Formulation, Characterization and Optimization of Valsartan Self-Microemulsifying Drug Delivery System Using Statistical Design of Experiment

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The aim of the present research was to systematically investigate the main, interaction and the quadratic effects of formulation variables on the performance of self-microemulsifying drug delivery system (SMEDDS) of valsartan using design of experiment. A 17-run Box–Behnken design (BBD) with 3-factors and 3-levels, including 5 replicates at the centre point, was used for fitting a 2nd-order response surface. After the preliminary screening, Labrafil M 2125 CS as oil, Tween 20 as surfactant and Capryol 90 as co-surfactant were taken as independent variables. The dependent factors (responses) were particle size, polydispersity index (PDI), dissolution after 15 min and equilibrium solubility. Coefficients were estimated by regression analysis and the model adequacy was checked by an *F*-test and the determination coefficient (R^2). All the responses were optimized simultaneously by using desirability function. Our results demonstrated marked main and interaction effects of independent factors on responses. The optimized formulation consisted of 26.8% (w/w) oil, 60.1% (w/w) surfactant and 13.1% (w/w) co-surfactant, and showed average micelle size of 90.7 nm and 0.246 PDI, 91.2% dissolution after 15 min and 226.7 mg/g equilibrium solubility. For the optimized formulation, predicted value and experimental value were in close agreement. After oral administration, the optimized formulation gave more than 2-fold higher area under curve (*AUC*) and about 6-fold higher C_{max} in rats than valsartan powder ($p<0.05$). The BBD facilitated in the better understanding of inherent relationship of formulation variables with the responses and in the optimization of valsartan SMEDDS in relatively time and labor effective manner.

Key words Box–Behnken design; design of experiment; desirability function; optimization; valsartan; self-microemulsifying drug delivery system

During early 2000s, formulators and regulators realized that suboptimal quality management and resource-intensive regulatory barriers were hampering the continuous improvement in formulation manufacturing.[1,2] In order to improve and streamline the outmoded regulatory processes, U.S. Food and Drug Administration (FDA) and equivalent agencies worldwide have been emphasizing in the implementation of Quality by design (QbD) approach.[1] The QbD approach requires comprehensive understanding of the formulation and manufacturing processes for the identification and selection of critical quality attributes and process parameters that can be fine-tuned and controlled to consistently produce quality drug products.[3] The core element of QbD is 'design space' which is defined by the FDA/International Conference on Harmonization (ICH) as "the multidimensional combination and interaction of input variables (*e.g.*, material attributes) and process parameters that have been demonstrated to provide assurance of quality."[4] Once the design space is identified, movement within it is not considered change and doesn't require regulatory post approval.[4] However, defining and determining the design space for a particular formulation is prohibitively cost, labor, and time intensive *via* traditional and intuitive one-factor-at-a-time (OFAT) method in which factor influence is studied by changing one factor or variable at a time while keeping all other factors constant. Simultaneous changes in multiple factors may produce interactions that are difficult to break up into individual effects. OFAT method cannot assess factor interactions and cannot encompass the entire feasible factor space or design space.[5] Therefore, the FDA recommends that the design space be determined by using statistical Design of Experiments (DoE).[4]

DoE is one of the core tenets of QbD initiative. DoE, a matrix based multi-factor method constructs useful predictive model of all critical responses and allows all potential variables to be identified and evaluated simultaneously, systematically and rapidly. DoE approach as a systematic alternative to single-variable experimentation also helps to optimize the levels of each critical variable and comes up with the best possible (optimum) combination of excipients and the processes within the total multidimensional experimental region.[6] This is in contrast to the traditional OFAT method of optimization which doesn't take interaction and quadratic effects of formulation and/or process variables into consideration and is generally based on trial-and-error method. DoE is also used in testing robustness of the manufacturing process.[5] Hence, the value of DoE for screening, investigating and optimizing experimental parameters, minimizing operational cycle times, including time to obtain regulatory approval, and direct cost saving cannot be disputed.

In the present study, self-microemulsifying drug delivery system (SMEDDS) of valsartan, a Biopharmaceutical Classification System (BCS) class II, antihypertensive drug was chosen as a formulation system and Box–Behnken design (BBD), a statistical DoE which uses response surface methodology (RSM) was used to understand and optimize the formulation system. Self-microemulsifying drug delivery system

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(SMEDDS) has been extensively investigated to overcome the poor and variable oral bioavailability of drugs attributed primarily to poor solubility and dissolution, and low gut permeability.[7] SMEDDS comprises of non-aqueous components of microemulsion: natural or synthetic oil(s), surfactant(s) and co-surfactant(s) or co-solvent(s) incorporated with lipophilic drug in suitable proportion. It readily disperses upon dilution in gastrointestinal (GI) fluid even with mild agitation (provided by gastric motility) and forms fine, microheterogeneous, thermodynamically stable oil–water microemulsion (20–200 nm).[8] Microemulsion formed presents lipophilic drugs to GI tract in highly solubilized form.[9,10] Besides, the lipid component of SMEDDS can increase the translymphatic drug transport while surfactant component of SMEDDS can increase the transcellular permeability of GI membrane by disrupting the structural organization of the lipid bilayer, thereby increasing the bioavailability.[11,12] Examples of some successful commercialized SMEDDS formulations include cyclosporine (Neoral), ritonavir (Norvir), saquinavir (Fortovase).[13,14]

Here, SMEDDS of valsartan was characterized and formulated by using BBD. BBD combines two level factorial designs with incomplete block designs. It excludes the corners where all the variables are simultaneously at the maximum or minimum levels. Rather, design points are at the midpoints of the edges of the design space and at the centre so the region of experimentation is polyhedron, approximating a sphere so that the precision of predications is independent of direction from centre. This also makes BBD rotatable or nearly rotatable and also provides a choice of orthogonal blocking for four and five factors. For small number of factors (four or less), BBD requires fewer runs than other designs like central composite design and is economically and experimentally convenient.[15–18]

The aim of the present research work was to systematically investigate the main, interaction and the quadratic effects of formulation variables (independent variables) of SMEDDS on desired responses; and to develop a model that would yield an optimized SMEDDS of valsartan by using BBD. A 17-run BBD with 3-factors and 3-levels, including 5 replicates at the centre point, was used for fitting a 2nd-order response surface. Estimation of the coefficients for the second order polynomial model was performed by regression analysis and the model adequacy was checked by an *F*-test and the determination coefficient (R^2). All the responses were optimized simultaneously by using desirability function. An *in-vivo* bioavailability study was performed in rats and pharmacokinetic parameters of the optimized SMEDDS were compared to that of valsartan powder.

Experimental

Materials Valsartan was supplied by Hanmi Pharm. Co. (Suwon, South Korea). Polyglycolyzed glycerides (Capryol PGMC, Cremophor EL, Labrafac CC, Labrafac lipophile 1349, Labrafil WL 2609, Labrafil M 1944 CS, Labrafil M 2125 CS) were obtained from Gattefosse (Saint-Priest Cedex, France). Castor oil, corn oil, cotton seed oil, mineral oil, sesame oil, sunflower oil and peanut oil were supplied by Sigma (St. Louis, MO, U.S.A.). Polysorbate 20 (Tween 20), sorbitan monolaurate 20 (Span 20) and sorbitan monooleate 80 (Span 80) were purchased from DC Chemical Co. (Seoul, South Korea). Deionized water was used and was freshly prepared using Milli Q-water purification system (Millipore, MA, U.S.A.) whenever required. All other chemicals were of reagent grade and used as received without further purification.

Methods. HPLC System HPLC system (Hitachi, Tokyo, Japan) consisted of Hitachi L-2130 pump and Hitachi L-2400 UV-Vis detector with Ez chrom elite (version 318a) computer software. Column used was Inertsil ODS-3 reverse-phase C18 column (0.5 μm, 15 cm×0.46 cm) (GL Science). Mobile phase consisted of acetonitrile and distilled water (60 : 40 v/v) adjusted to pH 3.0 with 10% phosphoric acid. Mobile phase was filtered through 0.45 μm membrane filter and eluted at a flow rate of 1.0 mL/min. The effluent was monitored at a UV absorption wavelength of 247 nm. All standard curves showed excellent linearity with R^2=*ca.* 0.999 and relative standard deviation at different concentrations and time was less than 3%.

Solubility Studies An excess amount of valsartan (about 100 mg) was vortex-mixed with 1 mL of each of the selected vehicles (oils, surfactants or co-surfactants) in 2 mL micro tube (Axygen MCT-200). The mixture was then shaken for 6 d at 25°C in isothermal shaker (100 strokes per min) for equilibration. Then, the equilibrated samples were centrifuged at 3000×***g*** for 15 min (Eppendorf, U.S.A.). Supernatant was filtered through a membrane filter (0.45 μm) and diluted with acetonitrile for quantification of valsartan by HPLC system.

Construction of Ternary Phase Diagram Based on the results of saturation solubility studies in Table 1 and preliminary studies, Labrafil M 2125 CS, Tween 20 and Capryol 90 were selected as oil, surfactant and co-surfactant, respectively. A ternary phase diagram was constructed for the system containing oil-surfactant-co-surfactant. The grading method reported by Craig *et al.*[19] was modified and adopted in this study. A series of self-microemulsifying systems were prepared with varying weight percentage of Labrafil M 2125 CS, Tween 20 and Capryol 90. Since the drug incorporated in the SMEDDS may have some effect on self-microemulsion boundary,[20] every system in the series also consisted of 10% w/w valsartan. 0.2 mL of each formulation was introduced into 200 mL of water in a glass beaker maintained at 37°C and was mixed gently with a magnetic stir bar (200 RPM). The tendency to emulsify spontaneously and the progress of emulsion droplets spread were observed. The tendency to form an emulsion was judged as 'good' when droplets spread easily in water and formed a fine milky or slightly bluish emulsion within 1 min. It was judged 'bad' when there was poor, slow or no emulsion formation or when oil droplets coalesced when stirring was stopped or when dull, grayish white emulsion was formed. All studies were repeated thrice. The extreme and middle level of the independent variables consisting of oil, surfactant and co-surfactant were selected for further study.

Preparation of Valsartan-Loaded SMEDDS The BBD matrix for the selected independent variables is shown in the Table 3. Three batches of 17 blank SMEDDS (without drug) were prepared according to the design matrix and valsartan (10% w/w of the blank SMEDDS) was added. The mixture was vortexed for about 3 min. Then, the final formulation was equilibrated in water bath at 37°C for 48 h before carrying out the droplet size, PDI and dissolution. The final drug content of the liquid SMEDDS 8.6–9.7% w/w. Optimized formulations were prepared by the same method.

Experimental Design. Box–Behnken Experiment Design A 3-factor, 3-level BBD was used to explore and optimize the

Table 1. Solubility of Valsartan in Various Vehicles

Vehicles	Solubility at 25°C (mg/mL)[a)]
Oils tested:	
Sesame oil	2.33±0.36
Castor oil	35.29±19.40
Cotton seed oil	9.69±0.17
Corn oil	6.98±0.10
Labrafil M 1944 CS	110.64±47.98
Labrafil WL 2609 BS	185.75±12.25
Labrafil M 2125 CS	141.15±10.11
Surfactants tested:	
Labrasol	479.92±61.39
Capryol PGMC	392.72±24.33
Tween 20	240.77±9.64
Tween 80	282.13±24.46
Brij 92	416.08±12.72
Capryol 90	702.62±44.67

a) Each value represents the mean±S.D. (n=3).

main effects, interaction effects and quadratic effects of the formulation ingredients on the *in-vitro* performance of liquid SMEDDS.[21)] A total of 17 experimental runs, including 5 replicates at the centre were generated and evaluated by using Design-Expert software (V. 8.0.4, Stat-Ease Inc., Minneapolis, U.S.A.). The purpose of the replication was to estimate experimental error and increase the precision by computing a model independent estimate of the process standard deviation. Preliminary experiments on ternary phase diagram containing oil, surfactant and co-surfactant were performed to identify the efficient self-microemulsifying regions. Based on the feasibility of microemulsion formation at extreme values, the range for the each component was selected as follows: oil (5–30%), surfactant (50–90%), co-surfactant (5–35%). Water content was taken as a "slack variable" as it is in considerable excess in GI tract. All the 17 experimental runs were carried out in random order. The significant response factors studied for assessing the quality of the SMEDDS formulation were droplet size (Y_1), polydispersity index (Y_2), dissolution after 15 min (Y_3) and equilibrium solubility (Y_4). The data obtained after the each response was fitted to quadratic polynomial model explained by the following non linear Eq. 1.[22)] At 5% significance level, a model was considered significant, if the p-value (significance probability value) is less than 0.05. And also, for each response which generates higher F value was identified as the fitted model.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1 X_2 + \beta_5 X_2 X_3 + \beta_6 X_1 X_3 + \beta_7 X_1^2 + \beta_8 X_2^2 + \beta_9 X_3^2 + E \quad (1)$$

where Y is the response of the dependent variables; β_0–β_9 are the regression coefficients; and X_1, X_2, X_3 are independent variables.

Optimization by Using Desirability Functions All four responses were optimized by using the desirability function approach introduced by Derringer and Suich.[23)] Each response is associated with its partial desirability function (d_i), scaled from 0 (furthest from the target value) to 1 (closest to the assigned target) and a utility function was computed to provide the overall or global desirability. For the response to be maximized, the desirability functions can be defined as:

$$d_i = (Y_i - Y_{\min}) / (Y_{\max} - Y_{\min})$$

where d_i is individual desirability of the response to be maximized; Y_i is the experimental result, and $Y_{\min}$ and $Y_{\max}$ represent the minimum and maximum possible value. If Y_i is equal to or less than $Y_{\min}$, then, d_i=0, if Y_i is higher or equal to $Y_{\max}$, d_i=1. For the response to be minimized, the desirability function is defined as:

$$d_i = (Y_{\max} - Y_i) / (Y_{\max} - Y_{\min})$$

If Y_i is higher than or greater than $Y_{\max}$ then d_i=0; and if Y_i is less than or below minimum then d_i=1. Here, lower and upper limits for the responses were set from the highest and lower limits of the observed responses. After obtaining the individual desirability value for each response, the results were combined together usually together to give overall desirable function (D) as the geometric mean which is given by the following equation:

$$D = (d_1 \cdot d_2 \cdot d_3 \cdot d_4 \cdot d_5 \cdots d_n)^{1/n}$$

where n specifies the number of responses.

Characterization of SMEDDS. Particle Size and Polydispersity Index Seventeen different formulations each containing 10% (w/w) valsartan were prepared. One hundred microliters was diluted in 100 mL de-ionized water in a beaker maintained at 37°C and gently stirred using magnetic stirrer. The stirring rate and time was fixed at 200 rpm and 5 min, respectively. The droplet size of the resulting emulsion was determined by Zetasizer Nano ZS (Malvern Instruments, Malvern, U.K.), dynamic light scattering particle size analyser at a wavelength of 633 nm and at a scattering angle of 90° at 25°C. The z-average diameter of the microemulsion was derived from cumulated analysis by the Auto measure software (Malvern Instruments) supplied by the manufacturer. All the studies were carried out in triplicate.

Equilibrium Solubility For equilibrium solubility studies, excess amount of valsartan was added to the blank SMEDDS and vortexed for 3 min. The mixture was then equilibrated at 37°C in a shaking water bath (100 rpm) for 6 d. The resulting mixture was centrifuged at 3000×***g*** for 15 min (Eppendorf, U.S.A.). Supernatant was filtered through polyvinylidene difluoride (PVDF) Syringe filter (0.45 μm) and diluted with acetonitrile for quantification of valsartan by HPLC system.

Dissolution Studies Drug release studies from different SMEDDS formulations were performed using United States Pharmacopeia (USP) XXIII, dissolution apparatus II (Universal Scientific Co., model: TW-SM) with 900 mL of simulated gastric fluid (without enzymes) USP as medium at 37±0.5°C. The rotating speed of the paddle was adjusted to 100 rpm. 0.5 g of each valsartan-loaded SMEDDS formulations (equivalent to 50 mg of valsartan) were filled in hard gelatin capsules size '0.' Capsule sinkers were used to avoid the capsule flotation in the medium. At predetermined time intervals, an aliquot (3 mL) of sample was collected, filtered (0.45 μm PVDF syringe filter) and analyzed for the content of valsartan by validated HPLC method as mentioned above. An equivalent volume (3 mL) of the fresh dissolution medium maintained at 37°C was immediately added to compensate for the loss due to sampling. The dissolution studies were carried out in triplicates. The dissolution profile of the optimized formulation was assessed by the same method.

***In Vivo* Pharmacokinetic Study** Male Sprague-Dawley (SD) rats (7 weeks) weighing 280±20 g were purchased from Orient Bio Inc. (Sungnam, South Korea) and used after one week of quarantine and acclimatization. The animals were housed in individual cages in Animal Centre of Yeungnam University (Gyeongsan, South Korea) under adequate temperature and humidity control with a 12-h light/12-h dark cycle. All surgical and experimental procedures involving animals were in accordance with the Guiding Principles in the Use of Animals in Toxicology, as adopted by the Society of Toxicology.[24] The procedures were also reviewed and approved by the Institute of Laboratory Animal Resources of Yeungnam University. SD rats were fasted for 12 h prior to the experiments but allowed access to water *ad libitum*. Twelve rats were randomly divided into two groups. The rats were administered the drug powder or the optimized SMEDDS at a dose of 10 mg/kg as a valsartan.

Oral Administration and Blood Collection The right femoral artery of each rat, anaesthetized in an ether-saturated chamber was cannulated with heparinized polyethylene cannula (PE-50) fitted with a three-way stopcock. The accurately weighed drug powder was placed in small hard capsules (#9, Suheung Capsule Co., Seoul, South Korea). After rats recovered from anaesthesia, the rats were dosed using oral sonde. Then, about 0.3 mL of blood was collected into heparinised Eppendorf tubes from the femoral artery at designated time interval. The blood samples were centrifuged at 3000×***g*** for 10 min and the plasma was immediately stored at −20°C until further analysis.

Plasma Drug Concentration Analysis To 125 μL of plasma, 25 μL of flurbiprofen solution (50 μg/mL) in acetonitrile was added as an internal standard (IS) and vortexed for 3 min. 0.35 mL of acetonitrile was added for extraction and deproteinization, and the sample was vortexed vigorously for 15 min, followed by centrifugation at 10000×***g*** for 15 min. The supernatant (25 μL) was taken and assayed for drug concentration by HPLC. The HPLC conditions were same as described above except for mobile phase. The mobile phase consisted of acetonitrile and aqueous 2.5% (v/v) phosphoric acid solution (1 : 1, v/v). The calibration curve for valsartan concentration in plasma was linear (R^2=0.998) over the range of 40–5000 ng/mL. The lowest standard on the calibration curve with 40 ng/mL concentration was identified as the lower limit of quantification (LLOQ) with identifiable and reproducible valsartan and IS peaks. In our study, any peak below LLOQ was supposed to be zero concentration.

Standard non-compartmental analysis were performed to determine all the pharmacokinetic parameters including maximum plasma concentration (C_{max}), time taken for its occurance (T_{max}), half-life ($T_{1/2}$), area under curve (AUC), elimination rate constant (K_e) for each rats by using WinNonlin™ standard edition, version 2.1 (Pharsight Corp., Mountain View, CA, U.S.A.) program. Moreover, Students *t*-tests were performed to evaluate the significant differences between the optimized test formulation drug powder. All the values are reported as mean±S.D. and the data were considered statistically significant at $p<0.05$.

Results and Discussion

Solubility Study The components used in the SMEDDS formulation should solubilize the maximum amount of the drug and posses the large efficient self-microemulsification region in ternary phase diagram. Selection of the vehicles was also done considering the safety and compatibility of the excipients with gelatin capsule. The solubility of the drug in the various vehicles is presented in Table 1.

Initially, the vehicles (oil, surfactant and co-surfactant) in which valsartan showed the highest solubility were selected as the components of SMEDDS. Valsartan showed highest solubility in Labrafil WL 2609 BS, Labrasol and Capryol 90 among the oils, surfactants and co-surfactants screened. However, the ternary diagram with these three components had a very small self-emulsification zone which was not feasible for designing purpose (data not shown). Labrasol was replaced with Brij 90 which showed second highest solubility among the surfactants tested. But, during ternary phase construction study, the microemulsions formed with these components were found to be very unstable. Thus, finally, Labrafil M 2125 CS (141.15±10.11 mg/mL), Tween 20 (240.77±9.64 mg/mL) and Capryol 90 (702.62±44.67 mg/mL) were chosen as oil (X_1), surfactant (X_2), and co-surfactant (X_3), respectively. They showed large self-emulsification domain and solubility of valsartan in this system was found to be capable of meeting the needs of medical use. In addition, Labrafil M 2125 CS and Tween 20 show good compatibility with soft gelatin capsule and have been used in the commercial products: Sandimmune soft gelatin capsule and Targretin soft gelatin capsule, respectively.[25] Capryol 90 has comprehensive regulatory, toxicity and handling dossiers (including Type IV *N*,*N*-dimethylformamide (DMF)) and is also compatible with gelatin and HPMC capsules.[10]

Construction of Ternary Phase Diagram Based on the preliminary experiments on the solubility of the drug in various vehicles, ternary phase diagram (Fig. 1**)** was constructed by taking Labrafil M 2125 CS as oil phase, Tween 20 as a surfactant and Capryol 90 as co-surfactant. The blue-shaded region in the diagram represents the efficient self-emulsifying region where desired visual observation characteristics were observed *i.e.* clarity of the solution, no phase separation, rapidity and spontaneity of the emulsion formation. During emulsification, surfactant molecules migrate to the oil/water interface and lower the interfacial tension. By adding co-surfactant, the interfacial tension further decreases and the induction of ideal curvature of interfacial film takes place. The droplet size decreases and the net outcome is negative value for free energy of microemulsion formation which means spontaneous microemulsion formation. From the self microemulsifying domain in the ternary diagram (Fig. 1), the range and level for each component (independent variables) was selected as: oil (5–30%), surfactant (50–90%), co-surfactant (5–35%), as shown in Table 2. The red lines in the Fig. 1 indicate the boundary of the level used in the BBD study and the polygonal area bounded by all the red lines indicate the region from which optimum formulation is to be selected. As the water is always in considerable abundance and oil volume fraction is low, it was safely supposed that only oil/water emulsion was formed, and no other dispersed and bicontinuous pseudophases were formed.

Statistical Analysis of the Designed Experiment Since the range and level of oil (X_1), surfactant (X_2) and co-surfactant (X_3) were delimited as independent variables, BBD was performed to optimize SMEDDS with constraints on droplet

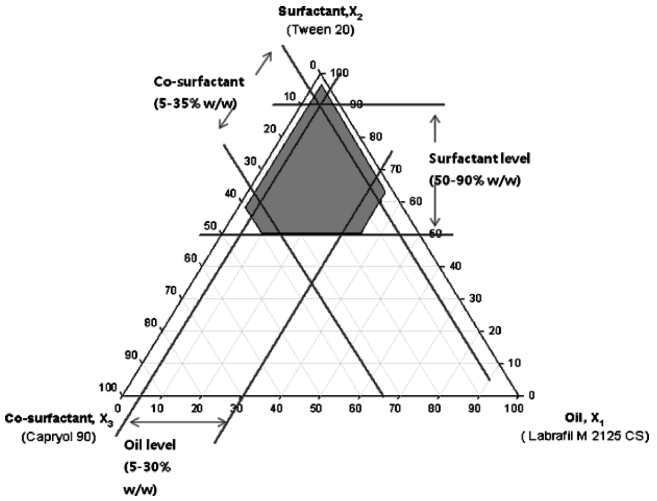

Fig. 1. Ternary Phase Diagram of the SMEDDS Formulation

Blue-shaded region represents the self-microemulsifying domain and the red line indicates the levels taken in the BBD.

Table 2. Variables in BBD

Independent variables[a)]	Levels		
	Low (−1)	Middle (0)	High (+1)
X_1: Amount of oil added (mg)	5	17.5	30
X_2: Amount of surfactant added (mg)	55	70	85
X_3: Amount of co-surfactant added (mg)	10	20	30
Dependent variables	**Constraints**		
	Range		Goal
Y_1: Particle size (nm)	In the range		Minimize
Y_2: Polydispersity index (PDI)	In the range		Minimize
Y_3: Dissolution after 15 min (%)	In the range		Maximize
Y_4: Equilibrium solubility (mg/g)	In the range		Maximize

a) Oil: Labrafil M 2125 CS; Surfactant: Tween 20; Co-surfactant: Capryol 90.

size, PDI, dissolution after 15 min and equilibrium solubility. Based on the experimental matrix of BBD, total 17 experiments were then conducted and the observed responses are summarized in Table 3.

All the data were fitted to the second order quadratic model and validation of the model was carried out by analysis of variance (ANOVA) test, lack of fit test and correlation coefficient (R^2). Various statistical evaluations of models for each response are depicted in the Tables 4 and 5. ANOVA was used to test the statistical significance of the ratio of mean square variation due to regression and mean square residual error. As shown in Table 4, at 5% significance level, it was observed that for responses Y_1, Y_3 and Y_4, quadratic fitting was significant (p-value <0.05). But the response Y_2, PDI showed better fit in linear model (p-value <0.05). The corresponding large value of F indicates that most of the variation in the response can be explained by the regression equation. At 5% significance level, the model was considered to be significant, if significance p-value is less than 0.5 and carried insignificant lack of fit. The lack-of-fit measures the failure of the model to represent data in the experimental domain at points which are not included in the regression. Insignificant lack of fit is one of the desirable statistical parameter to prove the model fitting on the responses. From Table 4, it can be seen that all models show insignificant lack of fit. While calculating the correlation coefficient (R^2) for the responses Y_1, Y_2, Y_3 and Y_4, the confidence that the regression equations would predict the observed value better than mean were more than 95%, 89%, 94% and 83%, respectively (Table 5). The corresponding coefficients which showed the quantitative effects of independent variables (X_1, X_2, and X_3) and their interactions on the responses are shown in the Tables 6 and 7. The coefficients (Factor intercepts) with more than one term ($X_1 \cdot X_2$, $X_1 \cdot X_3$ and $X_2 \cdot X_3$) and those with the higher order terms (X_1^2, X_2^2 and X_3^2) indicate the interactions and quadratic effects, respectively.

Table 3. BBD Matrix and the Observed Responses

Run	X_1 (mg)	X_2 (mg)	X_3 (mg)	Y_1	Y_2	Y_3	Y_4
1	17.5	55	30	144.9±6.0	0.209±0.040	31.33±4.3	200.33±7.7
2	5.0	70	30	134.2±14.2	0.369±0.030	70.49±7.7	214.78±21.5
3	17.5	85	30	98.2±3.2	0.269±0.018	80.45±3.7	151.76±10.0
4	17.5	70	20	63.9±2.7	0.269±0.038	70.24±4.4	184.92±2.8
5	17.5	55	10	61.9±3.1	0.391±0.029	88.96±6.6	247.52±36.8
6	17.5	70	20	64.5±1.4	0.348±0.023	85.74±7.9	188.62±15.9
7	5.0	70	10	62.4±7.0	0.403±0.193	82.09±6.8	257.25±10.3
8	30.0	70	30	118.2±3.6	0.159±0.019	71.35±8.1	154.89±5.3
9	17.5	85	10	30.0±2.1	0.316±0.009	47.83±6.2	243.38±25.0
10	17.5	70	20	61.2±1.3	0.296±0.033	96.97±4.5	202.33±15.4
11	30.0	85	20	72.8±2.6	0.229±0.008	85.99±2.9	163.55±26.9
12	30.0	55	20	144.1±4.4	0.153±0.012	84.46±5.0	216.90±18.5
13	17.5	70	20	47.6±2.0	0.361±0.009	88.03±5.7	194.92±11.3
14	30.0	70	10	95.9±1.9	0.216±0.004	97.02±3.6	250.46±16.7
15	5.0	85	20	69.4±4.3	0.504±0.046	79.36±5.6	267.06±35.7
16	17.5	70	20	67.3±6.3	0.343±0.056	75.61±5.0	175.46±16.0
17	5.0	55	20	90.0±4.4	0.457±0.023	69.59±5.6	264.04±19.9

Y_1: particle size; Y_2: polydispersity index; Y_3: dissolution after 15 min; Y_4: equilibrium solubility (mg/g).

The positive sign represents the synergistic effect of the factor where as negative sign represents the antagonist effect of the factor on the response.

Response Surface Analysis The three-dimensional response surface plots and two-dimensional contour plots are graphical representations of the regression equation and express two independent variables at once against the response (Figs. 2 to 5). Thus, the statistically significant relationship between the dependent and independent variables was further interpreted by using response surface analysis. In all the response surface and contour plots, the factors showing the least significant values were fixed at their middle levels.

Figure 2 shows the response surface and contour plots for effects of surfactant (Tween 20, X_2) and co-surfactant (Capryol 90, X_3) on droplet size (Y_1) at middle level of oil (Labrafil 2125 CS, X_1). Droplet size determines the rate and extent of drug release. From Tables 6 and 7, it can be seen that all independent variables showed significant main effects ($p<0.05$) for droplet size; the most prominent effect being the amount of co-surfactant (X_3) added ($p=0.0001$). The interaction effects

Table 4. Analysis of Variance in the Regression Models

Source		DF	Sum of squares	Mean square	*F* Value	*p*-Value
Y_1: Micelle size (nm)	Model	9	17654.15	1961.57	15.73	0.0007*
	Residual	7	872.98	124.71		
	Lack of fit	3	631.80	210.60	3.49	0.1292**
	Pure error	4	241.19	60.30		
	Cumulative total	16	18527.14			
Y_2: Polydispersity index (PDI)	Model	3	0.13	0.044	21.92	<0.0001*
	Residual	13	0.026	2.027E-003		
	Lack of fit	9	0.020	2.249E-003	1.47	0.3768**
	Pure error	4	6.113E-003	1.528E-003		
	Cumulative total	16	0.16			
Y_3: Dissolution after 15 min (%)	Model	9	3964.35	440.48	6.57	0.0106*
	Residual	7	469.05	67.01		
	Lack of fit	3	24.23	8.08	0.073	0.9716**
	Pure error	4	444.82	111.20		
	Cumulative total	16	4433.40			
Y_4: Equilibrium solubility (mg/g)	Model	9	22585.04	2509.45	13.57	0.0012*
	Residual	7	1294.54	184.93		
	Lack of fit	3	881.85	293.95	2.85	0.1689**
	Pure error	4	412.69	103.17		
	Cumulative total	16	23879.58			

Table 5. Correlation Coefficients for Four Responses

Quadratic model	R^2	Adjusted R^2	Predicted R^2	Adequate precision	S.D.	%CV
Y_1	0.9529	0.8923	0.4340	12.143	11.17	13.31
Y_3	0.8942	0.7582	0.7558	10.643	8.19	10.66
Y_4	0.9458	0.8761	0.3821	11.845	13.60	6.46
Linear model						
Y_2	0.8350	0.7969	0.6959	14.835	0.045	14.46

Table 6. Factor Coefficients and Their Corresponding *p*-Values

Factors	Y_1		Y_2		Y_3		Y_4	
	Coefficient	*p*-Value	Coefficient	*p*-Value	Coefficient	*p*-Value	Coefficient	*p*-Value
Intercept	60.894		0.311294		83.3173		189.2510	
X_1	9.3775*	0.0492	−0.122**	<0.0001	4.65813	0.1515	−27.1659**	0.0008
X_2	−21.3187**	0.0010	0.0135	0.4117	2.4116	0.4322	−12.8807*	0.0316
X_3	30.6821**	0.0001	−0.04*	0.026	−7.7864*	0.0311	−34.6066**	0.0002
$X_1 \cdot X_2$	−12.6785	0.0575			−2.0594	0.6303	−14.0923	0.0769
$X_1 \cdot X_3$	−12.3642	0.0624			−3.5166	0.4187	−13.2756	0.0918
$X_2 \cdot X_3$	−3.73	0.5255			22.5637**	0.0009	−11.109	0.1463
X_1^2	26.0555**	0.0020			7.3152	0.1093	23.6164**	0.0092
X_2^2	7.1263	0.2317			−10.7815*	0.0305	15.0185	0.0578
X_3^2	15.7463*	0.0232			−10.3933*	0.0352	6.4794	0.3608

Significant model terms at: $^{**}p<0.01$, $^{*}p<0.05$.

were not very pronounced ($0.05<p<0.6$), though the amounts of oil and co-surfactant showed significant quadratic effects on droplet size ($p<0.1$). With the increasing surfactant (coefficient is negative) in the formulation, droplet size decreased. Zhao *et al.*, also reported similar effect of surfactant on the droplet size.[26] This phenomenon may be explained by the availability of more surfactant for the formation of more closely packed surfactant film with reduced curvature at the oil/water interface. The increment in the droplet size is more marked with increasing amount of co-surfactant (coefficient is positive). Similar effect of co-surfactant was reported by Gao *et al.*, with microemulsion system containing Captex 355, Cremophor EL, Transcutol P and saline. This increment may be due to the expansion of interfacial film by the co-surfactant.[27]

Figure 3 exhibits the response surface and contour plots for effects of oil (Labrafil 2125 CS, X_1) and co-surfactant (Capryol 90, X_3) on polydispersity index (PDI, Y_2) at middle level of surfactant (Tween 20, X_2). PDI is the ratio of standard deviation to the mean droplet size and depicts the size distribution of droplets in microemulsion. Narrow, unimodal size distribution of microemulsion is preferable for the sake of reproducible bioavailability. Among the formulation variables, amount of oil added (Labrafil 2125 CS, X_1) showed significant effect ($p<0.0001$) followed by the amount of co-surfactant added (Capryol 90, X_3), which also showed significant effect ($p<0.05$) on PDI (Tables 6, 7). The software suggested linear model fitting, which means that interaction and quadratic effects are very insignificant. PDI decreased markedly with the increasing amount of oil. PDI also decreased with increasing amount of co-surfactant, though the decrease was not as prominent as with oil.

Furthermore, it was found that the level of significance for amount of co-surfactant added (Capryol 90, X_3) is below 0.5 and that its coefficient is negative, implying that co-surfactant has main and negative effect in determining the drug dissolution after 15 min (Tables 6, 7). There was also high interaction between the surfactant (Tween 20, X_2) and the co-surfactant ($p<0.05$). The surfactant and co-surfactant also exhibited quite high negative quadratic effects ($p<0.05$). Figure 4 shows the effects of oil and co-surfactant on dissolution profile of valsartan SMEDDS in simulated gastric fluid USP (pH 1.2) without enzymes at middle level of surfactant (Tween 20, X_2).

Tables 6 and 7 show the coefficient and p-value for all the independent variables. It was seen that all the independent variables (X_1, X_2 and X_3) showed significant effect on equilibrium solubility ($p<0.05$). The most dominant main effect was shown by the amount of co-surfactant (Capryol 90, X_3)

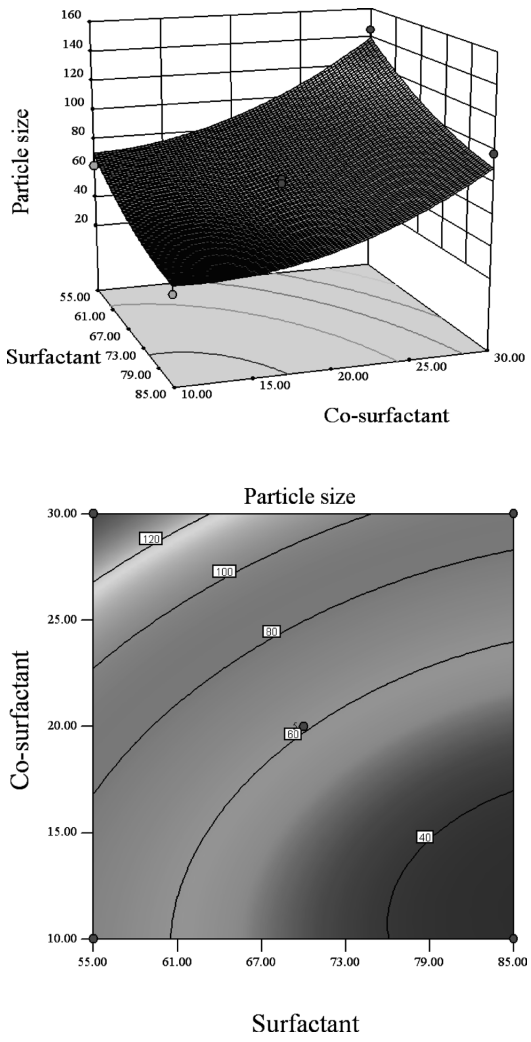

Fig. 2. Response Surface and Contour Plots Showing the Effects of Surfactant and Co-surfactant on Particle Size (Oil Is Constant at 17.5%)

followed by oil (Labrafil M 2125 CS, X_1) added ($p<0.01$). Though no significant interaction effect was seen, oil exhibited marked quadratic effect ($p<0.01$). Figure 5 shows the effects of oil and co-surfactant on equilibrium solubility at middle level of surfactant (Tween 20, X_2). This was to be expected as the drug showed highest solubility in co-surfactant (Capryol 90) in the preliminary solubility studies (Table 1) as well.

Optimization by Using Desirability Function After

Table 7. The Pooled Results of Significant Coefficients Only

Factors	Coefficients of Y_1	Coefficients of Y_2	Coefficients of Y_3	Coefficients of Y_4
Oil (X_1)	9.3775	−0.122		−27.1659
Surfactant (X_2)	−21.3187			−12.8807
Co-surfactant (X_3)	30.6821	−0.04	−7.7864	−34.6066
Interaction between surfactant & co-surfactant ($X_2 \cdot X_3$)			22.5637	
Quadratic effect of oil (X_1^2)	26.0555			23.6164
Quadratic effect of surfactant (X_2^2)			−10.7815	
Quadratic effect of co-surfactant (X_3^2)	15.7463		−10.3933	

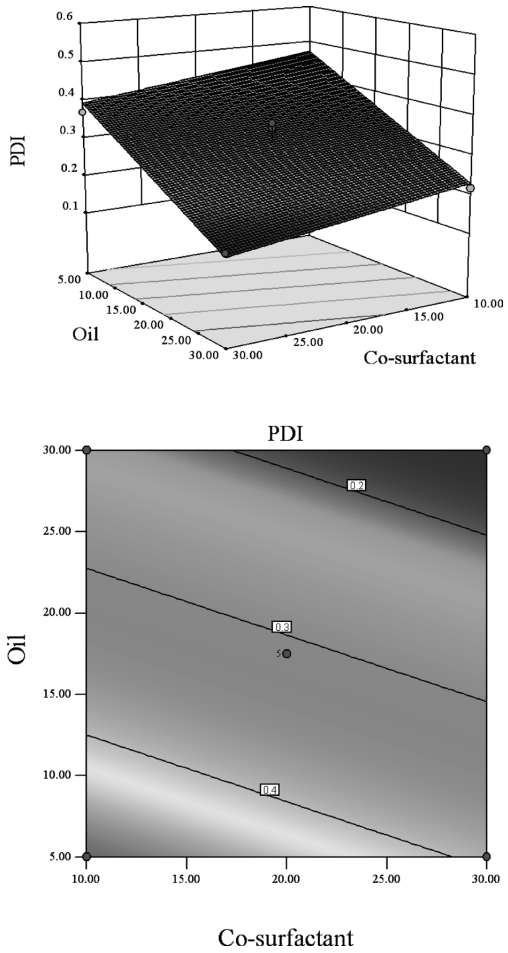

Fig. 3. Response Surface and Contour Plots Showing the Effects of Oil and Co-surfactant on Particle PDI (Surfactant Is Constant at 70%)

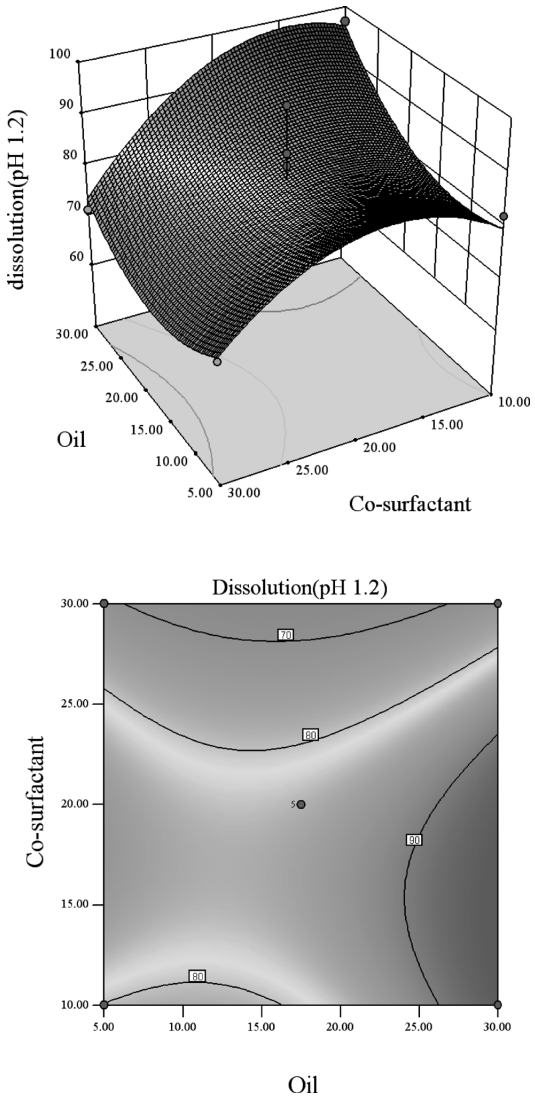

Fig. 4. Response Surface and Contour Plots Showing the Effects of Oil and Co-surfactant on Dissolution at 15 min (Surfactant Is Constant at 70%)

generating the model polynomial equations to relate the dependent and independent variables, the process was optimized for all four responses simultaneously by using desirability function. Multiple responses including Y_1, Y_2, Y_3 and Y_4 were transformed into individual desirability scale d_1, d_2, d_3 and d_4, respectively. Factors were set within the range. Constraints were set to the all the responses. Y_1 and Y_2 were to be minimized, while Y_3 and Y_4 were set to be maximized. Equal weight and importance were provided to all the responses. The global desirability value was calculated by combining all the individual desirability functions as the geometric mean by using extensive grid and feasibility search over the domain. The suggested optimized formulation consisted of 26.8% oil, 60.1% surfactant and 13.1% co-surfactant with the corresponding desirability (D) value of 0.722. This factor level combination predicted the response as Y_1=85.8 nm, Y_2=0.256, Y_3=96.6% and Y_4=238.5 mg/g. To confirm the model adequacy for the prediction, three batches of the optimized formulations were prepared and all the responses were evaluated for each formulation (Table 8). The optimized valsartan-loaded SMEDDS had particle size of 90.7±3.5 nm, PDI of 0.246±0.027, dissolution after 15 min of 91.2±3.9% and equilibrium solubility of 226.7±8.6 mg/g, respectively. It can be concluded that the experimental values were in close agreement with predicted values, indicating the success of the design to evaluate and optimize the SMEDDS formulation.

***In Vitro* Dissolution** The *in vitro* dissolution profile of optimized SMEDDS in pH 1.2 was compared with drug powder, as shown in the Fig. 6. It can be seen that the release of valsartan from SMEDDS is significantly higher than that of the drug powder. It could be suggested that spontaneous micro-emulsification resulted in the faster rate of drug release into the aqueous phase in the form of small and monodispersed droplets.[28] Furthermore, the drug release kinetics from optimized valsartan SMEDDS was investigated using various models including zero order, first order, Higuchi and Korsmeyer–Peppas equations.[29] The best fit was obtained with Higuchi equation (Q=10.45$t^{1/2}$−10.50, r^2=0.947). This greater availability of dissolved valsartan from the optimized formulation could lead to higher absorption and enhanced bioavailability.

Pharmacokinetic Study Figure 7 shows mean plasma concentration profile of valsartan after oral administration of valsartan powder and the optimized SMEDDS to rats at a dose of 10 mg/kg. The total plasma concentrations of the drug with SMEDDS formulation were significantly higher

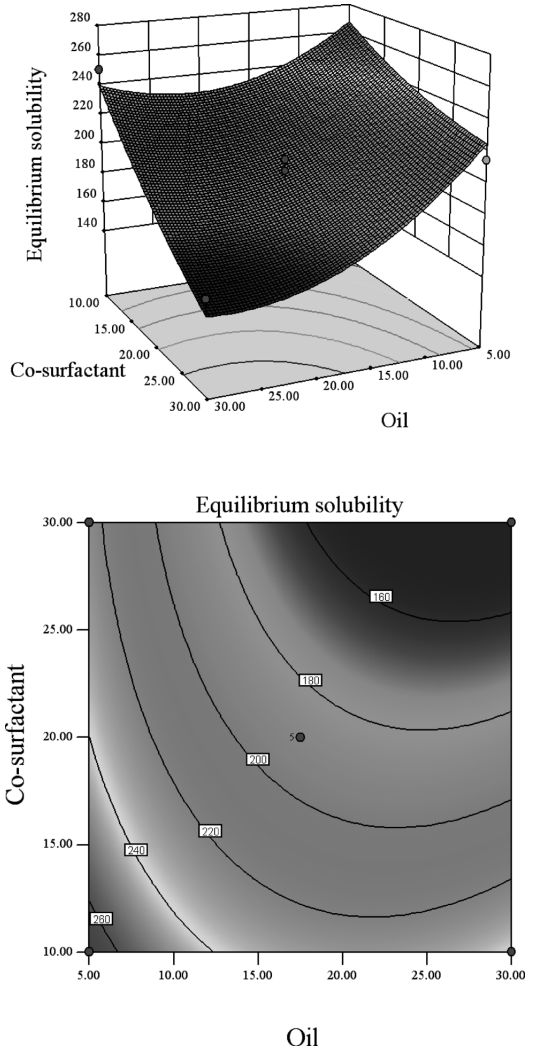

Fig. 5. Response Surface and Contour Plots Showing the Effects of Oil and Co-surfactant on Equilibrium Solubility (Surfactant Is Constant at 70%)

than those with valsartan powder. The higher initial plasma concentrations of valsartan might have been due to the increased initial dissolution rate and permeability of the drug with SMEDDS. The pharmacokinetic parameters of valsartan were shown in Table 9. The C_{max}, K_{el} and $t_{1/2}$ values of the SMEDDS were significantly different from that of powder. The optimized SMEDDS formulation gave significantly higher *AUC* and C_{max} of drug than did valsartan powder ($p<0.05$). In particular, the AUC values of SMEDDS were more than two-fold greater than that of the powder ($p<0.05$), showing the enhanced bioavailability of valsartan.

Conclusion

In this work, the effects of three formulation factors (Labrafil M 2125 CS as oil, Tween 20 as surfactant and Capryol 90 as co-surfactant) on the four main characteristics valsartan SMEDDS were investigated using 3-level, 3-factor BBD. Of the factors studied, all three factors showed significant effect on particle size and equilibrium solubility while the amount of co-surfactant exhibited main effect on dissolution profile after 15 min. The amount of oil and co-surfactant used had main effect on PDI. Except for PDI, the

Table 8. Predicted and Measured Values of Responses and Corresponding Biasness

Responses	Predicted value	Measured value	Biasness %
Particle size (nm)	85.8	90.7±3.5	5.72%
PDI	0.256	0.246±0.027	3.91%
Dissolution after 15 min (%)	96.6	91.2±3.9	5.59%
Equilibrium solubility (mg/g)	238.4	226.7±8.6	4.91%

Biasness %=(predicted value-measured value)×100/predicted value.

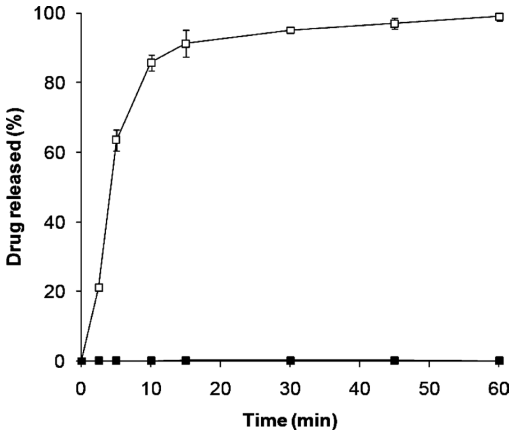

Fig. 6. Dissolution Profiles of Pure Powder (■) and Valsartan-Loaded SMEDDS (□)

Each value represents the mean±S.D. ($n=3$).

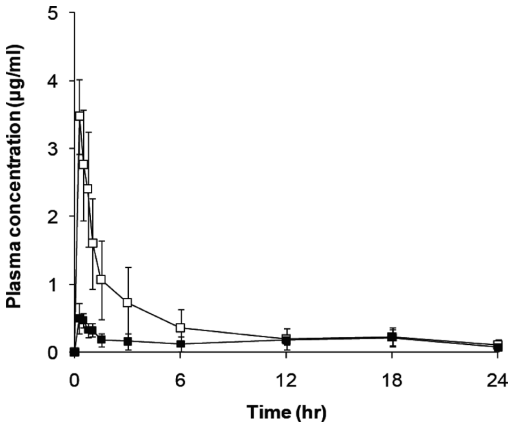

Fig. 7. Plasma Concentration–Time Profiles of the Drug after Oral Administration of Pure Powder (■) and Valsartan-Loaded SMEDDS (□) at a Dose of 10 mg/kg to Rats

Each value represents the mean±S.D. ($n=6$). *$p<0.05$, compared to the powder.

Table 9. Pharmacokinetic Parameters of Valsartan after Oral Administration of the Drug Powder and the Optimized SMEDDS

	Powder	SMEDDS
C_{max} (μg/mL)	0.56±0.16	3.60±0.57*
T_{max} (h)	0.25±0.00	0.29±0.09
AUC (h·μg/mL)	4.09±1.54	11.00±3.79*
$t_{1/2}$ (h)	17.04±7.43	5.72±0.80*
K_e (h^{-1})	0.04±0.03	0.12±0.01*

Each value represents the mean±S.D. ($n=6$). *$p<0.05$, compared to the powder.

formulation factors also had interaction and quadratic effects on the responses studied. An optimized formulation was successfully developed by using desirability function, and the experimental values were found to be in close agreement with the predicted values. Furthermore, *in vitro* dissolution study of the optimized formulation revealed significant increase in release (about 95% release after 15 min). The optimized formulation showed significantly increased bioavailability compared to that of valsartan powder. Therefore, it was concluded that BBD facilitated in the better understanding of inherent relationship of formulation variables with the responses and in the optimization of valsartan SMEDDS in relatively cost, time and labor effective manner, as demonstrated by the present study.

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