*Drug Development and Industrial Pharmacy*, 33:927–931, 2007

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040601003733

informa
healthcare

# Application of Mixture Experimental Design to Simvastatin Apparent Solubility Predictions in the Microemulsifion Formed by Self-Microemulsifying

**Jian Meng and Liangyuan Zheng**
*Department of Pharmaceutics, Shenyang Pharmaceutical University, Shenyang, Liaoning, P.R. China*

**Self-microemulsifying drug delivery systems (SMEDDS) are useful to improve the bioavailability of poorly water-soluble drugs by increasing their apparent solubility through solubilization. However, very few studies, to date, have systematically examined the level of drug apparent solubility in o/w microemulsion formed by self-microemulsifying. In this study, a mixture experimental design was used to simulate the influence of the compositions on simvastatin apparent solubility quantitatively through an empirical model. The reduced cubic polynomial equation successfully modeled the evolution of simvastatin apparent solubility. The results were presented using an analysis of response surface showing a scale of possible simvastatin apparent solubility between 0.0024 ~ 29.0 mg/mL. Moreover, this technique showed that simvastatin apparent solubility was mainly influenced by microemulsion concentration and, suggested that the drug would precipitate in the gastrointestinal tract due to dilution by gastrointestinal fluids. Furthermore, the model would help us design the formulation to maximize the drug apparent solubility and avoid precipitation of the drug.**

**Keywords** self-microemulsifying; simvastatin; apparent solubility; mixture experimental design

## INTRODUCTION

The low water solubility of lipophilic drugs always results in poor and variable drug absorption after oral administration (Gursoy et al., 2004; Gershanik et al., 2000). However, the concentration of the drug in the gastrointestinal fluids may be increased by orders of magnitude by means of self-microemulsifying, the basic principle of which is to self-microemulsify rapidly in the gastro-intestinal fluids and form o/w microemulsion under the gentle agitation given by gastrointestinal motion. In such a system, the lipophilic drug is present in microemulsion. The large interfacial area promotes drug to diffuse into intestinal fluids quickly (Charman, 1992).

Address correspondence to Liangyuan Zheng, Department of Pharmaceutics, Building 4, Lane 899, Zuchongzhi Rd., Pudong District, Shanghai 201203, P.R. China. E-mail: goodwill_123@163.com

In spite of their potential as drug delivery vehicles, very few studies, to date, have systematically examined the level of drug solubilized in o/w microemulsion. This was rather surprising since Self-microemulsifying drug delivery systems (SMEDDS) could improve the bioavailability of poorly water-soluble lipophilic drugs by increasing their apparent aqueous solubility (Constantinides, 1995). However, the microemulsion concentration would decrease after it was diluted by gastrointestinal fluid so that the drug would precipitate in gastrointestinal tract (Gao, 2003). This would limit the bioavailability enhancement.

In this study, a mixture experimental methodology was set up since it had been proven to be an efficient approach for solving such a kind of formulation challenge (Béatrice et al., 2003; Gao et al., 2004). Simvastatin, a lipophilic drug, was selected as a model drug on the basis of Kang Bok Ki's report (Kang et al., 2004), in which the bioavailability of simvastatin-SMEDDS was enhanced but the drug solubility study was excluded. Therefore, planned mixture experiments were performed in order to establish an empirical model which could estimate simvastatin apparent solubility as a function of composition factors such as microemulsion concentration and the surfactant/oil ratio. This step led to define a polynomial equation estimating the response variable (simvastatin apparent solubility) as a function of the compositions. The value of this model representing the response surface was subsequently determined.

## MATERIALS AND METHODS

### Materials

Simvastatin was purchased from Hisun pharmaceutical Co., Ltd. (China). Cremophor EL® (polyoxyl 35 castor oil) was gifted from BASF (Germany). The Labrafac CC® (caprylic and capric triglycerides) and Lauroglycol 90® (propylene glycol monolaurate 90%) were obtained from Gattefosse (France). Deionized water was prepared by ultrapure water system from Liyuan Co., Ltd. (China). Acetonitrile (HPLC grade, Dima, USA.) was used in the present study.

## Pseudo-Ternary Phase Diagram Study

Labrafac CC® (L-CC) was mixed with Lauroglycol 90® (L-90) in a fixed weight ratio (6:4) and then aliquots of the L-CC/L-90 mixture (L-CC/L-90) mixed with Cremophor EL® (Cre EL). Finally each mixture was titrated with simulated gastrointestinal fluid (SGF) containing 0.05 M NaCl and 0.01 M HCl (pH 2.0) and mixed by magnetic stirring at 37°C until equilibrium was reached. The equilibrated samples were assessed visually and determined as being clear and transparent microemulsions, or crude emulsions or gels (Gao, 1998).

The physical states were represented on a pseudo-ternary diagram (Figure 1) with one axis representing SGF, another representing Cre EL and the third representing L-CC/L-90. According to the proportions of the components, the system showed complete different phases, which were indicated on the pseudo-ternary phase diagram. The microemulsion region was selected as the restricted domain for further solubility study.

## Methodology of Experimental Design

To explore simvastatin apparent solubility, a mixture experimental design was set up within the restricted domain. In this specific case, the restricted experimental region was represented by a subregion from the pseudo-ternary phase diagram, namely, a triangle according to the constraints on the component proportions (Figures 1 and 2). In this case, three variables, corresponding to the amount of SGF, Cre EL, and L-CC/L-90, respectively $X_1$, $X_2$, and $X_3$ were considered. These were also some so-called nonindependent variables subject to the constraint that their sum is 100% (Table 1). The number of experiments to be carried out depends on the number of coefficients to be estimated (Lewis et al, 1999). Consequently, 17 experiments were selected within the restricted domain (Figure 2, Table 2), which allowed for the fitting of a reduced cubic model, a check for lack of fit and an estimate of experimental error. The experiments were carried out randomly to minimize systematic errors and the experimental results were analyzed with design –expert® software (version 6).

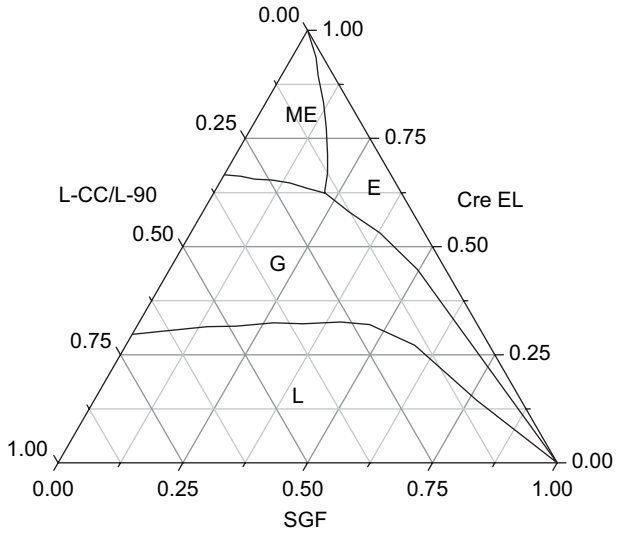


FIGURE 1. Pseudo-ternary diagram composed of Cre EL, L-CC/L-90 and SGF. (ME, o/w microemulsion; E, crude emulsion; G, gel; L, isotropic region).

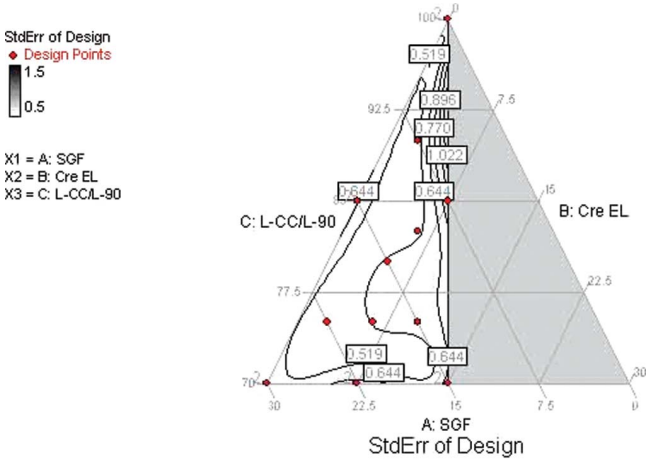


FIGURE 2. Schematic of three-component mixture experimental design. (Dots with "2" indicate points that were repeated).

TABLE 1
Experimental Ranges for Independent Variables and Constraints

| | Experimental Ranges | | |
|---|---|---|---|
| Factors | Low Values (%) | High Values (%) | Constraints |
| $X_1$ (percent of SGF) | 70 | 100 | $X_3/X_2<1.00$ |
| $X_2$ (percent of Cre EL) | 0 | 30 | $X_1+$ |
| $X_3$ (percent of L-CC/L-90) | 0 | 30 | $X_2+X_3=100$ |

## Preparation of Model Formulations

A series of SMEDDS were prepared as shown in Table 2. Briefly, the mixing oil, L-CC/L-90, and surfactant, Cre EL, were accurately weighed into glass vials and the components were mixed well by gentle stirring at 37°C in water bath. Then, the proper amount of simvastatin was added and the system was stirred continuously until simvastatin was completely dissolved. The mixture was stored at room temperature.

## HPLC Analysis of Simvastatin

The HPLC analysis of simvastatin was slightly modified based on the literature (Serajuddin et al., 1991). The instruments

TABLE 2
Three-component Mixture Design and Results

| Standard Order | Random Order | SGF (%) | Cre-EL (%) | L-CC/L-90 (6:4) (%) | Solubility (mg/mL) | Comments |
|---|---|---|---|---|---|---|
| 1 | 7 | 85.00 | 15.00 | 0.00 | 8.43 | Identical to Std. #17 |
| 2 | 10 | 70.00 | 15.00 | 15.00 | 20.08 | Identical to Std. #15 |
| 3 | 9 | 80.00 | 15.00 | 5.00 | 15.60 | |
| 4 | 12 | 100.00 | 0.00 | 0.00 | 0.0024 | Identical to Std. #13 |
| 5 | 5 | 70.00 | 30.00 | 0.00 | 14.42 | Identical to Std. #14 |
| 6 | 8 | 85.00 | 7.50 | 7.50 | 21.98 | |
| 7 | 11 | 70.00 | 22.50 | 7.50 | 24.52 | Identical to Std. #16 |
| 8 | 2 | 75.00 | 22.50 | 2.50 | 20.60 | |
| 9 | 1 | 90.00 | 7.50 | 2.50 | 2.13 | |
| 10 | 13 | 75.00 | 15.00 | 10.00 | 19.78 | |
| 11 | 6 | 75.00 | 18.75 | 6.25 | 17.75 | |
| 12 | 14 | 82.50 | 11.25 | 6.25 | 15.18 | |
| 13 | 17 | 100.00 | 0.00 | 0.00 | 0.00246 | |
| 14 | 4 | 70.00 | 30.00 | 0.00 | 13.85 | |
| 15 | 15 | 70.00 | 15.00 | 15.00 | 21.05 | |
| 16 | 3 | 70.00 | 22.50 | 7.50 | 23.07 | |
| 17 | 16 | 85.00 | 15.00 | 0.00 | 7.90 | |

consisted of a RP-C18 5-μm column (250 × 4.6 mm) (Dikma, Beijing, China), LC-10$AT_{VP}$ pump, SPD-10$A_{VP}$ UV-Vis detector, and an integrator Shimadzu C-R6A chromatopac (Shimadzu, Japan). The mobile phase consisted of a mixture of acetonitrile-$H_2O$ (80:20, v/v), the flow-rate was 1 mL/min and the wave-length was set at 238 nm. The standard linear calibration curve was applied and the linear relationship in the concentration range of 0.0012–0.024 mg/mL was attained.

### Determination of Simvastatin Apparent Solubility in Microemulsion

The self-emulsifying formulations and SGF were accurately weighed into glass vials which were then tightly closed and stirred in a water bath at 37°C to equilibrate for 24 h. Excess drug were separated by 5 min centrifugation at 2000*g*. Of the supernatant solutions, 0.5 mL was properly diluted and then subjected to HPLC analysis. The drug apparent solubility in microemulsion was calculated according to the standard linear calibration.

## RESULTS AND DISCUSSION

Phase studies were performed to define the restricted domain of the experimental design. According to the system compositions, different phases have been shown on the phase diagram as shown in Figure 1. SMEDDS formed oil in water microemulsion with gentle stirring, upon being introduced into aqueous media. Since the free energy of the microemulsion is very low, the formation is thermodynamically spontaneous. Cremophor EL formed a layer around the droplet of microemulsion, which not only reduced the interfacial energy but also provided a mechanical barrier to coalescence. The ratio of Labrafac CC and Lauroglycol 90 was set at 6:4 (w/w) because at this ratio the maximum proportion of oil was incorporated in microemulsion according to our experiments (data not shown). Generally, high proportion of oil in microemulsion may result in high solubilization for poorly water-soluble drugs. However, no microemulsion was formed when SMEDDS of high proportion of oil were diluted. Therefore, only the SMEDDS with the ratio of L-CC/L-90 to Cre EL lower than 1:1 were studied. According to the proportions of the components in the microemlsion region, the domain was defined. It was described as a triangle which composed of SGF (70 ~ 100%), Cre EL (0 ~ 30.0%), and L-CC/L-90 (0 ~ 15.0%). The ratio of L-CC/L-90 to Cre EL was kept lower than 1:1 (Figure 2, Table 1). The domain covered most of the microemulsion region and the experiments were selected within this domain.

Table 2 shows the results in term of simvastatin apparent solubility corresponding to the 17 experiments carried out. It should be noted that microemulsion equilibrated with excess solid drug was obtained after centrifugation before analysis. This identified that the presence of simvastatin did not affect the stability of microemulsion and the system was saturated with simvastatin. As shown in Table 2, the wide variation of response (S) indicated that the different compositions resulted in different drug apparent solubility. The analysis of response variables was carried out with experimental design software. As shown in Table 3, the reduced cubic model with a natural

TABLE 3
Model Summary Statistics

| Source | SD | $R^2$ | Adjusted $R^2$ | Predicted $R^2$ | PRESS |
|---|---|---|---|---|---|
| Linear | 1.74 | 0.6941 | 0.6504 | 0.4912 | 70.64 |
| Quadratic | 0.42 | 0.9862 | 0.9799 | 0.9666 | 4.64 |
| Special cubic | 0.041 | 0.9999 | 0.9998 | 0.9996 | 0.059 |
| Cubic | 0.034 | 0.9999 | 0.9999 | 0.9998 | 0.034 |

log transform was the most suitable to approximate to response value (S) due to its small standard deviation and predicted residual sum of squares (PRESS), as well as large predicted R-squared. The coefficients of the reduced cubic model were estimated from the results of the experiments, and the model with natural log transform was as follows:

$$\mathrm{Ln(S)} = -0.0601X_1 - 0.952X_2 - 3.24X_3 + 0.0169X_1X_2 + 0.0487X_1X_3 + 0.207X_2X_3 - 0.00288X_1X_2X_3$$

As shown in Table 4, the *p* value of the lack of fit of the model was 29.30, which implied that the lack of fit is not significant relative to the pure error. In order to evaluate the adequacy of the model, an analysis of the residuals was performed. Figure 3 shows the analysis of the residuals obtained from the difference between the predicted simvastatin apparent solubility and the observed solubility. The residuals were normally distributed. Therefore, it can be concluded that the reduced cubic model fits the observed simvastatin apparent solubility.

Figure 4 corresponds to the plotting of the response surface inside the experimental domain, where the effect of each component on the drug apparent solubility could be determined. Each line or plateau of simvastatin apparent solubility descends from bottom to top sharply which indicate that the percentage of SGF or the microemulsion concentration is a major factor to affect the drug solubility. When SGF proportion was increased, simvastatin apparent solubility strongly decreased. This phenomenon could be explained by the solubilization of

TABLE 4
ANOVA and Related Statistic for Simvastatin Solubilization

| Source | Sum of Squares | DF | Mean Square | *F* value | *p*-value |
|---|---|---|---|---|---|
| Model | 138.83 | 6 | 23.14 | 13980.98 | <0.0001 |
| Residual | 0.017 | 10 | 0.0017 | | |
| Lack of fit | 0.01 | 5 | 0.002 | 1.67 | 0.2930 |
| Pure error | 0.0062 | 5 | 0.0012 | | |
| Cor total | 138.85 | 16 | | | |

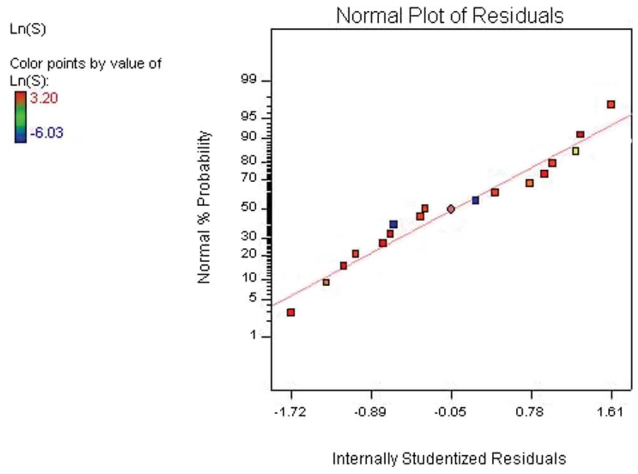


FIGURE 3. Normal probability plot of the residuals obtained from the reduced cubic model for simvastatin apparent solubility.

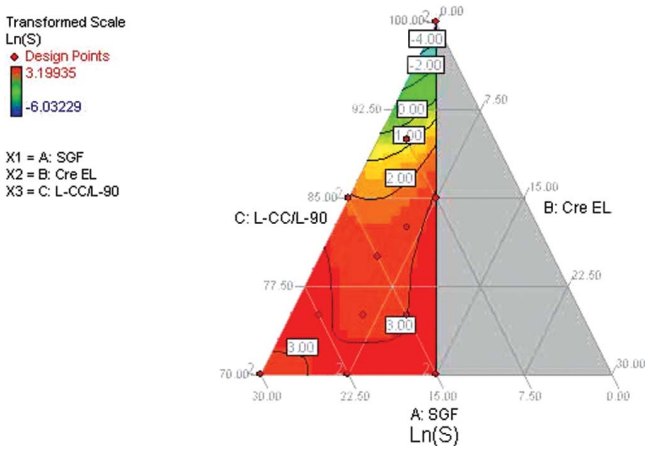


FIGURE 4. The triangular-dimensional contour diagrams illustrating the effects of SGF ($X_1$), Cre EL ($X_2$) and L-CC/L-90 ($X_3$) on simvastatin apparent solubility.

microemulsion, which contributed significantly to the lipophilic drug solubility. Microemulsion concentration decreased as the amount of SGF in the system increased. Therefore some simvastatin precipitated out from the system. This phenomenon suggested that simvastatin would also precipitate in gastrointestinal tract as the SMEDDS were diluted by gastrointestinal fluids. Figure 4 also shows the response, i.e. simvastatin apparent solubility, increases as oil increases in the microemulsion when the microemulsion concentration is low. This may be explained by the fact that the oil incorporated in the microemuslion improves solubilization for the drug. However, simvastain apparent solubility decreases to a minimal value as the oil increases in the microemulsion when the microemulsion concentration is high. The similar results were

TABLE 5
Verification of the Model Predictions (Three Test Points)

| | Weight Fraction of Excipient | | | Simvastatin Solubility (mg/mL) | | |
|---|---|---|---|---|---|---|
| | SGF (%) | Cre EL (%) | L-CC/L-90 (%) | S Estimated (CI)[a] | S Measured | CV (%)[b] |
| 1 | 82.50 | 15.00 | 2.50 | 2.44 (2.39–2.48) | 2.47 | −1.21 |
| 2 | 85.00 | 11.25 | 3.75 | 2.09 (2.06–2.13) | 2.07 | 0.97 |
| 3 | 78.75 | 13.12 | 8.13 | 2.95 (2.90–3.00) | 2.96 | −0.34 |

[a]CI: confidence interval.
[b]CV: coefficient of variance.

also found by Malcolmson etc. (Malcolmson et al., 1998) who attributed these findings to the change in the structure of the microemuslion at high oil level.

In order to verify the model predictions, three additional experiments selected from the restricted domain were carried out, and the comparison of simvastatin apparent solubility estimated based on the model with that observed are listed in table 5. Values of the test points fall in the confidence intervals for the given statistical significance ($\alpha$ = 5%). Thus, it is a good method obtained to estimate simvastatin apparent solubility in the microemlsion with a good reproducibility.

At this point, the pharmaceutical potentialities of the SMEDDS were evaluated using previous results. The SMEDDS have been described as a useful dosage form to improve the oral bioavailability of poorly water-soluble drugs. However, this function may be reduced by the drug precipitation as the SMEDDS are diluted by gastrointestinal fluid after oral administration. The proposed mathematical tool helps us select the formulation to avoid the drug precipitation. It is obvious that the drug in the microemulsion is reduced as it is diluted, though its volume is increased. Therefore it is wise to design the formulation according to the drug solubility in the diluted system (such as 0.2% microemulsion concentration provided that the weight of SMEDDS and the volume of the gastrointestinal fluids are 0.5 g and 250 mL, respectively) so as to maximize simvastatin apparent solubility and avoid the drug precipitation in gastrointestinal tract.

## CONCLUSION

Finally, the modeling of simvastatin apparent solubility in the micoemulsion was possible using a reduced cubic polynomial equation for each factor. The model led us to predict the quantitative influence of each component on simvastatin apparent solubility. Moreover, the results showed a considerable effects of SGF and L-CC/L-90 on simvastatin apparent solubility and a considerable decrease in simvastatin apparent solubility due to a decrease in microemuslion concentration as it was diluted. Furthermore the model also recommends us to design the formulation according to simvastatin apparent solubility in the diluted system so as to maximize the drug solubility, and avoid the drug precipitation in gastrointestinal tract.

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