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# Formulation of Self-Microemulsifying Drug Delivery System (SMEDDS) by D-optimal Mixture Design for Enhancing Oral Bioavailability of DN200428 (Cathepsin K Inhibitor)

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서울대학교 대학원

약학과 약제과학 전공

Visetvichaporn Voradanu

## Abstract

# Formulation of Self-Microemulsifying Drug Delivery System (SMEDDS) by D-optimal Mixture Design for Enhancing Oral Bioavailability of DN200428 (Cathepsin K Inhibitor)

Voradanu Visetvichaporn

Department of Pharmaceutics, College of Pharmacy

The Graduate School

Seoul National University

DN200428 is a new compound designed and synthesized to treat osteoporosis as a cathepsin K inhibitor. Its poor aqueous solubility is expected to result in low bioavailability after oral administration. The objective of this study is to formulate self-microemulsifying drug delivery systems (SMEDDS) for enhancing the oral bioavailability of DN200428. Solubility studies of DN200428 were performed to select the suitable oil, surfactant and cosurfactant. Pseudoternary phase diagrams were plotted to identify the microemulsion region and to determine the range of components in isotropic mixture. The D-optimal mixture design and

the desirability function was introduced to optimize the formulation of SMEDDS with the optimum physicochemical characteristics, *i.e.*, high drug concentration at 15 minutes after dilution in simulated gastric fluid (SGF) and high solubilized capacity. Pharmacokinetic study was performed in rats, and the drug concentration in plasma samples was assayed using the high-performance liquid chromatography with fluorescence detector (HPLC-FL). The optimized DN200428-loaded SMEDDS formulation consisted of 5.0% of Capmul MCM EP (oil), 75.0% of Tween 20 (surfactant) and 20.0% of Carbitol (co-surfactant). The droplet size of microemulsion formed by the optimized formulation was 10.7 ± 1.6 nm. Transmission electron microscopy (TEM) analysis was confirmed the spherical shape of the microemulsion. Pharmacokinetic studies showed that the relative oral bioavailability of DN2000428-loaded SMEDDS increased up to 3-fold when compared with its solution in DMSO:PEG400 (8:92). Thus, it can be concluded that the formulation of SMEDDS could be a promising approach to improve oral bioavailability of DN200428.

**Keywords:** SMEDDS, D-optimal design, Cathepsin K Inhibitor

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# Abbreviation and Acronyms

DOE: Design of experiment

SMEDDS: Self-microemulsifying drug delivery system

SGF: Simulated gastric fluid

DS: Droplet size

PDI: Polydispersity index

DIL: Drug concentration at 15 min after diluted with simulated gastric fluid (1:250 dilution)

SC: Solubilized capacity

# 1. Introduction

In recent years, poor drug solubility in water issue has been wide spread as emerging of the new drug candidates from the latest drug design tends to have hydrophobic properties and low bioavailability (Sandeep and Vijaykumar, 2015). The solubility and/or dissolution rate in the gastrointestinal (GI) tract is the main reason for the limitation of the absorption of these drugs. Several techniques were developed to improve the absorption of poorly water-soluble drug by enhancing the solubility of the compound including pH modification, salt formation, using of co-solvent, nanosuspensions, polymeric nanoparticles, solid dispersions, cyclodextrin complexes, and lipid-based formulation such as proliposome and self-emulsifying drug delivery system (Sandeep and Vijaykumar, 2015; Yeom et al. 2015).

Self-Microemulsifying Drug Delivery System (SMEDDS) has been successfully used as a promising strategy to enhance oral bioavailability for several poor aqueous soluble compounds (Kamboj and Rana, 2016; Yeom et al., 2015; Zhang et al., 2007). Many commercial products of SMEDDS preparation are available on the market in capsules such as Neoral® (Cyclosporine A) Fortovase® (Saquinavir), and Agenerase® (amprenavir) (Porter et al., 2008). SMEDDS is an isotropic mixture of oil, surfactant and co-surfactant, which can spontaneous form fine oil-in-water (o/w) emulsions after dilution in the GI fluid with the gentle agitation from GI tract motility. Spontaneous formation of the microemulsion in the GI tract influence the drug to maintain in solubilized form. The small droplet size also provides a large interfacial surface area which promotes rapid drug release,

consequently, enhance the drug absorption by transporting the drug through the unstirred water layer to the GI membrane. In addition, one of the SMEDDS advantage as lipid-based formulation is this delivery system can avoid the hepatic first-pass effect by its uptake via the lymphatic transport (Wu et al., 2015; Zhang et al., 2008). There are many studies demonstrated that enhanced drug release from SMEDDS could improve the bioavailability (Wu et al., 2015; Yeom et al., 2015, Zhang et al., 2007).

DN200428 is a new compound designed and synthesized to treat osteoporosis as a cathepsin K inhibitor. Osteoporosis is a bone disease characterized by low bone mass with a consequent increase in bone fragility and risky of broken bone (Stoch and Wagner, 2008). Osteoporosis could be prevented or treated by either inhibiting bone resorption and/or increasing bone formation. Cathepsin K is the cysteine protease expressed in the osteoclast and has a critical role in osteoclastic bone resorption (Boonen et al., 2012; Stoch and Wagner, 2008). Therefore, Cathepsin K inhibitors were expected to be novel target for developing new drug candidates to treat osteoporosis by direct inhibition of the enzyme cathepsin K which is primarily responsible for bone matrix degradation by osteoclasts (Boonen et al., 2012). Like several new drug candidates, DN200428 is practically insoluble in water. Also, its poor aqueous solubility is expected to result in low bioavailability after oral administration. To improve the solubility, SMEDDS was applied to the formulation of DN200428.

Design of experiment (DOE) is a systemic experimental approach used for identification of the correlation between independence variables (input variables) and dependence variables (responses) according to the statistics. The advantage of

DOE is fewer experiments were performed to achieve the optimum formulation. The mathematical models were involved and the precise estimates of each response were calculated based on the polynomial equations without doing experiment (Singh et al., 2011). The magnitude of the coefficients also demonstrates significance of each independent variable to each dependent variable (Kamboj and Rana, 2016). Due to time-consuming of development process based on the trial and error, many experimental designs have been used for optimization of SMEDDS such as Box-Behnken design, factorial design and D-optimal mixture design. (Chaji and Lodha, 2008; Cho et al., 2013; Holm et al., 2006; Kamboj and Rana, 2016; Yeom et al., 2015). Unlike other design, D-optimal mixture design considered total mixture as 100% which is suitable for development of SMEDDS (Yeom et al. 2015).

The aim of this study was to develop and optimize DN200428-loaded SMEDDS formulation by using the D-optimal mixture design to improve its oral bioavailability.

# 2. Materials and Methods

## 2.1 Materials

DN200428 was supplied by Hanlim Pharmaceutical Co., Ltd. (Seoul, South Korea). Etodolac was purchased from Tokyo Chemical Industry (Tokyo, Japan). Capmul MCM EP (Glyceryl caprylate/caprate) was gifted from Abitec. (Wisconsin, United States). Capryol 90 (Propylene glycol monocaprylate), Labrafil M1944 CS (Oleoyl polyoxyl-6 glycerides), Labrafil M2125 CS (Linoleoyl polyosyl-6 glycerides), Labrafil M2130 CS (Lauroyl polyoxyl-6 glycerides), and Labrasol (Caprylocaproyl polyoxyl-8 glycerides) were purchased from Gattefossé (Saint Priest, France). Cotton seed oil, Soybean oil, Sunflower seed oil, Tween 20, Tween 80, Polyethylene glycol 400 (PEG400), Solutol HS 15 (Poly-oxyethylene esters of 12-hydroxystearic acid), Carbitol (Diethylene glycol ethyl ether) and Span 80 (Sorbitan monooleate) were purchased from Sigma Aldrich (St. Louis, MO, USA). All other chemicals and solvents were of analytical grade.

## 2.2 Methods

### 2.2.1 Solubility studies

The solubility of DN200428 was determined in various natural oils (Cotton seed oil, Sunflower seed oil, Soybean oil), synthetic/semi synthetic oils (Capmul MCM EP, Labrafil 1944 CS, Labrail M2125 CS, Labrafil M2130 CS, Ethyl oleate), and in various surfactants/co-surfactant (Tween 20, Tween 80, PEG 400, Labrasol, Solutol

HS 15, Carbitol, Span 80). The excess amount of DN200428 were added into 1 mL of each oil/surfactant and vortexed until the drug was totally dispersed. The samples were kept at a constant temperature in shaking water bath (Lab Companion, BS-21, JEIO TECH) at 50 rpm and 37±0.5°C for 24 hours. Then, the samples were centrifuge (Centrifuge 5415 R, Eppendorf, Germany) at 13200 rpm for 5 minutes. The supernatants were collected and filtered through 0.45 µm syringe filter (Minisart®-RC, Sartorius, Germany). The filtrates were analyzed by HPLC-FL after appropriate dilution with dimethyl sulfoxide (DMSO): acetonitrile (ACN) (50:50 v/v).

### 2.2.2 HPLC analysis of DN200428

Quantification of DN200428 was validated on high-performance liquid chromatography (HPLC) system. The samples obtained from solubility, dilution test, solubilized capacity, and *in vivo* animal pharmacokinetic study were determined for DN200428 concentration using an isocratic HPLC system. The HPLC system included a pump (We2695; Waters Corporation, Milford, MA, USA), fluorescence detector (W2475; Waters Corporation, Milford, MA, USA), and chromatographic XBridge Shield RP18 column (4.6×250 mm, 5 µm; Waters Corporation, Milford, MA, USA) that was maintained at a flow rate of 1.0 mL per minute at 25°C. The solvent consists of ACN and water (65:35 v/v) was used as mobile phase for *in vitro* studies. Whereas ACN:10mM phosphate buffer pH 2.5 (57:43 v/v) was used as mobile phase for *in vivo* studies. The combination of DMSO and ACN (50:50 v/v) was used as diluting solvent. For fluorescence

detection, the excitation and emission wavelengths of DN200428 were set at 295 and 395 nm, respectively. And the excitation and emission wavelengths of etodolac as an internal standard were set at 235 and 345 nm, respectively. The injection volume was 20 µL. The chromatograms were evaluated with Empower 2 Software (Waters Corporation, Milford, MA, USA)

### 2.2.3 Construction of pseudoternary phase diagram

Based on the solubility and hydrophilic-lipophilic balance (HLB) value, Capmul MCM EP, Tween 20 and Carbitol were chosen as oil, surfactant and co-surfactant, respectively. To determine the microemulsion existence area, pseudoternary phase diagrams were constructed employing the water titration method at 37 °C. Mixtures of surfactant and co-surfactant (S-mix) in different ratios by volume (3:1, 2:1, 1:1, 1:2, 1:3) were prepared. Each ratio of S-mix was combined with oil in different ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 with a total volume of 1 mL. The prepared mixtures were vortexed and then titrated with water dropwise under gentle agitation by magnetic stirrer (75 rpm) up to 100 mL (1:100 dilution). After each addition, the emulsion was observed visually (turbid or clear). Each experimental component of blank SMEDDS were marked as opened circles (microemulsion; clear) and closed circles (macroemulsion; turbid). The microemulsion region was figured out by constructing a pseudoternary phase diagram using the Sigma Plot® software (Sigma Plot, USA). Additional point of blank SMEDDS were performed to fulfill the appropriate region to select the design space.

### 2.2.4 Preparation of DN200428 suspension, solution and SMEDDS formulation

DN200428 suspension (2.5 mg/mL) was prepared by dispersing the drug in PEG400 (1.0%) and then water was added. The suspension was vortexed to make homogenous mixture. DN200428 solution in DMSO:PEG400 (8:92) (2.5 mg/mL) was prepared by dissolving the drug in DMSO and then added PEG400 into the mixture. The solution was vortexed and stirred at the room temperature for 1 hour. A blank SMEDDS formulation was prepared by mixing oil, surfactant, and cosurfactant. And DN200428 was added into blank SMEDDS to make 2.5 mg/mL DN200428-loaded SMEDDS. Then the mixture was vortexed until the drug was totally dispersed. The mixtures were kept at a constant temperature in shaking water bath at 50 rpm and 37±0.5°C for 3 hours to obtain a clear homogenous solution.

### 2.2.5 Optimization of DN200428-loaded SMEDDS formulations using D-optimal mixture design

Amount of oil, surfactant and co-surfactant were chosen as the independent variables based on the solubility and the ability of the previously prepared pseudoternary phase diagram to form microemulsion. Range of Capmul MCM EP (oil; $X_1$), Tween 20 (surfactant; $X_2$), and Carbitol (co-surfactant; $X_3$) using in the design were set as 5-15%, 55-75%, and 15-35%, respectively. The total combination of each formulations was 100%. Drug concentration at 15 minutes after diluted

with simulated gastric fluid (SGF) (1:250 dilution) (DIL; $Y_1$), and solubilized capacity (SC; $Y_2$) were evaluated to determine the optimal SMEDDS formulation for best physiochemical characteristics. Design Expert® Software version 7 (Stat-Ease Inc, Minneapolis, MN, USA) was used for developing and evaluating the experimental design. Sixteen formulations were obtained from the program to fit a cubic model, to check for lack of fit, and to estimate experimental error in the responses. The responses were fitted in the linear, quadratic, cubic and special cubic polynomial model. The equations were generated for each response using the software. The suitable mathematical fitting model was selected based on the comparison of various statistical parameters provided by the analysis of variance (ANOVA) such as sequential p-value, lack of fit p-value, r-squared and adequate precision. The selected model for each response were used for predicting the desirable results of the optimized independent factors by using the desirability function.

### 2.2.6 *In vitro* evaluation and optimization of SMEDDS formulation

**Droplet size (DS) and polydispersity index (PDI)**

Aliquot (20 µL) of DN200428-loaded SMEDDS formulation was reconstituted with 1000 mL double-distilled water. After gently shaking, microemulsions were transferred into optical polystyrene cuvette and were measured the DS and PDI by Zeta-potential & Particle size Analyzer (Photal, ELSZ, Otsuka Electronic, Japan)

**Drug concentration at 15 min after diluted with SGF (1:250 dilution) (DIL)**

Aliquot (100 µL) of DN200428-loaded SMEDDS formulation was added into 25 mL of SGF in a constant temperature at 37°C and gently stirred at 75 rpm with magnetic stirrer for 15 minutes. Samples 1 mL were collected and filtered through 0.2 µm membrane filtration. The filtrate was centrifuged at 13200 rpm for 2 minutes. The supernatant was collected and diluted with diluting solvent, after which 100 µL of the sample was analyzed. SGF in this study was prepared by dissolving 2.0 g of sodium chloride in 7.0 mL of hydrochloric acid and adjusted the volume to 1000 mL with water according to the method in United States Pharmacopoeia (USP), 39$^{th}$ edition without addition of the purified pepsin.

**Solubilized capacity (SC)**

Blank SMEDDS of each formulation was prepared by mixing Capmul MCM EP, Tween 20, Carbitol. The excess amount of DN200428 was added into 1 mL of blank SMEDDS and vortexed until the drug was totally dispersed. The samples were kept at a constant temperature in shaking water bath at 50 rpm and 37±0.5°C for 24 hours. Then, the samples were centrifuge at 13200 rpm for 10 minutes. The supernatants were collected and filtered through 0.45 µm syringe filter (Minisart®-RC, Sartorius, Germany). The filtrates were analyzed by HPLC-FL after appropriate dilution with DMSO:ACN (50:50 v/v).

**Morphology of microemulsion**

The morphology of the microemulsion droplet formed from optimized SMEDDS formulation was observed using energy-filtering transmission electron microscope (TEM; LIBRA 120; Carl Zeiss, Jena, Germany) at 80 kV. 20 μL of SMEDDS formulation was added into 1000 μL of water (1:50 dilution) and sample drop was placed on a copper grid. The sample was subsequently stained with uranyl acetate solution for 10 seconds and washed with water for 1 second for two times. The excess was drawn off with a filter paper.

## 2.2.7 *In vivo* studies

**Oral administration and plasma collection**

The bioavailability of DN200428 solution and DN200428-loaded SMEDDS were assessed in rats. The animal experiments were performed in accordance with the National Institute of Health guidelines on principles of laboratory animal care (National Institute of Health publication 85-23, revised 1996) and were approved by the Institutional Animal Care and Use Committee of the College of Pharmacy, Seoul National University, Seoul, Korea. Male Sprague-Dawley rats (weight 300 g, age 7-9 weeks) were purchased from Orient Bio (Kyunggi-Do, Korea). The rats were fasted for approximately 12 hours with free access to water before experiment. The DN200428 solution and DN200428-loaded SMEDDS optimized formulation were administered at doses of 5 mg/kg with or without 1 mL of water after

administration. Blood samples (approximately 300 µL) were collected from the cannulated femoral artery at predetermined time intervals after oral administration into polyethylene micro test tube. The samples were immediately centrifuged at 13200 rpm and 4°C for 3 minutes. The supernatants (100 µL) were collected and stored at -20°C until further analysis.

## Analysis of plasma drug concentration

The plasma samples (100 µL) were mixed with 1 mL of internal standard (Etodolac, 400 ng/mL in acetonitrile). Each sample was shaken for 5 minutes to completely dissolve the drug in acetonitrile and centrifuged at 13200 rpm and 4°C for 5 minutes. The supernatant was collected and evaporated to remove organic solvent under nitrogen gas on a pressured gas blowing concentrator (EYELA, MGS-2200, Tokyo Rikakikai, Japan). The residue was reconstituted with 100 µL of DMSO:ACN (10:90) and vortexed for 5 min. The concentration of DN200428 was determined by HPLC-FL as described above. The calibration curve of DN200428 concentration in plasma was prepared by spiking the known amount of drug into blank plasma. The curve was linear ($R^2$ = 0.9998) over the range of 40-2000 ng/mL Area of DN200428 peak for 40 ng/mL was approximately 65000 and was identified as the lower limit of quantification (LLOQ) with acceptable accuracy and precision.

**Pharmacokinetic parameters**

The plasma concentration data were analyzed by an noncompartmental model using WinNonlin (version 5.0.1, Pharsight, CA, USA) to obtain pharmacokinetic parameters. The area under the plasma concentration versus time curve from zero to 5 hours ($AUC_{0-5}$) was calculated using trapezoidal method. The maximum plasma concentration ($C_{max}$) and the time to reach $C_{max}$ ($T_{max}$) were obtained from the plasma data. The significance differences observed for the mean pharmacokinetic parameters after oral administration of DN200428 formulations to rats was determined using analysis of variance (ANOVA) at a significance level of $P < 0.05$ and $P < 0.01$. Tukey multiple comparisons was used as subsequent analysis by Graphpad PRISM® Software (Version 5.01). Results are presented as mean values ± standard deviation (SD).

# 3. Results and Discussion

## Selection of oil, surfactant and co-surfactant

Solubilization of drug in the excipients using in SMEDDS formulation is an important determination for developing of microemulsion. The materials showing high solubility of DN200428 should be selected. Solubility of DN200428 in excipients were shown in the Table 1. Among several types of oil, Capmul MCM EP showed the maximum solubility (0.239±0.04 mg/mL). Tween 20 and Carbitol showed the highest solubility among surfactant and co-surfactant of 7.461±0.76 mg/mL and 6.563±0.11 mg/mL, respectively. In addition, hydrophilic-lipophilic balance (HLB) values of surfactant was considered to use as a tool for categorizing between surfactant and co-surfactant. The water-soluble surfactant which has HLB values greater than 12 are recommended to use in SMEDDS formulation due to theirs easy forming micelle properties (Dokania and Joshi, 2015; Pouton and Porter, 2008). Also, surfactants with low HLB value can reduce the interfacial tension of the film formed by emulsion droplets and ensures the flexible of the film. In addition, combination of low HLB (HLB <10) and high HLB (HLB > 10) surfactant can prolonged the stability of the formulation. To form oil in water emulsion, the main emulsifier in SMEDDS should be water-soluble surfactant which has higher HLB. Thus, Capmul MCM EP, Tween 20 (HLB = 16.7) and Carbitol (HLB =4.2) were selected as oil, surfactant and co-surfactant in this study. The combination was further evaluated using pseudoternary phase diagram.

## Construction of pseudoternary phase diagram

The selected oil, surfactant and co-surfactant were investigated to confirm the microemulsion was formed by the mixture. Pseudoternary phase diagrams were plotted from Capmul MCM EP (oil), Tween 20 (surfactant), and Carbitol (co-surfactant) as shown in Figure 1. Capmul MCM EP, Tween 20, and Carbitol were marked as 100% at the apex of the diagram. Only three combinations of various ratios of S-mix and oil (marked as opened circle) showed the ability to form microemulsion among the experimental blank SMEDDS when diluted with water up to 100 times. However, the other combination of mixture showed turbid emulsion after diluted with water not more than 20 times (marked as closed circle). Six blank SMEDDS in different ratios were further performed to cover the previous three spots. The microemulsion region was shown in the light gray region as in Figure 1. This result was confirmed that concentration of oil using in SMEDDS is typically less than 20% (Dokania and Joshi, 2015).

## Statistical analysis using the D-optimal mixture design of DN200428-loaded SMEDDS

Design of experiment was introduced in development of SMEDDS in this study. Unlike other design, D-optimal design was applied in this study and optimized the SMEDDS formulation as it considered the system as 100 percent (Holm et al, 2006; Yeom et al, 2015). From the microemulsion region, the design space for D-optimal

mixture design of this study was selected as dark gray region as shown in Figure 1. The amount of oil, surfactant and co-surfactant were identified as the factors which influences the droplet size and *in vitro* dispersion of SMEDDS formulations (Kamboj and Rana, 2016; Yeom et al., 2015). Thus, these factors were set as the input variables. Droplet size and the size distribution are the most important characteristics affecting the *in vivo* test of emulsions (Liu et al., 2009). The rate and drug release depends on the size of emulsion droplet. Smaller droplet size and larger surface area is easier for drug absorption. However, the average microemulsion droplet size and polydispersity index after dilute DN200428-loaded SMEDDS with water (1:50 dilution) of the experimental formulations were small (10.3-11.9 nm) and homogenize (0.010-0.144) as shown in the Appendix Table I. In addition, no models were fitted to both responses indicating no significantly difference between all formulations. Thus, these two parameters were not included in this design. Table 2 shows the range independent variables and goal of dependent variables using in D-optimal design of this study. The results obtained from the various SMEDDS formulations and dependent variable of D-optimal mixture design are shown in Table 3. The results were inputted to Design Expert® 7 Software. The data were statistically fitted to different models and their polynomial equations of the responses were generated (drug concentration at 15 minutes after diluted with simulated gastric fluid (SGF) (1:250 dilution) (DIL; $Y_1$), and solubilized capacity (SC; $Y_2$)). The coefficients of $X_1$, $X_2$, $X_3$ were statistically correlated the response. A positive sign of coefficient represents a synergistic effect whereas a negative sign represents an antagonistic effect of the coefficients. The higher coefficient means

the independent variable has larger influence on the response. The model results statistic data are shown in Table 4.

**Drug concentration at 15 minutes after diluted with SGF (1:250 dilution) (DIL; $Y_1$)**

Drug precipitation is one of the problem found during the dispersion and digestion of lipid based formulation which may reduce the drug absorption and decreased the oral bioavailability (Khan et al., 2016). The drug concentration at 15 minutes after diluted 250 times with SGF represents the ability to maintain microemulsion formed by SMEDDS formulation. As shown in Table 3, formulation 7 showed the highest drug concentration (2.155 ± 0.11 µg/mL) and formulation 11 showed the lowest drug concentration (1.402 ± 0.10 µg/mL). The data were fitted to the quadratic model. The statistical fitting of model and ANOVA statistical results are shown in Table 4 and Table 5. The equation (1) was obtained from the program for DIL ($Y_3$);

$$\text{DIL } (Y_1) = +1.80\,X_1 + 2.47\,X_2 + 1.52\,X_3 - 1.57\,X_1X_2 - 0.84\,X_1X_3 - 0.66\,X_2X_3 \quad (1)$$

Based on the equation, the coefficients indicate the influential of the response. The magnitude of the coefficient was in the order $X_2 > X_1 > X_3$. The coefficients of $X_2$ had the most significantly high magnitude, indicating that amount of Tween 20 ($X_2$)

was a critical factor that affected $Y_1$. Although $X_2$ had a positive effect on $Y_1$, coefficients of combination of the independent variables ($X_1X_2$, $X_1X_3$, and $X_2X_3$) shows a negative effect on $Y_1$, suggesting the inverse relationship between $X_2$ and other parameters. A higher amount of $X_2$ resulted in higher $Y_1$. In other words, risk of precipitation was decreased with an increased in Tween 20 content.

**Solubilized capacity (SC; $Y_2$)**

The solubilized capacity of each formulation was shown in Table 3. The data were fitted to the linear model. The statistical fitting of model and ANOVA statistical results are shown in Table 4 and Table 5. The correlation between solubilized capacity and independent variables is shown in equation (2).

$$SC\ (Y_2) = +2.47\ X_1 + 6.36\ X_2 + 6.86\ X_3 \qquad (2)$$

Solubilized capacity is related to the ability to maintain the solubilized form of drug by SMEDDS formulation. The higher SC was expected to result in high drug absorption. The magnitude of the coefficient was in the order $X_3 > X_2 > X_1$. The positive sign of all coefficients indicated that solubilized capacity of DN200428 is higher as increasing the content of oil or surfactant or co-surfactant. However, the content of surfactant and co-surfactant may be mainly responsible for the solubilized capacity of the SMEDDS when compared to oil, which corresponding to the solubility of DN200428 in excipient from Table 1.

**Optimized SMEDDS formulation**

Desirability function of the Design Expert® program was used for optimization of all the responses. $Y_1$ and $Y_2$ were set to be maximized. After calculating by the combination of all the polynomial equations mentioned above, the program suggested the independent variables of 5.0% of Capmul MCM EP (oil), 75.0% of Tween 20 (surfactant) and 20.0% of Carbitol (co-surfactant) as the optimized formulation with desirability of 0.878. The droplet size and polydispersity index of the optimized formulation is small (10.7 ± 1.6 nm) and homogenize (0.006 ± 0.0). The optimized independent variables and the predicted responses are presented in Table 6. The percentage prediction error of $Y_1$ and $Y_2$ were similar to the predicted values, suggesting that the D-optimal design is suitable for optimizing the SMEDDS formulations. The TEM image of the optimized SMEDDS formulation showed the spherical shape of emulsion droplets which comprised with the measured droplet size as in Figure 6.

***In vivo* pharmacokinetic studies in Rats**

The plasma concentration-time profiles of DN200428 after oral administration (5 mg/kg) in rats are presented in Figure 7. The plasma concentrations of DN200428 in rats after administration of the optimized SMEDDS were significantly higher than those in rats receiving the DN200428 solution (DMSO:PEG400 (8:92) solution) and DN200428 suspension (data not shown). The pharmacokinetic parameters are listed in Table 7. Time to reach maximum plasma concentration ($T_{max}$) of the optimized

SMEDDS (with water) was significantly higher than those of the solution with and without giving water after administration. The delayed of $T_{max}$ may resulted from time to form microemultion of SMEDDS before drug absorption. However, the maximum plasma concentration ($C_{max}$) were identical for the DN200428 solution with/without water and the optimized SMEDDS with/without water. Nevertheless, the optimized SMEDDS formulation (without water) showed significantly higher AUC values than the solution (with water) (3.23-fold higher value; $P < 0.01$). Also, the optimized SMEDDS (with water) showed significantly higher AUC values than the solution (with and without water) (3.87-fold; $P < 0.01$ and 2.05-fold; $P < 0.05$ and, respectively). Increasing of AUC could be ascribed to the improvement in solubility and dissolution rate of DN200428 by the optimized SMEDDS. The oral bioavailability of DN200428 in optimized SMEDDS formulation was significantly higher than that of in the solution with and without water after administration. Oral bioavailability of DN200428 in the optimized SMEDDS (without water) was increased 1.63-fold and 2.85-fold compared to that of the solution (without water) and solution (with water), respectively. In addition, oral bioavailability of DN200428 in the optimized SMEDDS (with water) was increased 1.84-fold and 3.22-fold compared to that of the solution (without water) and solution (with water), respectively. From the plasma profiles, the solution with and without water were eliminated faster than the optimized SMEDDS, which could be attributed to the precipitation rate of DN200428. SMEDDS was designed to maintain the solubilized form of the drug for enhancement of drug absorption. Although the $T_{max}$ of the solutions were shorter than those of the optimized SMEDDS indicating faster absorption of DN200428 in the solutions, the precipitation rate of the solutions are

faster than the optimized SMEDDS. Moreover, plasma concentration of DN200428 suspension in all time point were lower than the LLOQ, which suggesting that the compound has low absorption in powder form (data not shown). Therefore, the optimized SMEDDS formulation developed in this study was effective in enhancing the oral absorption of DN200428 by improving the solubility of the compound.

# 4. Conclusion

Optimized DN200428-loaded SMEDDS formulation was successfully developed by using the desirability of D-optimal mixture design. The optimized SMEDDS formulation consisting of Capmul MCM EP (5.0%), Tween 20 (75.0%), and Carbitol (20.0%) showed small homogenous droplets in spherical shape of microemulsion after dilution, and had good solvent properties to maintain DN200428 in solubilized form before absorption. In addition, the desirable *in vitro* experimental responses of the formulation also close to the predicted value. Moreover, the *in vivo* pharmacokinetic studies showed higher bioavailability for optimized SMEDDS formulation than the solution. Thus, this study demonstrates that SMEDDS is a promising approach to enhance the oral bioavailability of DN200428.

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**Table 1 Solubility of DN200428 in selected excipients**

| Excipient | Solubility (mg/mL) |
|---|---|
| **Oil** | |
| Capmul MCM EP | 0.239±0.04 |
| Capryol 90 | 0.191±0.01 |
| Labrafil M2130 CS | 0.136±0.00 |
| Labrafil M2125 CS | 0.097±0.00 |
| Labrafil M1944 CS | 0.054±0.00 |
| Cotton seed oil | 0.004±0.00 |
| Soybean oil | 0.004±0.00 |
| Sunflower seed oil | 0.002±0.00 |
| Ethyl oleate | 0.002±0.00 |
| **Surfactant** | |
| Tween 20 | 7.461±0.76 |
| PEG400 | 5.022±0.20 |
| Labrasol | 3.306±0.20 |
| Tween 80 | 2.284±0.13 |
| Solutol HS 15 | 1.860±0.98 |
| **Co-surfactant** | |
| Carbitol | 6.563±0.11 |
| Span 80 | 0.015±0.01 |

Note: Values are presented as the mean ± SD (n=3)

**Table 2** Variables used in the D-optimal mixture design

| Independent variables | Range (%) | |
|---|---|---|
| | **Minimum** | **Maximum** |
| $X_1$ : Amount of oil (Capmul MCM EP) | 5 | 15 |
| $X_2$ : Surfactant (Tween 20) | 55 | 75 |
| $X_3$ : Co-surfactant (Carbitol) | 15 | 35 |
| **Dependent variables** | | **Goal** |
| $Y_1$ : Drug concentration at 15 min after diluted with SGF (1:250 dilution) (μg/mL; DIL) | | Maximize |
| $Y_2$ : Solubilized capacity (mg/mL; SC) | | Maximize |

**Table 3** Composition and observed responses from randomized runs in the D-optimal mixture design

| Formulation No. | Independent variables | | | Dependent variables (Responses) | |
|---|---|---|---|---|---|
| | Capmul MCM EP (%; $X_1$) | Tween 20 (%; $X_2$) | Carbitol (%; $X_3$) | DIL (μg/mL; $Y_1$) | SC (mg/mL; $Y_2$) |
| 1 | 5 | 65 | 30 | 1.799 ± 0.01 | 6.763 ± 0.04 |
| 2 | 5 | 70 | 25 | 1.893 ± 0.07 | 6.427 ± 0.07 |
| 3 | 5 | 75 | 20 | 2.142 ± 0.10 | 6.229 ± 0.05 |
| 4 | 5 | 75 | 20 | 2.195 ± 0.11 | 6.671 ± 0.06 |
| 5 | 5 | 60 | 35 | 1.576 ± 0.05 | 6.906 ± 0.10 |
| 6 | 5 | 60 | 35 | 1.620 ± 0.02 | 6.671 ± 0.11 |
| 7 | 10 | 75 | 15 | 2.155 ± 0.11 | 5.655 ± 0.04 |
| 8 | 10 | 75 | 15 | 2.048 ± 0.14 | 5.527 ± 0.11 |
| 9 | 10 | 65 | 25 | 1.610 ± 0.08 | 5.736 ± 0.07 |
| 10 | 10 | 60 | 30 | 1.531 ± 0.01 | 5.893 ± 0.10 |
| 11 | 15 | 55 | 30 | 1.402 ± 0.10 | 5.037 ± 0.04 |
| 12 | 15 | 55 | 30 | 1.419 ± 0.10 | 5.058 ± 0.06 |
| 13 | 15 | 60 | 25 | 1.613 ± 0.06 | 4.985 ± 0.02 |
| 14 | 15 | 65 | 20 | 1.597 ± 0.07 | 5.018 ± 0.05 |
| 15 | 15 | 70 | 15 | 1.819 ± 0.06 | 4.754 ± 0.01 |
| 16 | 15 | 70 | 15 | 1.808 ± 0.08 | 4.868 ± 0.02 |

Notes: DIL, Drug Concentration at 15 min after diluted (1:250 dilution) in SGF; SC, Solubilized capacity

**Table 4** Summary of statistical analyses and model equations for the measured responses

| Response | Model | Sequential p-value | Lack of fit p-value | SD | %CV | PRESS | R-squared | Adjusted R-squared | Predicted R-squared | Adequate precision |
|---|---|---|---|---|---|---|---|---|---|---|
| DIL ($Y_1$) | Quadratic | 0.0487 | 0.1596 | 0.06 | 3.10 | 0.69 | 0.9708 | 0.9561 | 0.9327 | 22.120 |
| SC ($Y_2$) | Linear | < 0.0001 | 0.9660 | 0.12 | 2.11 | 0.30 | 0.9779 | 0.9745 | 0.9652 | 37.055 |

**Table 5** Analysis of variance of measured responses

| Response | Source | Sum of squares | df | Mean square | F-value | P-value prob. > F | Remark |
|---|---|---|---|---|---|---|---|
| DIL ($Y_1$) | Model | 0.99 | 5 | 0.20 | 66.39 | < 0.0001 | Significant |
| | Linear mixture | 0.96 | 2 | 0.48 | 160.36 | < 0.0001 | |
| | $X_1X_2$ | 0.010 | 1 | 0.010 | 3.40 | 0.0952 | |
| | $X_1X_3$ | $2.88 \times 10^{-3}$ | 1 | $2.88 \times 10^{-3}$ | 0.97 | 0.3490 | |
| | $X_2X_3$ | 0.015 | 1 | 0.015 | 4.90 | 0.0513 | |
| | Residual | 0.030 | 10 | $2.982 \times 10^{-3}$ | | | |
| | Lack of Fit | 0.022 | 5 | $4.304 \times 10^{-3}$ | 2.59 | 0.1596 | Not significant |
| | Pure Error | $8.302 \times 10^{-3}$ | 5 | $1.660 \times 10^{-3}$ | | | |
| | Corrected total | 1.02 | 15 | | | | |
| SC ($Y_2$) | Model | 8.51 | 2 | 4.25 | 287.41 | < 0.0001 | Significant |
| | Linear Mixture | 8.51 | 2 | 4.25 | 287.41 | < 0.0001 | |
| | Residual | 0.19 | 13 | 0.015 | | | |
| | Lack of Fit | 0.052 | 8 | $6.530 \times 10^{-3}$ | 0.23 | 0.9660 | Not significant |
| | Pure Error | 0.14 | 5 | 0.028 | | | |
| | Corrected total | 8.70 | 15 | | | | |

Note: df, degrees of freedom

**Table 6** Predicted and experimental results of optimized DN200428-loaded SMEDDS formulation

| Response | Predicted value | Experimental value | Prediction error (%) |
|---|---|---|---|
| DIL (μg/mL; $Y_1$) | 2.17 | 2.34 ± 0.21 | - 7.83 |
| SC (mg/mL; $Y_2$) | 6.462 | 6.164 ± 0.06 | 4.61 |

Note: Prediction error (%) was calculated using the formula ([predicted value – experimental value]/predicted value) × 100; values are presented as the mean ± SD (n=3)

**Table 7** Pharmacokinetic parameters of DN200428 after oral administration of the solution and optimized SMEDDS formulation in rats.

| Parameters | Solution (without water) | Solution (with water) | Optimized SMEDDS (without water) | Optimized SMEDDS (with water) |
|---|---|---|---|---|
| $C_{max}$ (ng/mL) | 122.20 ± 38.80 | 108.13 ± 18.53 | 164.27 ± 55.87 | 162.99 ± 14.00 |
| $T_{max}$ (min) | 33.75 ± 7.50 | 33.75 ± 7.50 | 45.00 ± 0.00 | 63.75 ± 18.87[a,b] |
| $AUC_{last}$ (ng·min/mL) | 11044.56 ± 1765.64 | 5849.48 ± 2452.40 | 18868.64 ± 7944.91[b] | 22637.40 ± 3668.47[b,c] |
| $AUC_{inf}$ (ng·min/mL) | 15826.89 ± 2363.08 | 9043.41 ± 2651.75 | 25782.23 ± 9610.42[b] | 29087.39 ± 4946.50[b,c] |
| $t_{1/2}$ (min) | 79.80 ± 33.74 | 43.42 ± 12.56 | 110.76 ± 64.75 | 86.33 ± 19.21 |
| BA (%) | 6.78 | 3.87 | 11.04 | 12.46 |

Note: AUC, area under the curve; SMEDDS, Self-Microemulsifying Drug Delivery System. The data are presented as mean ± SD (n=4)

[a] $P < 0.01$ when compared with the parameter of solution (without water)

[b] $P < 0.01$ when compared with the parameter of solution (with water)

[c] $P < 0.05$ when compared with the parameter of solution (without water)

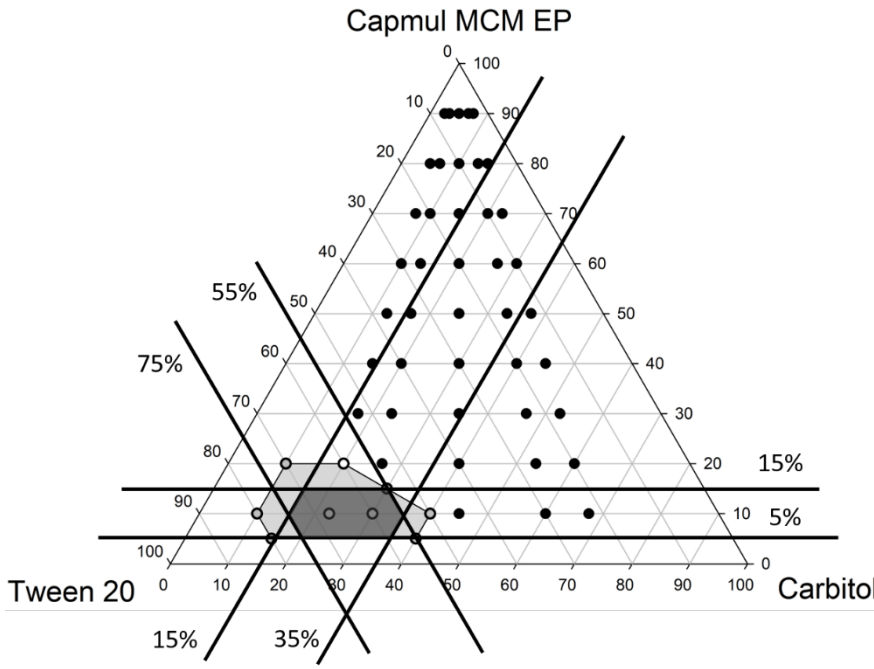



**Figure 1** Pseudoternary phase diagram of Capmul MCM EP (oil), Tween 20 (surfactant), and Carbitol (co-surfactant) and design space for D-optimal mixture design

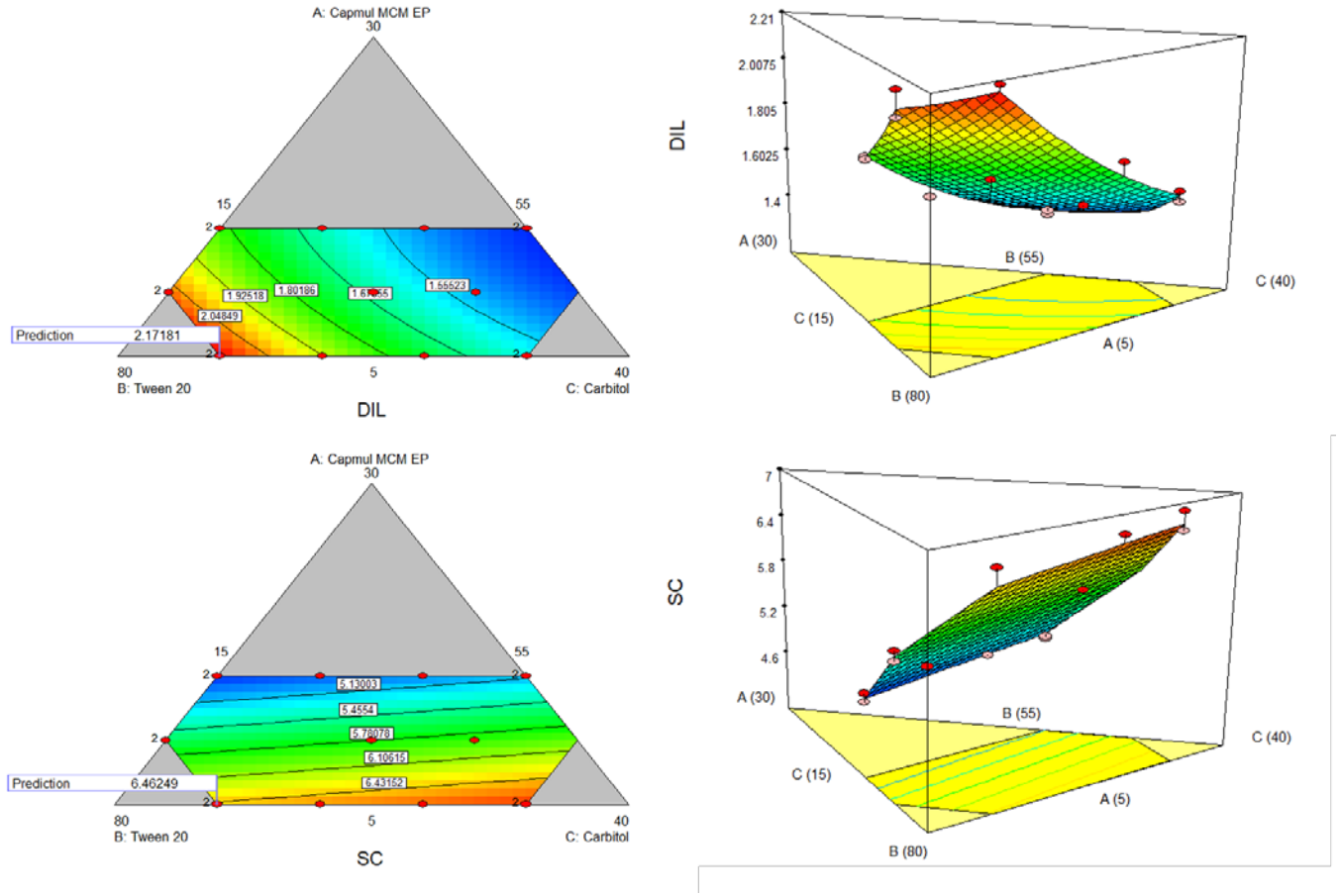



**Figure 2** Contour and response surface plots for DIL and SC

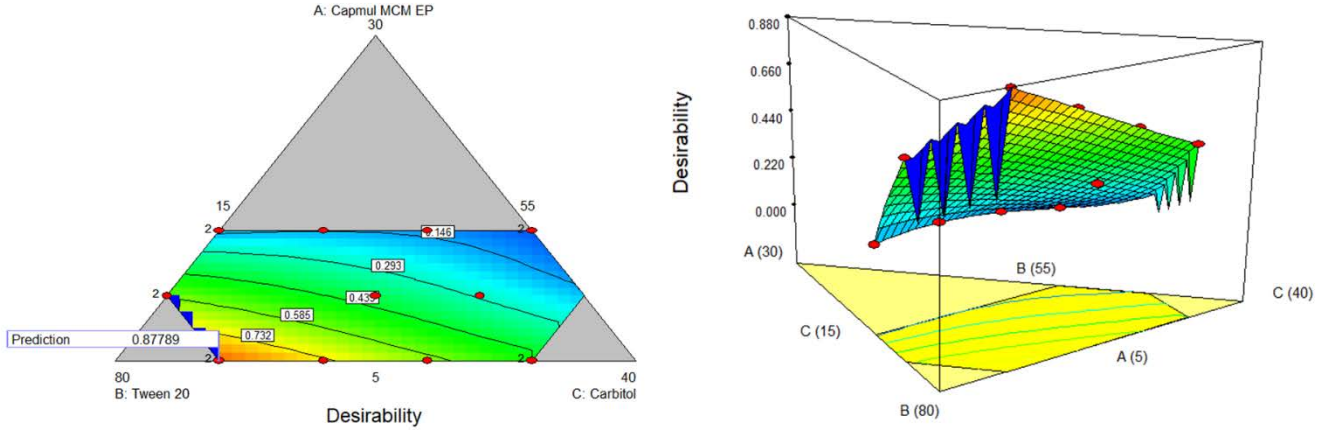
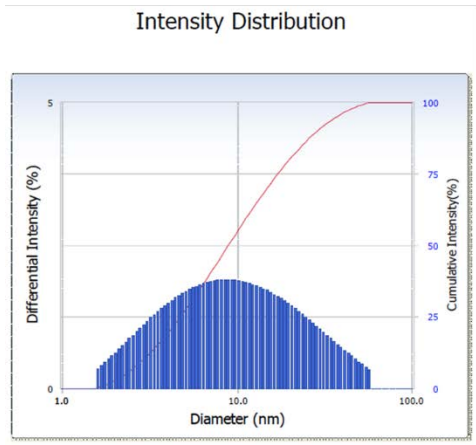



**Figure 3** Contour and response surface plots of numerical optimization of DN200428-loaded SMEDDS using desirability approach



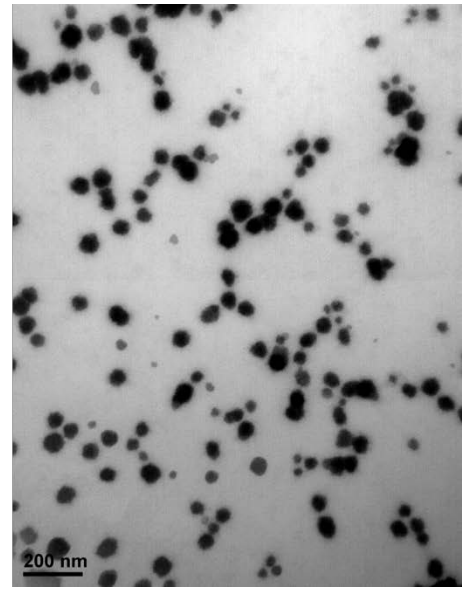
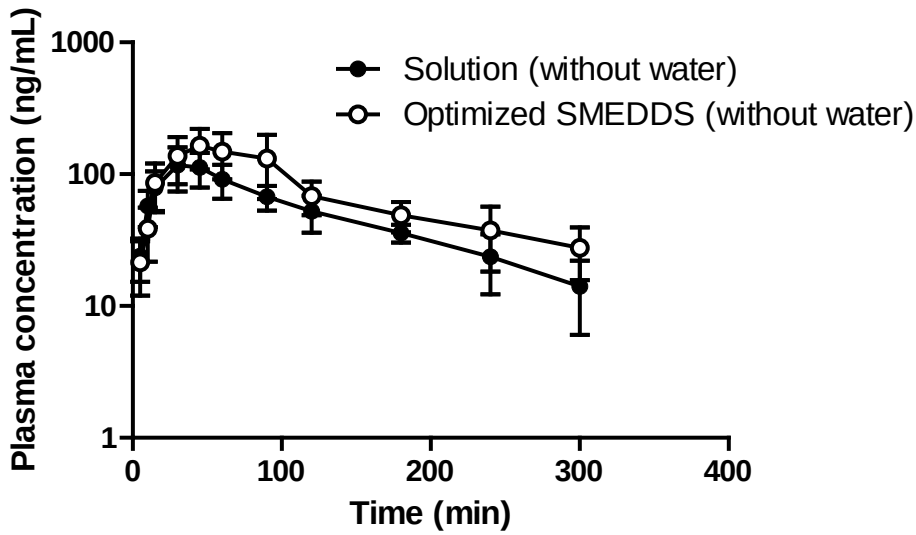




**Figure 4** Droplet size distribution and TEM image of reconstituted numerically optimized DN200428-loaded SMEDDS formulation in water (1:50 dilution)

(a)




(b)

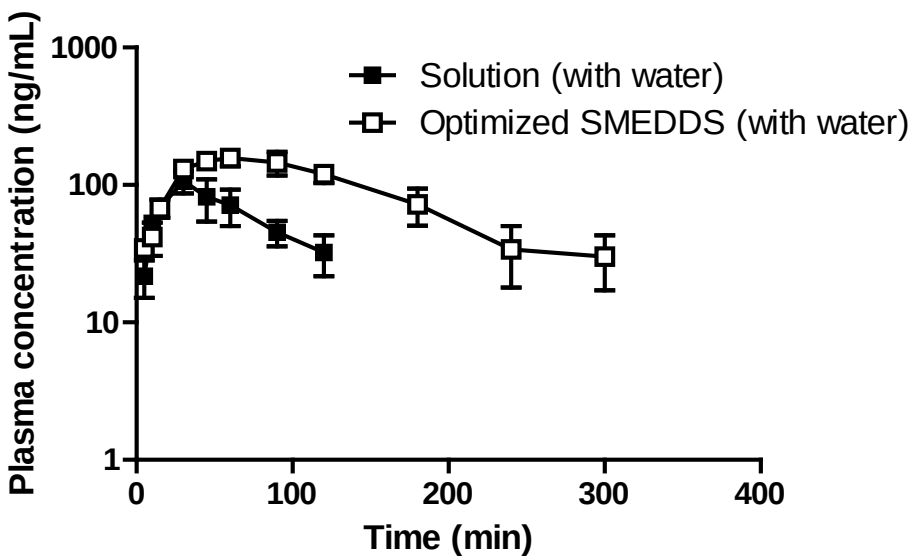



**Figure 5** *In vivo* pharmacokinetic profiles after oral administration of (a) the DN200428 solution (without water) (●) and the optimized DN200428-SMEDDS (without water) (○) (b) the DN200428 solution (with water) (■) and the optimized DN200428-SMEDDS (with water) (□)

# Appendix

**Table I** Droplet size and polydispersity index results from randomized runs in the D-optimal mixture design

| Formulation No. | Independent variables | | | Dependent variables (Responses) | |
|---|---|---|---|---|---|
| | Capmul MCM EP (%; $X_1$) | Tween 20 (%; $X_2$) | Carbitol (%; $X_3$) | DS (nm) | PDI |
| 1 | 5 | 65 | 30 | 12.2 ± 0.8 | 0.144 ± 0.2 |
| 2 | 5 | 70 | 25 | 10.7 ± 6.2 | 0.039 ± 0.0 |
| 3 | 5 | 75 | 20 | 13.3 ± 2.2 | 0.060 ± 0.1 |
| 4 | 5 | 75 | 20 | 11.9 ± 2.9 | 0.031 ± 0.1 |
| 5 | 5 | 60 | 35 | 13.9 ± 2.6 | 0.013 ± 0.1 |
| 6 | 5 | 60 | 35 | 12.7 ± 5.5 | 0.102 ± 0.1 |
| 7 | 10 | 75 | 15 | 12.6 ± 1.3 | 0.161 ± 0.1 |
| 8 | 10 | 75 | 15 | 11.6 ± 2.1 | 0.020 ± 0.0 |
| 9 | 10 | 65 | 25 | 12.7 ± 1.6 | 0.010 ± 0.0 |
| 10 | 10 | 60 | 30 | 10.3 ± 0.1 | 0.104 ± 0.0 |
| 11 | 15 | 55 | 30 | 12.8 ± 1.9 | 0.080 ± 0.0 |
| 12 | 15 | 55 | 30 | 13.4 ± 0.9 | 0.127 ± 0.1 |
| 13 | 15 | 60 | 25 | 12.0 ± 0.2 | 0.119 ± 0.0 |
| 14 | 15 | 65 | 20 | 12.2 ± 1.1 | 0.022 ± 0.0 |
| 15 | 15 | 70 | 15 | 11.7 ± 0.8 | 0.078 ± 0.0 |
| 16 | 15 | 70 | 15 | 11.7 ± 0.5 | 0.016 ± 0.0 |

Notes: DS, Droplet size; PDI, Polydispersity index

# 국문초록

DN200428 은 Cathepsin K 억제 기전으로 골다공증을 치료하기 위해서 설계한 신약후보 물질이다. 그러나, 낮은 용해도 문제로 인해 경구 생체 이용률이 매우 낮을 것으로 예상된다. 본 연구의 목적은 DN200428 의 Self-Microemulsifying Drug Delivery System (SMEDDS) 제형화를 통해 생체 이용률을 개선하는 것이다. 적합한 오일, 계면활성제 및 보조 계면활성제를 선정하기 위해 DN200428 의 용해도 연구를 수행하였으며, 마이크로에멀젼이 형성되는 범위를 알아내고 등장 혼합물에서 성분들의 범위를 결정하기 위해 위상 3 상 상평형도를 작성하였다. 또한 최적의 물리화학적 특성들을 갖는 SMEDDS 제형을 결정하기 위해 D-Optimal mixture design 과 기대함수 (Desirability function)를 도입하였다. 즉, 인공 장액에서 15 분간 희석한 후의 최고 약물 농도와 high solubilized capacity 등을 측정하였다. 체내 약물동태학적 연구는 랫트에서 수행하였으며, 고성능 형광 검출-액체 크로마토그래피 (HPLC-fluorescence)를 사용하여 혈중 약물의 농도를 측정하였다. 최적화된 DN200428 이 봉입된 SMEDDS 제형은 5.0%의 Capmul MCM EP (오일), 75.0%의 Tween 20 (계면활성제) 그리고 20.0%의 Carbitol (보조 계면활성제)로 구성되었다. 최적화된 제형에서 마이크로에멀젼의 크기는 10.7 ± 1.6 nm 로 관찰되었으며, 투과전자현미경 (TEM)을 통해 성상을 확인하였다. 체내 약물동태학적 연구에서, DN200428 이 봉입 된 SMEDDS

제형에서 DMSO:PEG400 (8:92)로 단순히 녹인 용액에 비해 2 배 이상 높아진 상대 경구 생체이용률이 관찰되었다. 결론적으로, DN200428 의 SMEDDS 제형화는 경구 생체이용률을 높일 수 있는 유익한 접근법이 될 것으로 생각된다

# Formulation of Self-Microemulsifying Drug Delivery System (SMEDDS) by D-optimal Mixture Design for Enhancing Oral Bioavailability of DN200428 (Cathepsin K Inhibitor)

**Voradanu Visetvichaporn**

Pharmaceutics, College of Pharmacy
Seoul National University

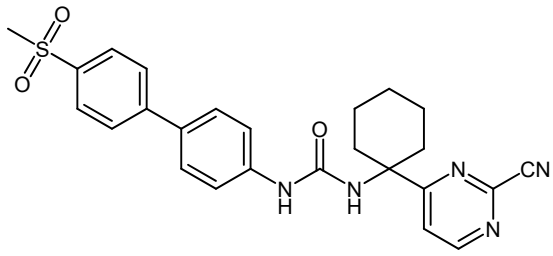
## Contents

I. Background
- DN200428
- Self-microemulsifying Drug Delivery System (SMEDDS)
- Design of Experiment (DoE)

II. Research Plan

I. Background

# DN200428



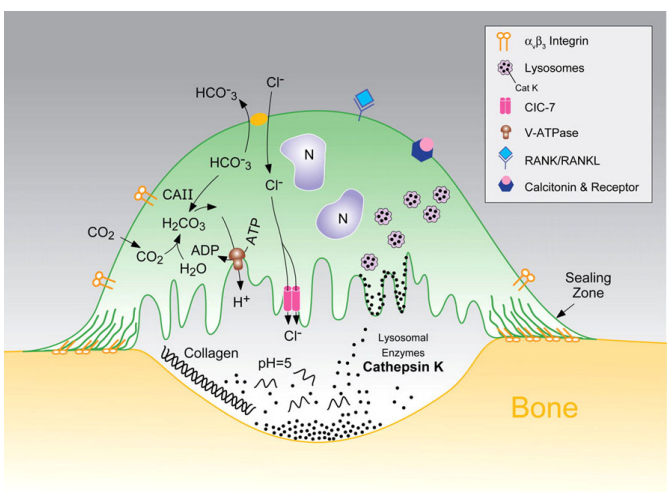
DN200428

- Cathepsin K inhibitor
- Treatment of Osteoporosis
- MW: 475.57
- Purity: 99.51%
- Soluble in DMSO, DMF
- Practically insoluble in water (< 0.1 µg/mL)

I. Background

# Cathepsin K Inhibitor

- Cathepsin K is a cysteine protease found in osteoclasts and involved in bone resorption.
- Inhibition of Cathepsin K decreases bone resorption and can be use for treatment of Osteoporosis




IBMS BoneKEy. 2008 January;5(1):16-24.

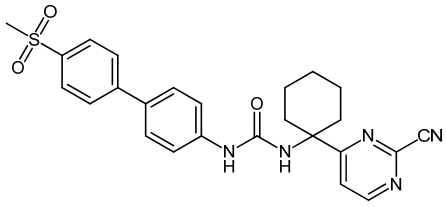
I. Background

## Formulation approaches to improve the oral bioavailability

- Conventional micronization
- Complexation with cyclodextrin
- Salt formation
- Use of surfactants
- Co-solvents
- Nano-and Micro-suspension
- Solid dispersion
- Microprecipitated bulk powder
- Lipid based formulations
  - Proliposome
  - Self-emulsifying Drug Delivery System (SEDDS)
  - Self-microemulsifying Drug Delivery System (SMEDDS)
  - Self-nanoemulsifying Drug Delivery System (SNEDDS)

I. Background

## Strategies for enhancing oral bioavailability of DN200428



DN200428

~~Proliposome~~

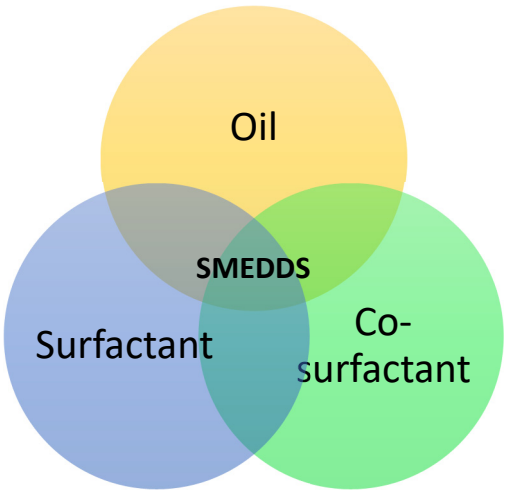
~~Solid Dispersion~~ (SD)

Microprecipitated Bulk ~~Powder~~ (MBP)

Self-microemulsifying Drug Delivery System (SMEDDS)

I. Background

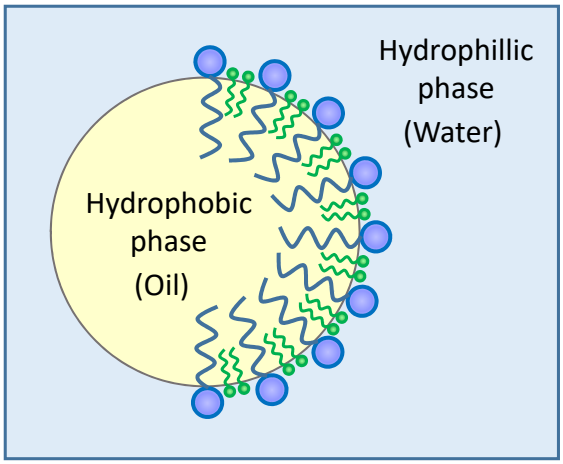
# Self-microemulsifying Drug Delivery System (SMEDDS)




- Isotropic mixture of Oil, Surfactant and Co-surfactant
- **No water in formulation**
- Oral administration:
  Form microemulsion (< 250 nm) in GI tract with mild agitation provided by gastric mobility

I. Background

# Function of Excipients in SMEDDS



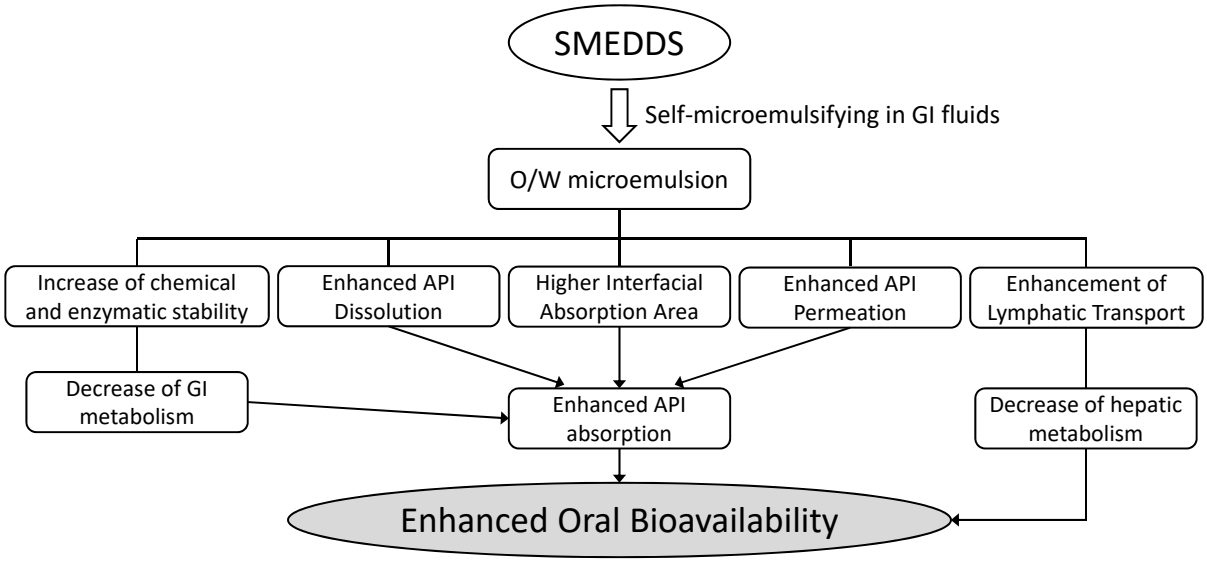

Oil : Solubilizing drug

Surfactant : Form interfacial film

Co-surfactant :
Ensure flexibility of interfacial film

I. Background

## Main factors that influence the bioavailability of SMEDDS




Adapted from Zanchetta et al., J Adv Chem Eng 2015, 5:3.

I. Background

## Design of Experiment (DoE)

Independent factors/ Input variables ⇨ Formulation ⇨ Dependent factors/ Responses

- Change more than one variable at a time
- Find the important and unimportant input variables.
- Predict the performance of formulations without preparing them

**Designs**

- Factorial designs (FD)
- Fractional factorial designs (FFD)
- Plackett–Burman designs
- Optimal designs
- Central composite designs (CCD)
- Box–Behnken designs (BBD)
- Taguchi designs
- Equiradial designs
- Mixture designs (MD)

## II. Research plan

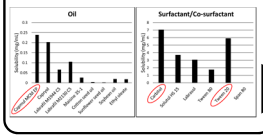
# Objective

- To develop and optimize DN200428-loaded SMEDDS formulation by using the D-optimal mixture design to improve its oral bioavailability.

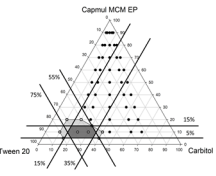
## II. Research plan: **Summary**

**Step 1** Solubility studies

- Capmul MCM EP (Oil)
- Tween 20 (Surfactant)
- Carbitol (Co-surfactant)




**Step 2** Construction of Pseudoternary Phase Diagram




**Step 3** Selection of Design of Experiment (DoE)

D-optimal mixture design

**Step 4** Preparation of DN200428 loaded SMEDDS based on DoE

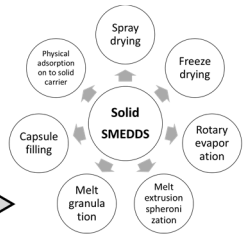
Blank SMEDDS + Drug
↓
Vortex mixing
↓
Shaking in water bath (37°C) for 3 hrs.

**Step 5** *In vitro* evaluation of SMEDDS and optimize formulation

- Drug Conc. at 15 min after diluted with SGF (1:250 dilution)
- Solubilized Capacity

**Step 6** *In vivo* studies

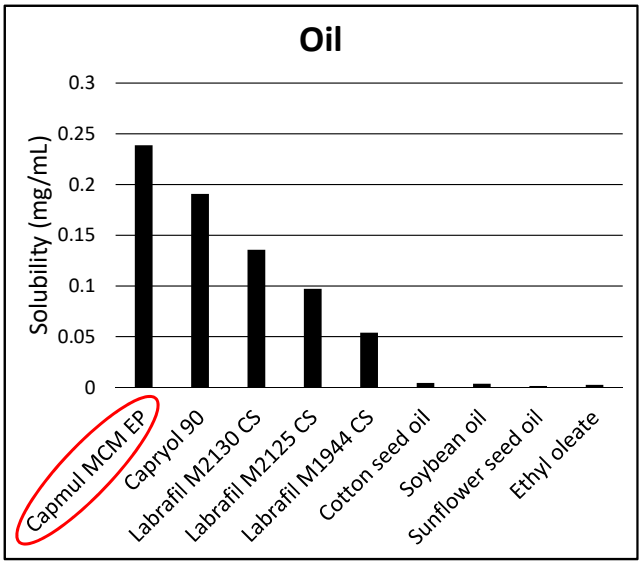
**Step 7** Further studies

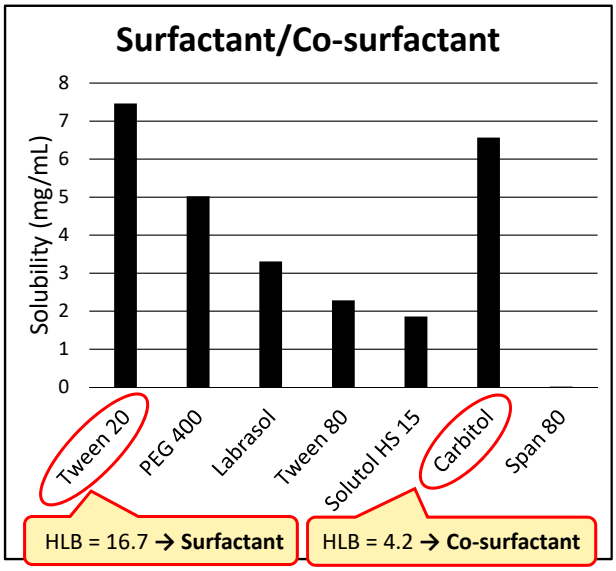


## II. Research plan: Step 1

Recommended HLB Value of Surfactant for SMEDDS: HLB > 12

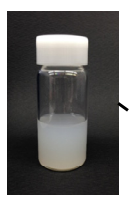
# DN200428: Solubility Studies

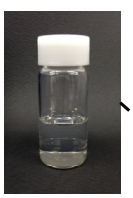



## II. Research plan: Step 3

# Selection of Design Space from Pseudoternary Phase Diagram








- Vary Ratio of Tween20:Carbitol (S-mix) (v/v) to 1:1, 1:2, 1:3, 2:1, 3:1
- Vary Ratio of S-mix:Oil (v/v) to 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1
- Water titration (dropwise) up to 100 times dilution (Visual inspection)

| Independent variables | Range (%) | |
|---|---|---|
| | Min | Max |
| $X_1$ : Amount of oil (Capmul MCM EP) | 5 | 15 |
| $X_2$ : Amount of surfactant (Tween 20) | 55 | 75 |
| $X_3$ : Amount of co-surfactant (Carbitol) | 15 | 35 |

Experimental domain
"Design space"

Microemulsion was formed after 100 times dilution

II. Research plan: **Step 3**

# Selection of Design

## Box-Behnken Design

Table 3 The BBD matrix and the observed responses

| Run | $X_1$ (mg) | $X_2$ (mg) | $X_3$ (mg) |
|---|---|---|---|
| 1 | 20 | 65 | 5 |
| 2 | 30 | 80 | 30 |
| 3[a] | 30 | 65 | 17.5 |
| 4 | 40 | 50 | 17.5 |
| 5 | 40 | 65 | 5 |
| 6 | 30 | 50 | 5 |
| 7 | 30 | 80 | 5 |
| 8[a] | 30 | 65 | 17.5 |
| 9[a] | 30 | 65 | 17.5 |
| 10 | 20 | 50 | 17.5 |
| 11 | 40 | 80 | 17.5 |
| 12 | 40 | 65 | 30 |
| 13[a] | 30 | 65 | 17.5 |
| 14 | 20 | 80 | 17.5 |
| 15 | 20 | 65 | 30 |
| 16 | 30 | 50 | 30 |
| 17[a] | 30 | 65 | 17.5 |

90 mg

112.5 mg

J Pharm Pharmacol. 2013 Oct;65(10):1440-50.

## D-optimal Design

- The D-optimal mixture design considers the total system of SMEDDS as 100%

| Mixture number | Capmul MCM (%; $X_1$) | Tween 20 (%; $X_2$) | Tetraglycol (%; $X_3$) |
|---|---|---|---|
| 1 | 18.30 | 38.24 | 43.46 |
| 2 | 12.20 | 26.49 | 61.32 |
| 3 | 13.71 | 56.29 | 30.00 |
| 4 | 20.00 | 44.44 | 35.56 |
| 5 | 5.71 | 24.29 | 70.00 |
| 6 | 5.11 | 60.00 | 34.89 |
| 7 | 5.46 | 33.57 | 60.97 |
| 8 | 5.11 | 60.00 | 34.89 |
| 9 | 7.71 | 49.47 | 42.82 |
| 10 | 19.38 | 20.00 | 60.63 |
| 11 | 13.71 | 56.29 | 30.00 |
| 12 | 5.71 | 24.29 | 70.00 |
| 13 | 19.38 | 20.00 | 60.63 |
| 14 | 20.00 | 44.44 | 35.56 |
| 15 | 20.00 | 29.10 | 50.90 |
| 16 | 10.80 | 38.83 | 50.37 |

100.00%

100.00%

Int J Nanomedicine. 2015 Jun 5;10:3865-77.

II. Research plan: **Step 3**

# Variables used in the D-optimal mixture design

| Independent variables | Range (%) | |
|---|---|---|
| | Minimum | Maximum |
| $X_1$ : Amount of oil (Capmul MCM EP) | 5 | 15 |
| $X_2$ : Amount of surfactant (Tween 20) | 55 | 75 |
| $X_3$ : Amount of co-surfactant (Carbitol) | 15 | 35 |

| Dependent variables | Goal |
|---|---|
| $Y_1$ : Drug concentration at 15 min after diluted with SGF (1:250 dilution) (DIL; μg/mL) | Maximize |
| $Y_2$ : Solubilized capacity (SC; mg/mL) | Maximize |

## II. Research plan: **Step 4**

| Formulation No. | Variables | | |
|---|---|---|---|
| | Capmul MCM EP (%; $X_1$) | Tween 20 (%; $X_2$) | Carbitol (%; $X_3$) |
| 1 | 5 | 65 | 30 |
| 2 | 5 | 70 | 25 |
| 3 | 5 | 75 | 20 |
| 4 | 5 | 75 | 20 |
| 5 | 5 | 60 | 35 |
| 6 | 5 | 60 | 35 |
| 7 | 10 | 75 | 15 |
| 8 | 10 | 75 | 15 |
| 9 | 10 | 65 | 25 |
| 10 | 10 | 60 | 30 |
| 11 | 10 | 55 | 30 |
| 12 | 15 | 55 | 30 |
| 13 | 15 | 60 | 25 |
| 14 | 15 | 65 | 20 |
| 15 | 15 | 70 | 15 |
| 16 | 15 | 70 | 15 |

Composition from randomized runs in the D-optimal mixture design by Design Expert®

Preparation of DN200428 loaded SMEDDS

Blank SMEDDS + Drug

↓

Vortex mixing

↓

Shaking in water bath (37°C) for 3 hrs.

⇩

**Step 5**

*In vitro* evaluation of SMEDDS and optimize formulation

## II. Research plan: **Step 5**

Composition and observed responses from randomized runs in the D-optimal mixture design

| Formulation No. | Independent variables | | | Dependent variables | |
|---|---|---|---|---|---|
| | Capmul MCM EP (%; $X_1$) | Tween 20 (%; $X_2$) | Carbitol (%; $X_3$) | Drug concentration at 15 min after diluted with SGF (1:250 dilution) (DIL) (µg/mL; $Y_1$) | Solubilized capacity (SC) (mg/mL; $Y_2$) |
| 1 | 5 | 65 | 30 | 1.799 ± 0.01 | 6.763 ± 0.04 |
| 2 | 5 | 70 | 25 | 1.893 ± 0.07 | 6.427 ± 0.07 |
| 3 | 5 | 75 | 20 | 2.142 ± 0.10 | 6.229 ± 0.05 |
| 4 | 5 | 75 | 20 | 2.195 ± 0.11 | 6.671 ± 0.06 |
| 5 | 5 | 60 | 35 | 1.576 ± 0.05 | 6.906 ± 0.10 |
| 6 | 5 | 60 | 35 | 1.620 ± 0.02 | 6.671 ± 0.11 |
| 7 | 10 | 75 | 15 | 2.155 ± 0.11 | 5.655 ± 0.04 |
| 8 | 10 | 75 | 15 | 2.048 ± 0.14 | 5.527 ± 0.11 |
| 9 | 10 | 65 | 25 | 1.610 ± 0.08 | 5.736 ± 0.07 |
| 10 | 10 | 60 | 30 | 1.531 ± 0.01 | 5.893 ± 0.10 |
| 11 | 15 | 55 | 30 | 1.402 ± 0.10 | 5.037 ± 0.04 |
| 12 | 15 | 55 | 30 | 1.419 ± 0.10 | 5.058 ± 0.06 |
| 13 | 15 | 60 | 25 | 1.613 ± 0.06 | 4.985 ± 0.02 |
| 14 | 15 | 65 | 20 | 1.597 ± 0.07 | 5.018 ± 0.05 |
| 15 | 15 | 70 | 15 | 1.819 ± 0.06 | 4.754 ± 0.01 |
| 16 | 15 | 70 | 15 | 1.808 ± 0.08 | 4.868 ± 0.02 |

**II. Research plan: Step 5** **Fitting model**

## Summary of the results of statistical analyses and model equations for the measured responses in the D-optimal mixture design

| Model selection criteria | < 0.05 | > 0.05 | | | Max | Max | > 4 | |
|---|---|---|---|---|---|---|---|---|
| **Model** | **Sequential p-value** | **Lack of fit p-value** | **Standard deviation** | **R-squared** | **Adjusted R-squared** | **Predicted R-squared** | **Adequate precision** | **Remark** |
| **Drug concentration at 15 min after diluted with SGF (1:250 dilution) (DIL) (µg/mL; $Y_1$)** | | | | | | | | |
| Linear | < 0.0001 | 0.6740 | 0.07 | 0.9379 | 0.9283 | 0.9112 | - | - |
| Quadratic | 0.0487 | 0.1596 | 0.06 | 0.9708 | 0.9561 | 0.9327 | 22.120 | Suggested |
| Special cubic | 0.6748 | 0.1201 | 0.06 | 0.9714 | 0.9523 | 0.9118 | - | - |
| Cubic | 0.0189 | 0.7338 | 0.04 | 0.9908 | 0.9803 | 0.9572 | - | Aliased |
| **Solubilized capacity (SC) (mg/mL; $Y_2$)** | | | | | | | | |
| Linear | < 0.0001 | 0.9660 | 0.12 | 0.9779 | 0.9745 | 0.9652 | 37.055 | Suggested |
| Quadratic | 0.8681 | 0.9049 | 0.13 | 0.9794 | 0.9690 | 0.9472 | - | - |
| Special cubic | 0.5842 | 0.8690 | 0.14 | 0.9801 | 0.9668 | 0.9383 | - | - |
| Cubic | 0.8759 | 0.6459 | 0.15 | 0.9808 | 0.9589 | 0.9094 | - | Aliased |

**II. Research plan: Step 5** "Relationship between input variables (X) and responses (Y)"

## Construction of Equations Based On The Results

When $X_1$= Capmul MCM EP
$X_2$= Tween 20
$X_3$= Carbitol

**$Y_1$ : Drug Concentration at 15 min after diluted with SGF (1:250 dilution)** **Model:** Quadratic

$$Y_1 = +1.80\,X_1 + 2.47\,X_2 + 1.52\,X_3 - 1.57\,X_1X_2 - 0.84\,X_1X_3 - 0.66\,X_2X_3$$

**$Y_2$ : Solubilized capacity (µg/mL)** **Model:** Linear

$$Y_2 = +2.47\,X_1 + 6.36\,X_2 + 6.86\,X_3$$

## II. Research plan: **Step 5**

# Optimization in Design Expert®

Criteria | Solutions | Graphs

Solutions 1

**Constraints**

| Name | Goal | Lower Limit | Upper Limit | Lower Weight | Upper Weight | Importance |
|---|---|---|---|---|---|---|
| Capmul MCM EP | is in range | 5 | 15 | 1 | 1 | 3 |
| Tween 20 | is in range | 55 | 75 | 1 | 1 | 3 |
| Carbitol | is in range | 15 | 35 | 1 | 1 | 3 |
| DIL | maximize | 1.402 | 2.195 | 1 | 1 | 3 |
| SC | maximize | 4.754 | 6.906 | 1 | 1 | 3 |

**Solutions**

| Number | Capmul MCM EP | Tween 20 | Carbitol | DIL | SC | Desirability | |
|---|---|---|---|---|---|---|---|
| 1 | 5.000 | 75.000 | 20.000 | 2.17181 | 6.46249 | 0.878 | Selected |

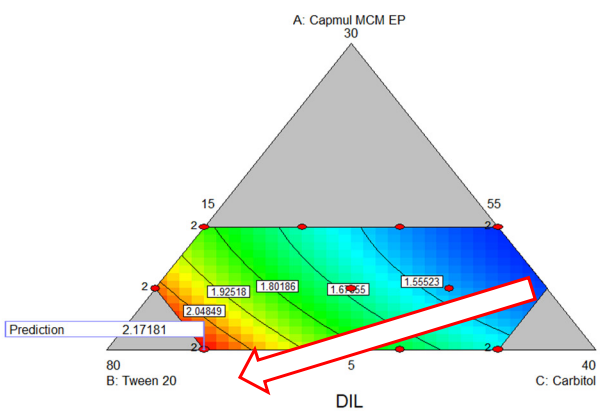
**1 Solutions found**

Optimized SMEDDS formulation

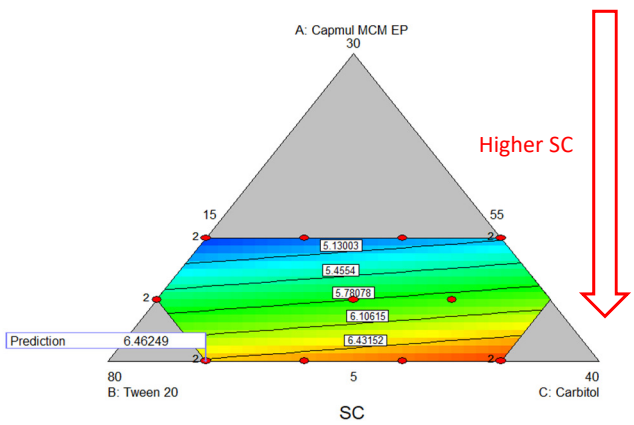
Predicted responses

## II. Research plan: **Step 5**

# Contour plots for DIL and SC




Higher DIL



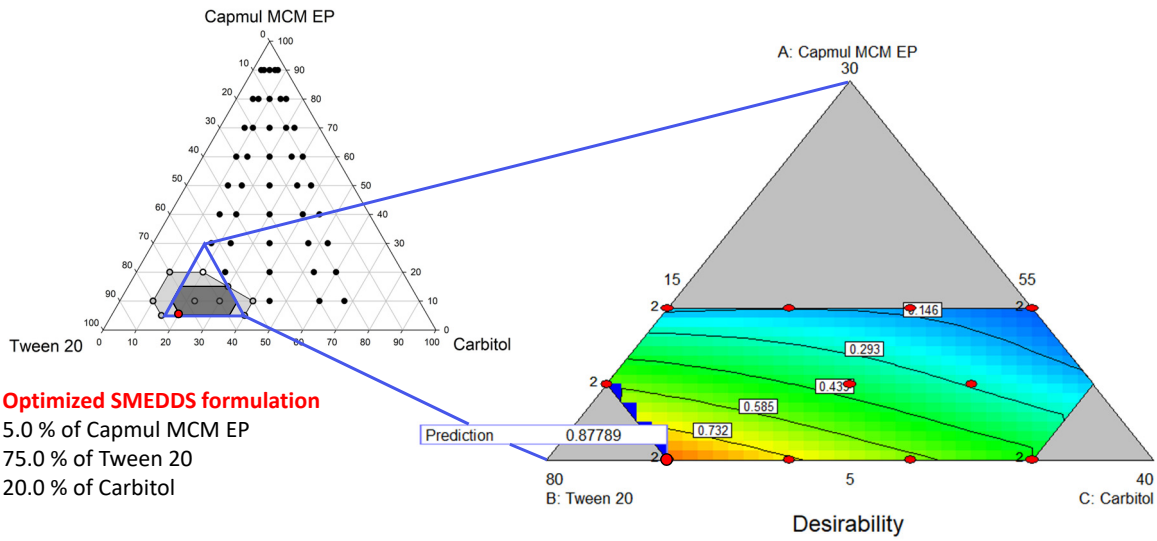

Higher SC

$$Y_1 = +1.80\,X_1 + 2.47\,X_2 + 1.52\,X_3 - 1.57\,X_1X_2 - 0.84\,X_1X_3 - 0.66\,X_2X_3$$

$$Y_2 = +2.47\,X_1 + 6.36\,X_2 + 6.86\,X_3$$

**II. Research plan: Step 5**

# Contour Plot for Desirability




**Optimized SMEDDS formulation**
5.0 % of Capmul MCM EP
75.0 % of Tween 20
20.0 % of Carbitol

**II. Research plan: Step 5**

# *In Vitro* Results of Optimized Formulation

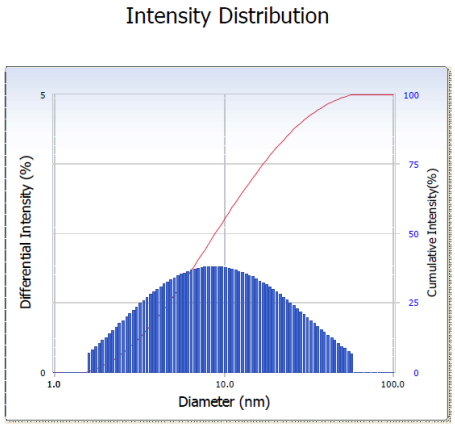
| Response | Predicted value | Experimental value | Prediction error (%) |
|---|---|---|---|
| **Droplet size (nm)** | - | 10.7 ± 1.6 | - |
| **Polydispersity index** | - | 0.006 ± 0.0 | - |
| **DIL (µg/mL; $Y_1$)** | 2.17 | 2.34 ± 0.21 | 7.26 |
| **SC (mg/mL; $Y_2$)** | 6.462 | 6.164 ± 0.06 | -4.83 |

Note: values are presented as the mean ± SD (n=3)

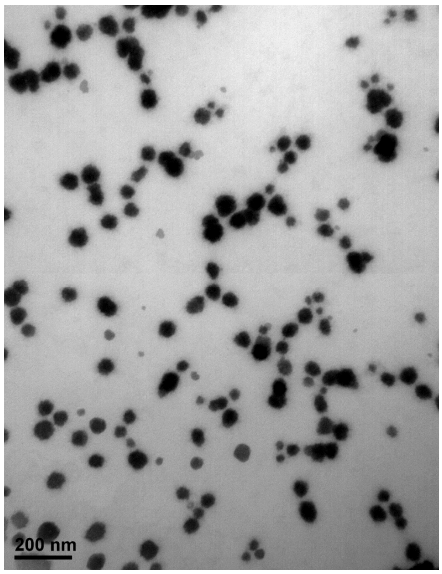
$$\text{Prediction error (\%)} = \frac{(\text{Predicted value} - \text{Experimental value})}{\text{Predicted value}} \times 100$$

## II. Research plan: Step 5

# Droplet size and morphology of reconstituted SMEDDS



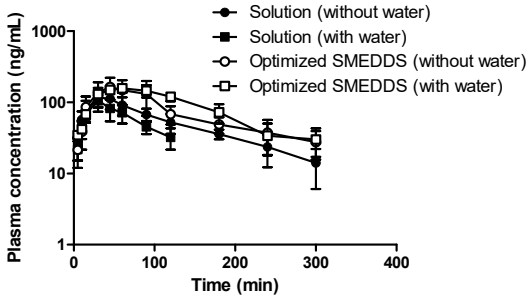

**a)** **Droplet size distribution** of reconstituted optimized SMEDDS formulation in water (1:50 dilution)

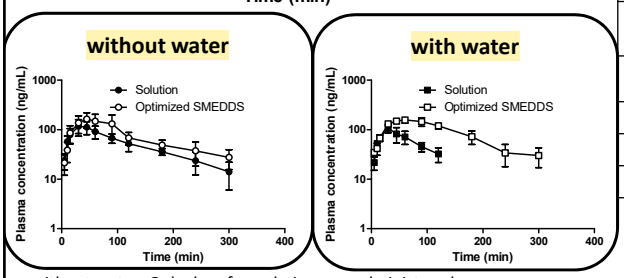



**b)** **TEM image** of reconstituted optimized SMEDDS formulation in water (1:50 dilution)

## II. Research plan: Step 6

# *In Vivo* Results of Optimized Formulation




without water: Only drug formulation was administered
with water: 1 mL of water was immediately administered after drug formulation

| Parameters | IV (5 mg/kg) | PO (5 mg/kg) | | | |
|---|---|---|---|---|---|
| | Solution (DMSO:PEG400) (8:92) | Solution (w/o water) | Solution (w/ water) | Optimized SMEDDS (w/o water) | Optimized SMEDDS (w/ water) |
| $C_{max}$ (ng/mL) | - | 122.20 ± 38.80 | 108.13 ± 18.53 | 164.27 ± 55.87 | 162.99 ± 14.00 |
| $T_{max}$ (min) | - | 33.75 ± 7.50 | 33.75 ± 7.50 | 45.00 ± 0.00 | 63.75 ± 18.87[a,b] |
| $AUC_{last}$ (ng·min/mL) | 226709.85 ± 24604.56 | 11044.56 ± 1765.64 | 5849.48 ± 2452.40 | 18868.64 ± 7944.91[b] | 22637.40 ± 3668.47[b,c] |
| $AUC_{inf}$ (ng·min/mL) | 233448.19 ± 28429.79 | 15826.89 ± 2363.08 | 9043.41 ± 2651.75 | 25782.23 ± 9610.42[b] | 29087.39 ± 4946.50[b,c] |
| $t_{1/2}$ (min) | 93.59 ± 44.86 | 79.80 ± 33.74 | 43.42 ± 12.56 | 110.76 ± 64.75 | 86.33 ± 19.21 |
| BA (%) | - | **6.78** | **3.87** | **11.04** | **12.46** |
| CL (mL/min/kg) | 21.66 ± 2.61 | | | | |
| Vss (mL/kg) | 1895.70 ± 386.86 | | | | |

BA increased 3.22-fold

Notes: [a] $P < 0.01$ when compared with the parameter of solution (w/o water)
[b] $P < 0.01$ when compared with the parameter of solution (w/ water)
[c] $P < 0.05$ when compared with the parameter of solution (w/o water)
DN200428 suspension profiles < LLOQ (data not shown)

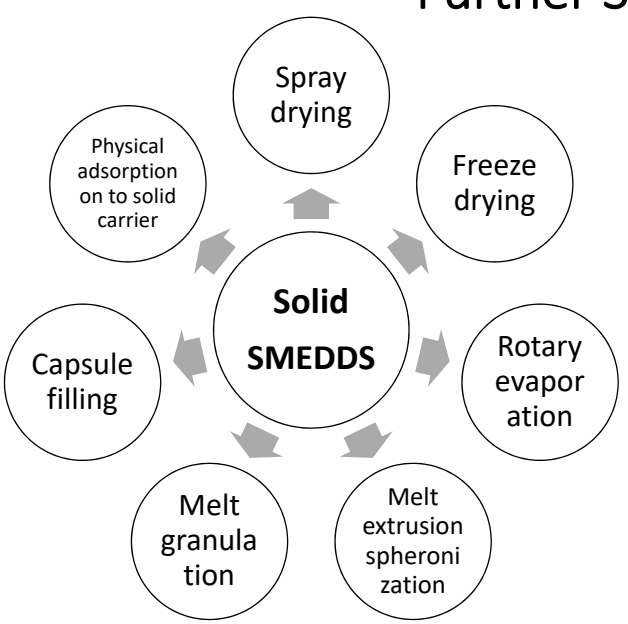
II. Research plan: **Step 6**

# Conclusion

- The result of this study demonstrates that SMEDDS is a promising approach to enhance the oral bioavailability of DN200428

II. Research plan: **Step 7**

# Further Studies




- Solid SMEDDS
- Thermodynamic stability studies
  - Heating Cooling Cycle
  - Centrifugation
  - Freeze Thaw Cycle

# Acknowledgement

This thesis would not have been possible without the guidance and support of many people.

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I will cherish all the memories forever!

January, 2018

Voradanu Visetvichaporn (보라다누)