 1.608 | 79.85 | 0.250 | -23.75 | 95.67 | 96.01 |

Figure 6 showed overlay plot obtained from Design Expert. Grayed area (Shaded areas) on the graphical optimization plot did not meet the selection criteria. Yellow area indicated the area in which the optimized formulation can be formulated. In yellow portion, the values of all variables i.e. GS, PDI, ZP, drug release at 15 minutes for Fenofibrate and Atorvastatin calcium were selected. The point indicating toggle flag showed the value of $X_1$ and $X_2$ for optimized formulation.

**Table 7: Optimized formulation of Fenofibrate and Atorvastatin Calcium SNEDDS (Batch OP1).**

| Material used | Quantity per Unit (mL) | Quantity per Unit (%) |
|---|---|---|
| Capmul MCM oil | 0.471 | 23.62% |
| Cremophor RH 40 | 1.206 | 57.27% |
| Transcutol-P | 0.402 | 19.11% |

**Check point batch analysis**

Two different check point batches (C1 and C2) of Fenofibrate and Atorvastatin Calcium SNEDDS were prepared (Table 8). The experimentally and theoretically computed values of GS, PDI, ZP, drug release at 15 minute for fenofibrate and Atorvastatin Calcium were presented and compared using student 't' test, the difference was found to be non-significant ($p<0.05$) in both cases.

**Table 8: Composition and results of Check point batches.**

| Batches | C1 | | C2 | |
|---|---|---|---|---|
| $X_1$ | 0.5 | | -0.5 | |
| $X_2$ | -0.5 | | 0.5 | |
| **Response** | **Predicted** | **Experimental** | **Predicted** | **Experimental** |
| Globule size (nm) [$Y_1$] | 132.39 | 127.3 | 7.42 | 11.56 |
| Polydispersity index [$Y_2$] | 0.288 | 0.291 | 0.203 | 0.194 |
| Zeta potential (mV) [$Y_3$] | -22.42 | -22.15 | -21.76 | -20.33 |
| Drug release at 15 minutes for Fenofibrate [$Y_4$] | 95.26 | 95.6 | 95.06 | 93.9 |
| Drug release at 15 minutes for Atorvastatin Calcium [$Y_5$] | 95.35 | 95.8 | 94.81 | 93.6 |

**Stability study of optimized SNEDDS formulation**

Stability study of optimized batch (OP1) was conducted up to 6 months at room temperature and accelerated condition of 40°C & 75% RH.

The GS, ZP, drug content and drug release at 15 minutes for Fenofibrate and Atorvastatin calcium were analysed at initial and after 1, 3 and 6 months. The results were recorded in Table-9-11.

**Table 9: Globule size and Zeta Potential of optimized batch at storage conditions.**

| | Average of Globule Size (nm) | | Zeta Potential (mV) | |
|---|---|---|---|---|
| **Storage Conditions** | **Room Temperature** | **Accelerated Conditions** | **Room Temperature** | **Accelerated Conditions** |
| **Initial** | 78.3 | 78.3 | -23.13 | -23.13 |
| **1 Month** | 79.2 | 79.8 | -22.38 | -22.45 |
| **3 Month** | 82.1 | 83.3 | -22.24 | -22.92 |
| **6 Month** | 82.5 | 83.9 | -21.79 | -21.47 |

**Table 10: Drug content of optimized batch at storage conditions.**

| | % Assay (±) SD | | | |
|---|---|---|---|---|
| | (Fenofibrate) | | (Atorvastatin Calcium) | |
| **Storage Conditions** | **Room Temperature** | **Accelerated Conditions** | **Room Temperature** | **Accelerated Conditions** |
| **Initial** | 100.2 ± 0.46 | 100.2 ± 0.46 | 100.4 ± 0.25 | 100.4 ± 0.25 |
| **1 Month** | 100.1 ± 0.27 | 100.0 ± 0.58 | 99.6 ± 0.46 | 99.4 ± 0.63 |
| **3 Month** | 99.7 ± 0.52 | 99.4 ± 0.65 | 99.3 ± 0.46 | 99.1 ± 0.82 |
| **6 Month** | 99.4 ± 0.38 | 99.1 ± 0.62 | 98.7 ± 0.58 | 98.3 ± 0.28 |

**Table 11: Drug content of optimized batch at storage conditions.**

| | % Drug release at 15 minutes | | | |
|---|---|---|---|---|
| | (Fenofibrate) | | (Atorvastatin Calcium) | |
| **Storage Conditions** | **Room Temperature** | **Accelerated Conditions** | **Room Temperature** | **Accelerated Conditions** |
| **Initial** | 96.2 ± 1.2 | 96.2 ± 1.2 | 97.1 ± 1.2 | 97.1 ± 1.2 |
| **1 Month** | 97.4 ± 1.7 | 97.1 ± 1.6 | 97.4 ± 1.7 | 97.1 ± 1.6 |
| **3 Month** | 97.7 ± 2.4 | 96.9 ± 2.1 | 97.7 ± 2.4 | 96.9 ± 2.1 |
| **6 Month** | 96.4 ± 1.9 | 96.1 ± 2.5 | 96.4 ± 1.9 | 96.1 ± 2.5 |

The results reveled the absence of any significant change in SNEDDS with respect to GS, ZP, drug content and drug release at 15 minutes for Fenofibrate and Atorvastatin calcium. It indicated that optimized SNEDDS remained stable during the storage conditions.

**Comparison of in vitro drug release between optimized batch, pure drug powder, and marketed product**

The Fenofibrate and Atorvastatin Calcium release profile of optimized batch was compared with pure drug powder and marketed capsule product. The marketed product was FENOSTAT of Ordain Health Care Global Pvt Ltd. which is a conventional capsule formulation.

**Table 12: Comparison of Fenofibrate release profile of batch OP1 with pure drug and Fenostat.**

| **Batches** | **% Drug Release (Fenofibrate) (Mean ± SD)** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Time (Minutes)** | | | | | | |
| | **0** | **10** | **15** | **20** | **30** | **45** | **60** |
| OP1 | 0±0 | 92.1±1.5 | 96.2±1.2 | 99.7±1.1 | 100.1±0.2 | 99.6±0.1 | 99.7±0.3 |
| Pure drug | 0±0 | 7.3± 0.7 | 17.6±0.8 | 19.1±0.5 | 27.4±1.1 | 38.7±0.4 | 48.2±0.5 |
| Fenostat | 0±0 | 14.1±0.2 | 22.8±0.7 | 23.2±0.8 | 32.4±0.5 | 47.5±1.4 | 59.3±1.4 |

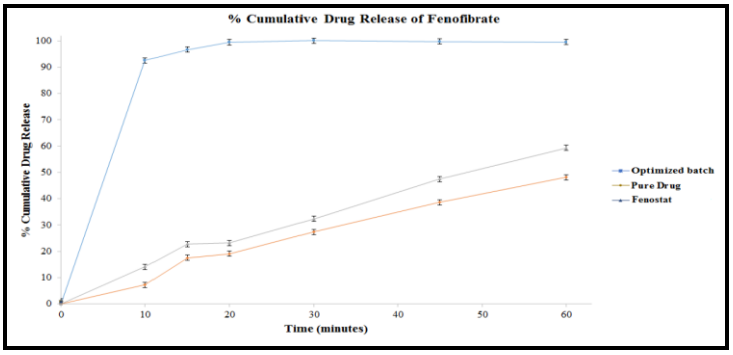



**Figure 7: Comparison of drug release profile of optimized batch with pure drug and Fenostat (Fenofibrate).**

**Table 13: Comparison of Atorvastatin Calcium release profile of batch OP1 with pure drug and Fenostat.**

| Batches | % Drug Release (Atorvastatin Calcium) (Mean ± SD) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Time (Minutes) | | | | | | |
| | 0 | 10 | 15 | 20 | 30 | 45 | 60 |
| OP1 | 0±0 | 92.4±1.5 | 97.1±1.2 | 99.3±1.1 | 100.0±0.2 | 99.8±0.1 | 99.7±0.3 |
| Pure drug | 0±0 | 6.3± 1.7 | 13.3±0.9 | 18.7±0.8 | 26.6±1.3 | 36.7±0.7 | 46.5±0.5 |
| Fenostat | 0±0 | 13.6±1.2 | 18.9±0.8 | 24.7±0.7 | 31.6±0.5 | 46.5±1.1 | 57.6±1.0 |

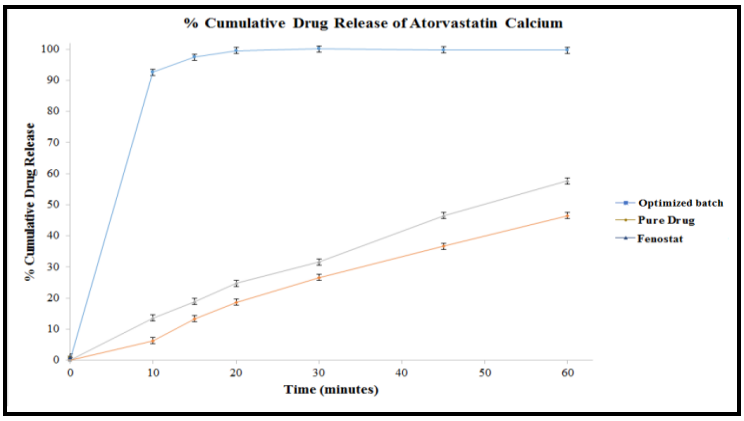



**Figure 8: Comparison of drug release profile of optimized batch with pure drug and Fenostat (Atorvastatin Calcium).**

Drug release from the optimized batch (OP1) was found to be better as compared with that of pure drug powder and marketed drug formulation (Figure 7, 8). It could be suggested that the optimized SNEDDS batch resulted in spontaneous formation of a nanoemulsion with a small globule size, which permitted a faster drug release into the aqueous phase than that of pure drug powder and marketed formulation. Thus, the greater availability of dissolved Fenofibrate and Atorvastatin Calcium from the SNEDDS formulation could lead to higher absorption and higher oral bioavailability.

## CONCLUSION

SNEDDS are isotropic mixtures made up of oil, surfactant and sometimes cosurfactant or cosolvent. In an aqueous environment a homogeneous, transparent (or at least translucent), isotropic and thermodynamically stable dispersion will result up on mild agitation. SNEDDS is best suited for dosage for development of poorly soluble drugs. Fenofibrate and Atorvastatin Calcium are BCS class II drugs having low solubility and high permeability. The present study was aimed to explore stable SNEDDS formulation development using $3^2$ factorial design for dissolution improvement compared to marketed formulation of Fenofibrate and Atorvastatin Calcium. On the basis of results of preliminary trials, Capmul

MCM as oil, Cremophor RH 40 as surfactant and Transcutol-P as cosurfactant were used for formulation development of Fenofibrate and Atorvastatin Calcium SNEDDS. The $3^2$ factorial design was employed using concentration of Capmul MCM oil and Cremophor RH 40: Transcutol-P (3:1) as independent variables. The Globule size (GS), Zeta potential (ZP), Polydispersity index (PDI) and drug release at 15 minute for Fenofibrate and Atorvastatin Calcium were selected as dependent variables. Multiple regression analysis, contour plot and response surface plot were used to study the main and interaction effects of the variables on the responses. Simple linear equation, or interactive equation or quadratic model was fitted by carrying out multiple regression analysis and F-statistic to identify statistically significant term. The optimized batch was selected on the basis arbitrary criteria using Design Expert employing overlay plot with desirability approach. The batch containing 0.471ml of Capmul MCM oil, 1.608ml of Cremophor RH 40: Transcutol-P (3:1) was selected as optimized SNEDDS formulation. The check point batches were prepared to validate the evolved equations. The optimized formulation was subjected to in vitro dissolution to evaluate drug release as compared to marketed product. The stability study for optimized batch was conducted at room temperature and 40°C & 75% RH. The optimized formulation was found stable and more than 90% drug dissolution was achieved within 15 minutes. The desirable goals can be achieved by systematic formulation approach in shortest possible time with reduced number of experiments for formulation development of Fenofibrate and Atorvastatin Calcium SNEDDS formulation using factorial design.

**ACKNOWLEDGEMENTS**

We are thankful to Dr. M.C. Gohel and Dr. Krutika Sawant for their guidance and suggestions in the present research work.

**REFERENCES**

1. Hauss DJ, Fogal SE, Ficorilli JV, Price CA, Roy T, Jayaraj AW, Keirns JJ. Lipid-based delivery systems for improving the bioavailability and lymphatic transport of a poorly water-soluble LTB4 inhibitor. J Pharm Sci. 1997; 87(2): 164–169.
2. Strickley RG. Currently marketed oral lipid-based dosage forms: Drug products and excipients. In: Hauss D, editor. Lipid-based formulations for oral delivery: Enhancing bioavailability of poorly water-soluble drugs. Informa Healthcare New York; 2007; 1–31.
3. Parke-Davis. Lipitor (atorvastatin calcium) tablets prescribing information. New York, NY; 2007.

4. Abbott Laboratories. TriCor (fenofibrate) tablets prescribing information. North Chicago, IL; 2004.
5. Kwang KK, Michael JQ, Seung HH, Wook JC, Jeong YA, Yiel HS, Eak KS. Additive Beneficial Effects of Fenofibrate Combined With Atorvastatin in the Treatment of Combined Hyperlipidemia, Journal of American College of Cardiology, 2005; 45(10): 1649-1653.
6. Limbani MD, Patel LD. Studies on Drug Solubilization and Role of Lipid Vehicle in Pseudo Ternary Phase Diagram in Formulation Development of SNEDDS containing Poorly Water Soluble drugs, Int. J. Pharm. Sci. Rev. Res., 2016; 40(2): 228-237.
7. Dabhi MR, Limbani MD, Sheth NR. Preparation and In Vivo Evaluation of Self-Nanoemulsifying Drug Delivery System (SNEDDS) Containing Ezetimibe, Current Nanoscience, 2011; 7(4): 616-627.
8. Gulshan C, Krishna C, Anil M, Kamla Pathak, Design and development of nanoemulsion drug delivery system of amlodipine besylate for improvement of oral bioavailability, Drug Dev. Ind. Pharm., 2011; 37(8): 907–916.
9. Deepan T, Paul Ambethkar K, Vijya Lakshmi G, Dhanaraju M. Analytical Method Development and Validation of RP-HPLC for Estimation of Atorvastatin Calcium and Fenofibrate in Bulk drug and Tablet dosage forms, Euro. J. App. Sci., 2011; 3(2): 35-39.
10. Vivek B, Hema N, Darshana H. Design and Evaluation of Self-Microemulsifying Drug Delivery System (SMEDDS) of Tacrolimus, AAPS Pharm Sci Tech, 2008; 9(1): 13-21.