Design and Optimization of Self Nano-Emulsifying Drug Delivery System Containing a New Anti-inflammatory Agent Pentagamavunon-0

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ABSTRACT

Pentagamavunon-0 is a potent anti-inflammatory drug. The oral bioavailability of PGV-0 is very low due to its low solubility in water. The aim of this study is to design and optimize SNEDDS formulation to improve dissolution of PGV-0 by Simplex Lattice Design. The independent variables were the amounts of oleic acid (X_1), Tween 20 and labrasol (X_2), and PEG 400 (X_3). The dependent variables were droplet size (Y_1), the concentration of PGV-0 dissolved in the 45th min (C_{45}) (Y_2) and solubility of PGV-0 (Y_3) fitted to a quadratic model. The equation, contour plots, and overlay plot were constructed to determine the optimum formulation and to understand the responses to various combinations of components. The optimum formulation of SNEDDS consists of 18.6% oleic acid, 51.4% Tween 20:labrasol 1:1 and 30% PEG 400. The C_{45} of the optimum formulation is 82.20%, significantly higher than unmodified PGV-0. The droplet size is 75.45 nm and solubility of PGV-0 is 31.80 mg/mL. The predicted values are not significantly different with the experimental values. The amount of oleic acid is the most influential factor to increase droplet size and decrease the C_{45}. The most-influential factor to increased C_{45} is the amount of PEG 400.

Keywords: *pentagamavunon-0; SNEDDS; simplex lattice design*

ABSTRAK

Pentagamavunon-0 merupakan obat anti-inflamasi yang poten. Ketersediaan hayati PGV-0 sangat rendah dikarenakan kelarutannya yang rendah dalam air. Tujuan studi ini adalah untuk mendesain dan mengoptimasi sebuah formula SNEDDS untuk meperbaiki disolusi PGV-0 dengan menggunakan Simplex Lattice Design. sebagai variabel tetap adalah jumlah asam oleat (X_1), Tween 20 dan labrasol (X_2), dan PEG 400 (X_3). Sebagai variabel tergantung adalah ukuran droplet (Y_1), konsentrasi PGV-0 yang terlarut pada menit ke-45 (C_{45}) (Y_2) dan kelarutan PGV-0 (Y_3) dipasangkan ke suatu model kuadratik. Persamaan, contour plot dan overlay plot dibuat untuk menentukan formula optimum dan untuk memahami efek berbagai kombinasi terhadap respon. Formula optimum SNEDDS terdiri dari 18,6% asam oleat, 51,4% Tween 20:labrasol 1:1 dan 30% PEG 400. C_{45} formula optimum 82,20%, secara bermakna lebih tinggi daripada PGV-0 yang belum diubah. Ukuran droplet 75.45 nm dan kelarutan PGV-0 31,80 mg/mL. Nilai perkiraan respon tidak berbeda bermakna dengan nilai respon hasil percobaan. Jumlah asam oleat merupakan faktor yang paling berpengaruh untuk meningkatkan ukuran droplet dan menurunkan C_{45}. Faktor yang paling berpengaruh untuk meningkatkan C_{45} adalah jumlah PEG 400.

Kata Kunci: *pentagamavunon-0; SNEDDS; simplex lattice design*

INTRODUCTION

Pentagamavunon-0 has potent anti-inflammatory and anti-allergic activity [1-3]. It is classified as practically insoluble drug in water and has low intestinal permeability [4]. Various strategies for improving solubility of PGV-0 and thereby the anti-inflammatory effect were PGV-0 salts synthesis and solid dispersion [5-7].

The previous techniques have shown limited utility. Salt formation of a drug candidate, especially if prepared with an organic solvent, often exhibit longer dissolution time because when the salt face aqueous environment from water or humidity, the salt become unstable or converted to free acid or base form [8]. Solid dispersion technique has a good potential but the amorphous state of the drug may undergo crystallization to achieve its more stable form during manufacturing and storage [9]. The crystallization process may cause a decrease in solubility and

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dissolution time [10].

SEDDS have minimal instability problem because after given orally, the drug will be dispersed spontaneously in the gastrointestinal fluid yielding micro- or nanoemulsion, lead to surface area expansion to facilitate drug absorption in high level quickly. Dissolution time and bioavailability enhancement of drug when formulated as oral SEDDS showed by β-lactamase and curcumin [11-12]. The intestinal absorption of curcumin-loaded SNEDDS was 3.86 times than curcumin-suspension. However, research about PGV-0-loaded SNEDDS was never yet reported.

Development of SNEDDS by following ~~a~~ a trial and error approach is highly time-consuming and cost ineffective. This approach is blending various excipients (factors) in different ratios, then the formulations studied for key performances. A most satisfactory result can be obtained, but better formulations may exist in other compositions, so an appropriate experimental design needed for optimization of SNEDDS.

Simplex lattice design (SLD) is one of standard mixture design that widely used for formulation optimization. The SLD for three components system represented by three-equilateral triangle. The design points (x) of a {q,m} SLD for q components and m levels are fall in the boundaries and lattices defined by following coordinate setting: x_i = 0, 1/m, 2/m, ..., 1 for I = 1, 2, ..., q. Typically in the SLD the fractions of independent factors must sum to one ($x_1 + x_2 + ... + x_q$ = 1). The responses of each formulation are fitted to a most suitable model. A mathematical equation can be obtained to predict the response of any mixture and determine the influence of relative proportion of each component on the response singly or in combination with other components. The SLD was successfully used to optimize curcumin-loaded SNEDDS and described the influence of the components on the dependent variables, as well as apigenin [12-13]. The aims of our study are to optimize PGV-0-loaded SNEDDS by applying the SLD and to investigate the main effect, interaction effect, and quadratic effect of the formulation factors (oil, surfactant, and cosurfactant amount) on two responses: droplet size and concentration of PGV-0 dissolved in the 45th min (C_{45}).

EXPERIMENTAL SECTION

Materials

PGV-0 was obtained from Curcumin Research Center UGM. Soybean oil, corn oil, VCO, olive oil, PEG 400, span 80, Tween 80, and Tween 20 were purchased from Bratachem (Purwokerto). Labrafil, labrasol, and transcutol were kindly provided by Gattefosse (France) via PT Mensa Group (Jakarta). Kolliphor and myritol were purchased from PT BASF Indonesia (Jakarta). All excipients were pharmaceutical grade.

Instrumentation

UV-Vis Spectrophotometer (Shimadzu) was used for solubility and dissolution studies. Particle size analyzer/PSA (Malvern zetasizer) was used to measure droplet size. A magnetic stirrer (Ika RH Basic I), a vortex mixer (Genie), and glassware were used for SNEDDS and sample preparation.

Procedure

Preliminary studies

Solubility studies

An excessive amount of PGV-0 that accurately weighed was added to an accurately measured volume of vehicles (oils, surfactants, or cosurfactants), vortexed for 5 minutes, and stirred for 24 hours. After allowed for 1 day at the room temperature, the mixture was separated by ultracentrifugation. The supernatant was separated by decantation. The precipitate was washed with the water until the rinse water became clear and colorless. The supernatant was diluted with methanol and the concentration of PGV-0 was quantified by UV-Vis spectrophotometric. This analysis was performed in triplicate. One or two of oil, surfactant, and cosurfactant that showed highest PGV-0 solubility capability was used to further study.

Compatibility test of oils-surfactants-cosurfactants combination

The compatibility of surfactant and cosurfactant in the ratio of 1:1, 1:2, 1:3, 2:3, 3:2, 3:1, and 2:1 were observed visually for 3 days. The surfactant-cosurfactant mixtures that showed most widely miscibility area were used to surfactant-cosurfactant-oil compatibility test. SNEDDS were prepared by mixed oil, surfactant, and cosurfactant using vortex mixer, in the concentration ratio of 30-65% surfactant, 30-65% cosurfactant, and 5-40% oil to construct a pseudoternary phase diagram.

Construction of the pseudoternary phase diagram

The compositions of SNEDDS which tend to form nanoemulsion predicted by pseudoternary phase diagram. Five hundred microliters of each SNEDDS was quickly added to 500 mL of aquadest then mixed by magnetic stirrer. The emulsification time was determined from the time of SNEDDS dripping until the SNEDDS emulsified completely that was marked by a change of turbid or cloudy emulsion to transparent or translucent one. The mixtures which showed

emulsification time ≤ 2 min and transparent or translucent without any immediate coalescence were considered as nanoemulsion.

Design of experiment for formulation

Design-Expert software (Design-Expert 7.1.5; Stat-Ease Inc., Minneapolis, Minnesota) was used as statistical tools to facilitate computation and evaluation of the three-factors Simplex Lattice Design. Based on pseudoternary phase diagram and the reported of the range concentration of oil-surfactant-cosurfactant formed SNEDDS [12], the composition of each component as independent factors were selected as follow: oil (X_1) 5-40% oil, surfactant (X_2) 30-65%, and cosurfactant (X_2) 30-65%.

Preparation of PGV-0 loaded SNEDDS

Solubility studies of PGV-0 in SNEDDS formulation

Oil, surfactant, and cosurfactant in various proportion predetermined by the design were mixed in a microtube using a vortex mixer to made SNEDDS blank formulations. The solubility of PGV-0 in each SNEDDS formulation was determined by UV-Vis spectrophotometric method.

Formulation of PGV-0 loaded SNEDDS

SNEDDS blank formulations were prepared as described above, then 16.35 mg of PGV-0 was added to the SNEDDS. The mixtures were homogenized by a vortex mixer for 1 min followed by sonication for 5 min until all particles of PGV-0 disappeared and the mixture became transparent.

Formulation optimization of PGV-0 loaded SNEDDS

Droplet size measurement

The nanoemulsion samples were prepared by adding 100 µL of formulations to 100 m of aquadest, mixed by magnetic stirrer until emulsification point achieved. Droplet size of the samples was measured by a dynamic light scattering particle size analyzer (Malvern Instrument Ltd) at a wavelength of 633 nm, 25 °C with a scattering angle of 90°.

Dissolution studies

PGV-0 release studies from SNEDDS were performed using dissolution apparatus type II with artificial gastric fluid (AGF) pH 1.2 as dissolution medium. Five hundred microliters of each formulation of PGV-0 loaded SNEDDS were filled in hard gelatin capsules size ”0”. The capsule was introduced to 500 mL of AGF at a temperature of 37 ± 0.5 °C and a revolution speed of the paddle at 50 rpm. At predetermined time intervals, 5 mL of sample aliquot was withdrawn and immediately replaced with an equivalent volume of fresh AGF (37 ± 0.5 °C). The sample was filtered through 0.45 µm membrane filter, then the PGV-0 content analyzed by spectrophotometer UV-VIS at a wavelength of 422 nm.

Analysis and modeling of mixture

The each response data were fitted to a canonical polynomial model explained by equation:

$$Y = b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3$$

where Y is the response, b_i is the expected coefficient for the factor X_i (i = 1,2,3 for pure mixture; 12, 13, 23 for binary mixture and 123 for ternary mixture). The optimum formulation area and the effects of the various combinations on the responses predict by the equation, contour plots, and overlay plot.

Prediction of optimum formulation based on desirability function

In the present study, two responses were optimized simultaneously. To obtain the most favorable compromising area for each of the responses objectively, a desirability function using Design-Expert software can be used since it uses the numerical optimization method.

Optimum formulation verification

Through the optimization process by the software, the point prediction of each response was obtained. The predicted values were verified experimentally by prepared the optimum formulation, measured its droplet size, C_{45} and solubility of PGV-0 and analyzed by one-sample t-test.

Data analysis

The response data (Y) of each composition (X_i) analyzed by Design-Expert software to obtain polynomial equations with an appropriate model for the system being studied. Ideally, the best suitable model should have the highest order polynomial where the additional terms are significant (p-value < 0.05) and the model is not aliased. It is obtained from the sequential model sum of squares (type 1) parameter. The selected model has an insignificant lack of fit too and the adjusted multiple correlation coefficients (R2) and predicted R2 are higher compared to the other models.

After selecting the best model, Design-Expert software will provide analysis of variance of the model and its lack of fit (Partial sum of squares - Type III), (R^2), and estimates the coefficient of the factor. The values of the coefficients are related to the effect of the

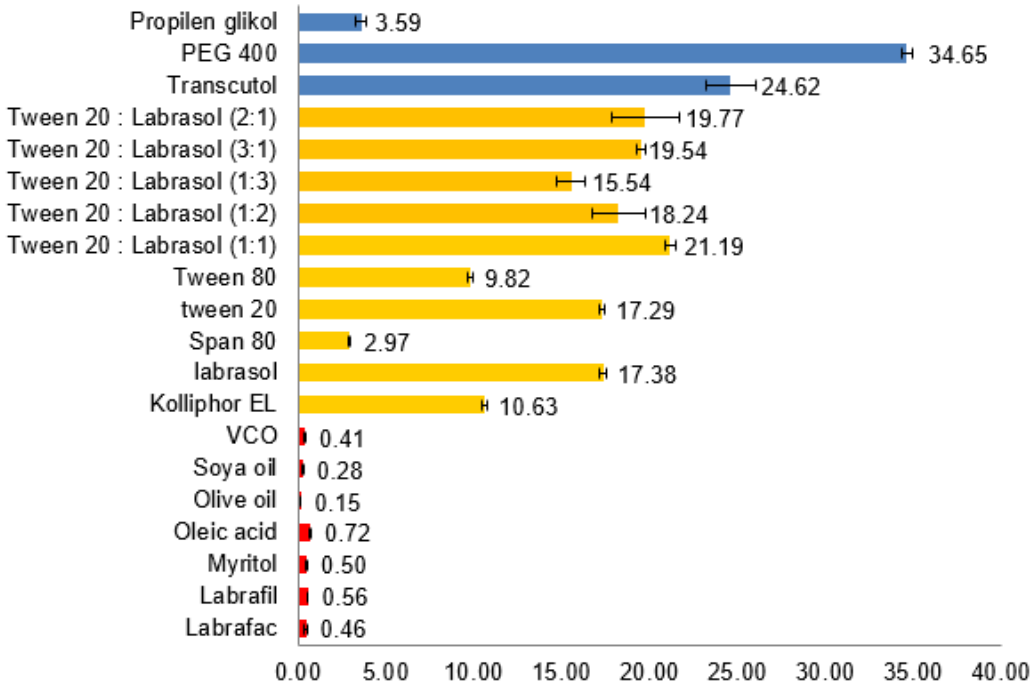

Fig 1. PGV-0 solubility in various vehicle

factors on the response. The larger coefficient means the more influential of the factor. Coefficients with only one term (b_1, b_2, and b_3) represent the main effects. Coefficients with more than one term (example b_1 and b_2) and higher order, means the interactive effect and quadratic effect, respectively. A positive sign indicates a synergistic effect, on the contrary, a negative sign indicates an antagonistic effect.

From the optimization process, the point prediction of the optimum formula will be obtained. The difference between experimental data and estimated data analyzed statistically by one sample t-test analysis using IBM SPSS statistic 23 software.

RESULT AND DISCUSSION

Preliminary Studies

The solubility of PGV-0 in oils, surfactants, and cosurfactants is presented in Fig. 1. In the recent study, among seven tested oils, oleic acid showed the highest capability to dissolve PGV-0. Therefore, oleic acid was chosen for further study. Although the solubility of PGV-0 in oleic acid was highest among the tested oils, it is still classified as very slightly soluble [14]. The solubility in oil is reflected by partition coefficient, logP, where logP > 3 means the compound is highly lipophilic [15]. Yuwono reported that apparent logP of PGV-0 was 1.84, so the solubility of PGV-0 in oils was low [16]. A similar result was reported by Cui. The highest solubility of curcumin among the tested oils (paraffin oil, peanut oil, castor oil and ethyl oleate) was in ethyl oleate, i.e. 0.357 mg/g (categorized as very slightly soluble) [12]. PGV-0 is a curcumin analog so it has structure similarity with curcumin. The amount of oleic acid content in ethyl oleate (oleic acid ethyl ester) also contributed to the similar result.

Five surfactants were tested for PGV-0 solubility. The result showed that PGV-0 had the highest solubility in Tween 20, followed by labrasol. In order to find out the best surfactant or mixture of surfactants for PGV-0, labrasol and Tween 20 were mixed in various combinations for further solubility tests. The mixture of Tween 20 and labrasol (1:1) showed the highest capability to dissolve PGV-0, hence it was chosen as surfactant. The same combination of surfactants was reported to have better microemulsion efficiency for capmul MCM. The viscosity of Tween 20 is high, so the inclusion of labrasol as a secondary surfactant could increase the fluidity of the interface and improve the self-emulsification [16]. Polyethylene glycol 400 (PEG 400) was chosen as cosurfactant because its superior capability to dissolve PGV-0 compared to transcutol and propylene glycol.

From compatibility test, the chosen vehicles from solubility study were miscible in one another and could be continued to pseudoternary phase diagram construction. The pseudoternary phase diagram was constructed to estimate concentration area where SNEDDS blank formulations formed nanoemulsion when introduced to water. The pseudoternary phase diagram consisted of oleic acid, Tween 20:labrasol

(1:1), and PEG 400 as presented in Fig. 2. The nanoemulsion area showed by gray spots in the diagram.

The self-nanoemulsion zone produced by the system achieved at highest content of oleic acid as 20%. At the range of oleic acid concentration as 20–40%, the monophasic were produced, but the time for emulsification was higher than 2 min, so they were excluded from self-nanoemulsion zone.

Drug solubility in vehicles, compatibility between vehicles and self-emulsification characteristics are crucial for an SNEDDS with a high content of drug, stable, and emulsified immediately when contact with the gastric liquid. Highest PGV-0 solubility in oil is used as a starting point to select surfactant and cosurfactant, because, in the aqueous environment, PGV-0 is expected to be loaded as much as possible in oil nanodroplet. To achieve nano/microdroplet size, an oil-water system need to lowering the interfacial tension and cosurfactant to further reduction and to stabilize the droplet. Oleic acid, a mixture of Tween 20:labrasol (1:1), and PEG 400 which showed the highest PGV-0 solubility in each group of oil, surfactant, and cosurfactant together with good compatibility and emulsification were chosen as the component to SNEDDS formulation. The high viscosity of Tween 20 help SNEDDS to prevent the precipitation of PGV-0 particles in aqueous solution, while the low viscosity of labrasol improve the homogeneity of the mixture and solubilization of the drug.

Design of Experiment for Formulation

A simplex lattice design was applied to optimize SNEDDS compositions effectively both cost and time compared to trial and error method. At the low and high limit of each of the three-factors, with an order to quadratic model fit and number of runs to replicate 4, SLD requires 14 experimental runs to determine experimental error and significance of quadratic fit. In this study, a total of 14 formulations were designed, generated, and analyzed using the Design-Expert software.

Droplet size measurement is important for assessing SNEDDS quality because it determines the solubility of the drug, the rate and extent of dissolution as well as absorption. Solubilization of maximum amount of PGV-0 in SNEDDS formulation is highly

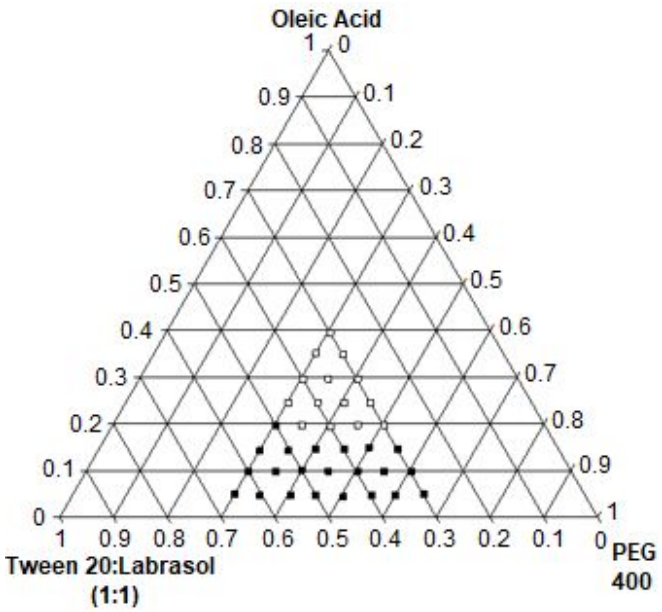

Fig 2. Pseudoternary phase diagram of SNEDDS consist of oleic acid, Tween 20:labrasol (1:1), and PEG 400

Table 1. Actual design of PGV-0 loaded SNEDDS optimization with response values

Std	Run	Component 1 A:Oleic acid %	Component 2 B:Tween 20 : Labrasol (1:1) %	Component 3 C:PEG 400 %	Response 1 Droplet size nm	Response 2 C_{45} %	Response 3 Solubility of PGV-0 mg/L
3	1	22.50	30.00	47.50	95.96	72.32	32.27
10	2	16.66	41.66	41.66	95.59	54.50	24.42
1	3	40.00	30.00	30.00	183.60	47.48	16.35
12	4	5.00	65.00	30.00	16.95	45.97	28.67
11	5	40.00	30.00	30.00	148.20	43.61	20.60
6	6	5.00	30.00	65.00	39.78	53.32	35.52
7	7	25.00	40.00	35.00	159.3	71.73	20.12
9	8	15.00	52.50	32.50	80.00	78.00	24.51
5	9	5.00	47.50	47.50	24.77	39.47	30.65
8	10	15.00	37.50	47.50	94.43	72.28	23.78
13	11	5.00	30.00	65.00	35.88	43.10	35.51
4	12	5.00	65.00	30.00	15.94	51.47	29.79
14	13	22.50	47.50	30.00	81.15	83.37	31.93
2	14	22.50	47.50	30.00	89.54	88.00	33.03

Table 2. Model summary statistics of responses

Model fit parameter	Suggested model	Statistics parameter			
		F value	Probability>F (p-value)	Adj-R^2	Pred-R^2
Y_1					
Sequential model sum of squares	Linear	40.20	<0.0001		
	Special cubic	6.07	0.0432		
Lack of fit	Linear	3.23	0.1370		
	Special cubic	2.54	0.1946		
Model summary statistics	Linear			0.8578	0.8227
	Special cubic			0.9024	-0.3614
Y_2					
Sequential model sum of squares	Quadratic	19.24	0.0005		
Lack of fit		3.67	0.1181		
Model summary statistics				0.8183	0.6854
Y_3					
Sequential model sum of squares	Special cubic	23.47	0.0019		
Lack of fit		3.69	0.1196		
Model summary statistics				0.8498	0.3809

Table 3. Significance of model and lack of fit from the partial sum squares (type III)

Response	Model	p-value	
		Model	Lack of fit
Y_1	Special cubic	0.0004	0.1946
Y_2	Quadratic	0.0012	0.1181
Y_3	Special cubic	0.0016	0.1196

desirable. This will allows minimizing the amount of SNEDDS dosage required for the therapeutically effect. This recent study was used the amount of drug released in minute 45 (C_{45}, %) as dissolution parameter. Dissolution is the most important parameter for assessing SNEDDS quality in this study because it represents the rate and extent of dissolved drug that released in gastrointestinal fluid. The actual design from the randomized runs for independent and dependent variables is presented in Table 1.

Preparation of PGV-0 Loaded SNEDDS

The solubility of PGV-0 in SNEDDS formulations is shown in Table 2. PGV-0 had the highest solubility in standard 6 (35.52 mg/mL), whereas in standard 1, PGV-0 showed lowest solubility. The smallest of PGV-0 solubility (16.35 mg/mL) was chosen as the PGV-0 content of all tested formulations.

Formulation Optimization of PGV-0 Loaded SNEDDS

Droplet size of nanoemulsion (Y_1) of all batches was <200 nm. The droplet size was increased as the higher content of oleic acid, but this phenomenon was not seen in the increase of mixsurf of Tween 20:labrasol (1:1), and PEG 400 content. So, the program suggested linear or special cubic as the suitable model for droplet size response. The percentage of drug released at minute 45 (Y_2) was in the range of 39.47–88% and solubility (Y_3) was 16.35–35.52%.

All of the responses were fitted to the quadratic model. The appropriate model was verified by ANOVA tests in a sequential model sum of squares (Type I) and lack of fit test, adjusted multiple correlation coefficients (Adj-R^2) and prediction multiple correlation coefficient (Pred-R^2) value in model summary statistics. Based on the statistics parameters, the model of best fit was suggested by the program. The model summary statistics of the three responses is showed in Table 2.

Although all of the responses are fitted to the quadratic model, only PGV-0 solubility response (Y_3) is fit with quadratic model. The suggested model for the droplet size responses (Y_1) is linear or special cubic. The special cubic model is the higher order than the linear model, had greater insignificance of lack of fit and greater adj-R^2 than the linear model, so the special cubic is chosen as the model.

For the C_{45} (Y_2) and PGV-0 solubility (Y_3) response, the program only suggested a quadratic and special cubic respectively. The p-value of each model is significant ($p<0.05$) and the lack of fit is not significant ($p>0.1$), so the model is suitable for each of response.

In order to confirm the fit of the model that previously suggested by the program and to obtain the polynomial equations of the chosen model, the ANOVA from the partial sum of squares - Type III and the coefficients of the factor were determined. The ANOVA result is showed in Table 3.

According to the ANOVA result in Table 3, the special cubic model for droplet size response is

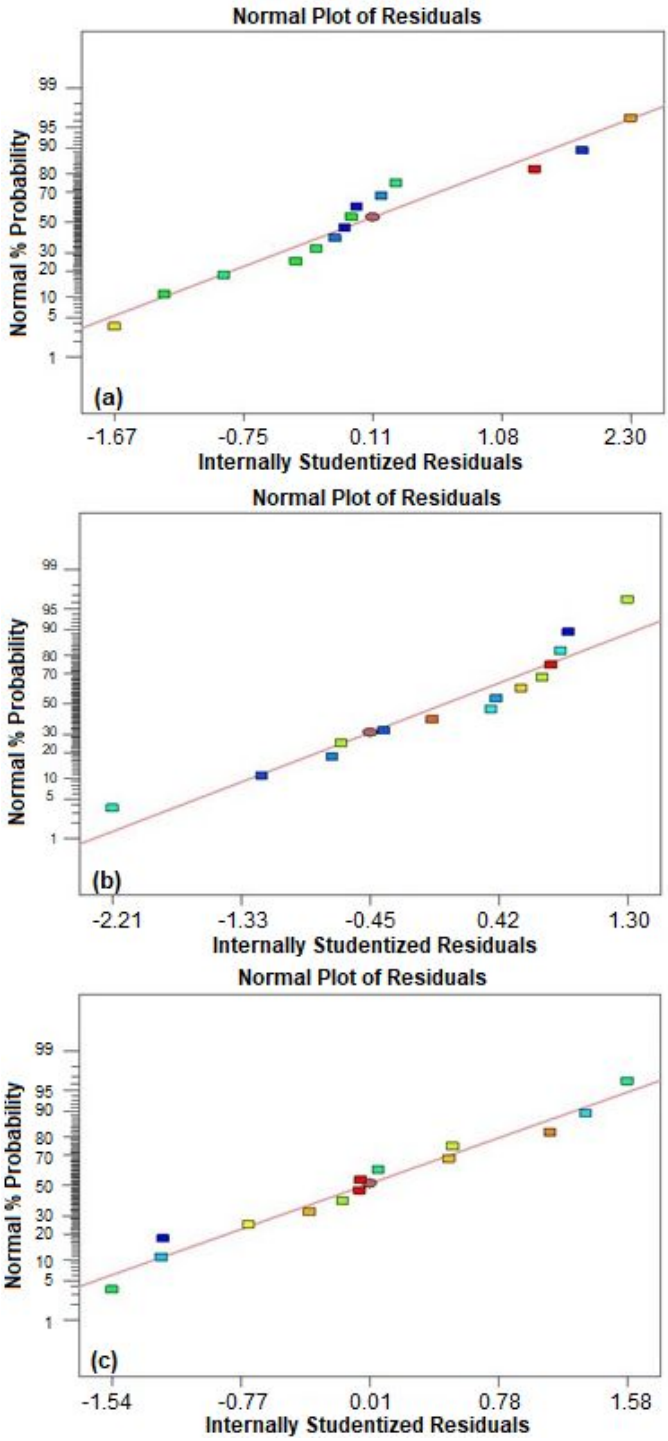

Fig 3. Normal plot of (a) droplet size residuals, (b) C_{45} residuals, and (c) PGV-0 solubility residuals, showed a linear pattern

significant ($p<0.05$) and the model is not aliased. Therefore, the special cubic is a good and appropriate model for describing the effect of a constituent on the droplet size response ($p<0.05$). The lack of fit is not significant ($p>0.1$) mean there are not significant differences between the observation results and estimated results from the model.

Normal probability plots of the studentized residuals of droplet size, C_{45}, and PGV-0 solubility response are shown in Fig. 3 (a), (b), and (c). The diagnostic plots show that all of the residuals points are distributed normally, followed a linear pattern. This is mean the model produce good predictive responses.

The predictive responses in any composition of factors calculated by the equation as showed in Table 4. By analyzing the coefficient of main effects and interactive effects in the equation, the influence of the factors on the responses could be described. The description further clarified visually by contour plot as showed in Fig. 4, 6, and 7.

Influence of Components Ratio on Droplet Size, C45, and Solubility

The polynomial equation of Y_1 shows the relationship between the proportion of components (X_i) and droplet size (Y_1) followed a special cubic model. The coefficient of the main effect of each component is positive. It is mean the increase of the proportion of oleic acid (X_1), mixsurf (X_2), or PEG 400 (X_3) in the system increase the droplet size.

The increased proportion of oleic acid independently increased the droplet size. Many earlier studies had same trend [12-13,17,22]. The more oil to surfactant ratio, the amount of surfactant become increasingly insufficient to cover the oil nanodroplet, so some of the droplets join together to minimize the interface area leading droplet size increase.

The droplet size is increase with the increased of mixsurf (Tween 20:labrasol). A similar result was reported by Basalious. The increased of mixsurf (cremophor/Tween 80) caused the increased of droplet size of lacidipine loaded SNEDDS [17]. This phenomenon could be occur if the increase in surfactant concentration mediate the excessive enhancement of water penetration into oil droplet, so that the interface disrupted, leading to the ejection of oil droplet into the aqueous phase [18].

Similar to many earlier reports [12-13,17,22], larger droplet size also observed on increased of PEG 400 proportion. Cosurfactant or cosolvent is added to provide solubilization of a large number of hydrophilic surfactant and leading to drug loading enhancement and improve the emulsification time [19]. The progressive addition of PEG 400 in SNEDDS system leads the less hydration of surfactant head group so the surfactant surface area becomes smaller, followed by micellar and droplet growth [20].

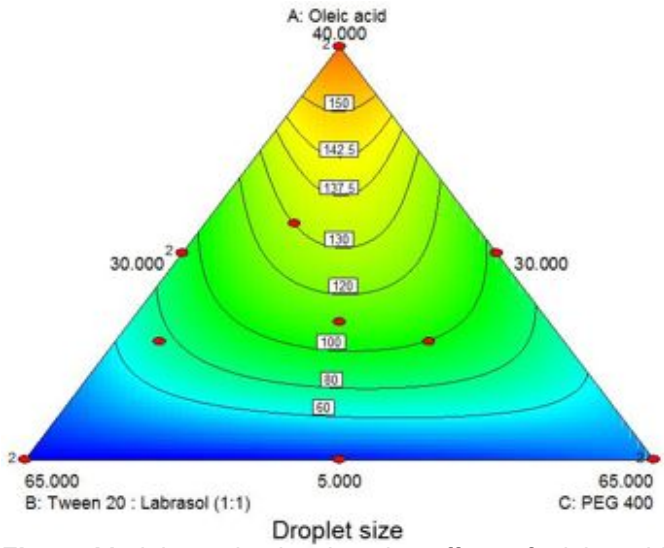

Fig 4. Model graph showing the effect of oleic acid, mixsurf (Tween 20:labrasol 1:1), and PEG 400 and their interaction on droplet size

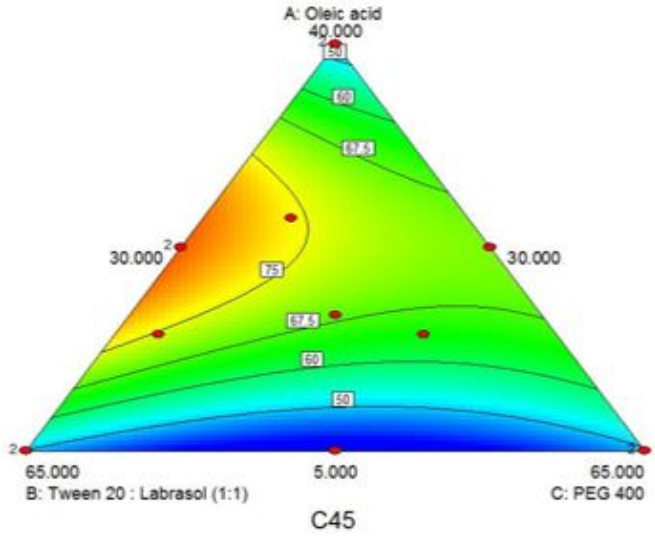

Fig 5. Model graph showing the effect of oleic acid, mixsurf (Tween 20:labrasol 1:1), and PEG 400 and their interaction on C_{45}

Table 4. Regression result of responses

	Coefficient Estimate		
	Y_1	Y_2	Y_3
X_1	167.98	45.43	18.39
X_2	16.31	49.60	28.93
X_3	37.08	49.08	35.59
X_1X_2	1.48	144.14	29.31
X_1X_3	-26.46	89.46	21.41
X_2X_3	-22	-50.57	-5.53
$X_1X_2X_3$	1159.46	-	-309.04

Oleic acid has the highest effect on droplet size, while the two other components have a more limited effect. This is illustrated more clearly in Fig. 4. The increase in the percentage of oleic acid from 5% to 40% shows a non-linear increase in droplet size from 16.31 nm to 167.98 nm.

The interaction effect of a binary mixture of oleic acid and mixsurf (X_1X_2) is positive, while oleic acid and PEG 400 (X_1X_3) and mixsurf and PEG 400 (X_2X_3) have antagonistic effect respectively. The effect of antagonistic factors are higher than the main effects, therefore the ratio enhancement of mixsurf and PEG 400 from 30% to 65% produce a non-linear decrease in droplet size as shown in Fig. 4. The interaction effect of the ratio of oleic acid and PEG 400 is the most influential factor to the reduction of droplet size. The interaction effect of tertiary mixture $X_1X_2X_3$ is synergistic, leading to increasing in droplet size.

In Table 3, a quadratic equation of PGV-0 released at minute 45 (Y_2) contains positive coefficient of main effects and interaction effect of X_1X_2 and X_1X_3. The contribution of X_2 and X_3 to the dissolution properties was similar to earlier report [17,22]. Dissolution properties of SNEDDS influenced by the solubility and the rate of self-emulsification. The addition of surfactant to the drug-oil-water system would decrease the interface tension, facilitated interaction between hydrophobic drug with oil and water leading the better solubilization. The addition of surfactant also enhances the water penetration, generate interface disruption and therefore decrease the droplet size and dissolution rate. This mechanism is further assisted by the addition of cosurfactant/cosolvent, which improves the solubility of drug and surfactant. The penetration of cosurfactant/cosolvent into the surfactant monolayer interface has the similar impact to water penetration, so it improves the self-emulsification and dissolution extent and rate. Otherwise, the interaction of amount of oleic acid and mixsurf gives the biggest contribution to the increase of the amount of PGV-0 released. As showed in Fig. 5, the area where the composition gave C_{45} equal to or higher than 75% is located on the edge of line 30% of PEG 400, in the middle level of oleic acid and mixsurf.

Table 4 shows the special cubic equation that describes the effect of oleic acid, mixsurf, PEG 400 and their interaction on the response of PGV-0 solubility. The each of the main effect has a positive coefficient, with the order from high to low: PEG 400 > mixsurf > oleic acid. This result is correspondent with preliminary solubility studies. The most significant antagonistic interactive effect is $X_1X_2X_3$, causes the effect of three factors together is less than the sum of the three factors taken independently of each other. In the other hand, the most significant synergistic interaction effect is the proportion of oleic acid and mixsurf. Fig. 6 shows

Table 5. One Way T-test of predicted and experimental values of PGV-0 loaded SNEDDS runs at the estimated optimal operating condition

Response	Prediction value	Experimental			p-value
		Value	Standard deviation	Standard error (%)	
Y_1 (nm)	75.45	78.63	11.52	6.65	0.680
Y_2 (%)	82.2	78.21	2.02	0.076	0.076
Y_3 (mg/mL)	31.80	30.57	1.82	1.05	0.362

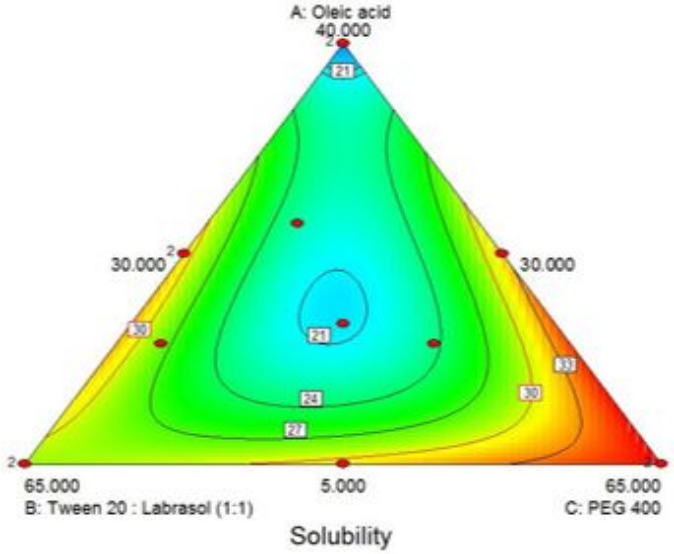

Fig 6. Model graph showing the effect of oleic acid, mixsurf (Tween 20:labrasol 1:1), and PEG 400 and their interaction on PGV-0 solubility

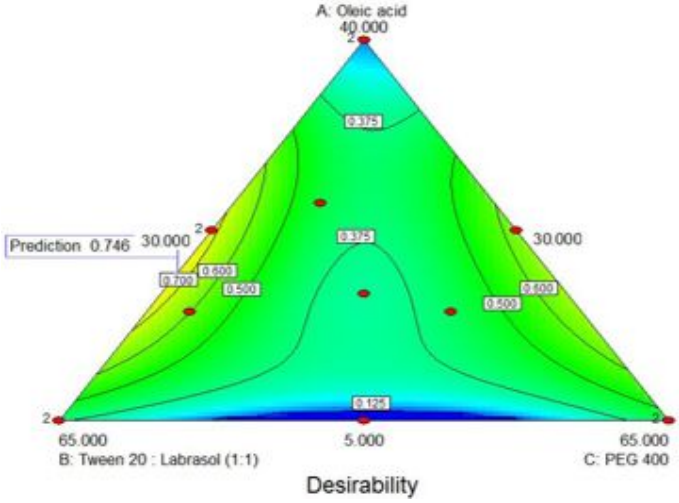

Fig 7. Overlay plot of desirability function

the maximum area of PGV-0 solubility located in around of the PEG 400 vertex (65% PEG 400). This result is reasonable because, from the previous solubility study, PGV-0 showed the highest solubility in PEG 400.

The increase proportion of oleic acid and mixsurf together causes the decrease in droplet size and increase in dissolution and solubility. Interaction between oleic acid and mixsurf generate the formation of micelles. PGV-0 soluble in oil is partially trapped in the center of micelles and some other is bounded in the hydrophilic head of surfactant because of the semipolarity of PGV-0. The more the formated micelles, the more PGV-0 soluble and dissolved.

The interaction effect of oleic acid-mixsurf to the droplet size is almost equal to the interaction effect of oleic acid-PEG 400. This is due to the micelles formation, as well as oleic acid-mixsurf. The interactive effect of oil with other components shows the highest significance, because the proportion of oleic acid independently as the main effect also has the highest effect, so a little change of oleic acid proportion shows a relatively major change on responses. The increase proportion of mixsurf and PEG 400 together is also decreased the droplet size. This is due to cosolvency in the mixsurf solubilisation of PGV-0 and oleic acid, so the droplet size becomes smaller. Cosurfactant molecules put itself on the sidelines of surfactant molecules leading the steric stabilization of micelles.

Formulation Optimization Using Desirability Function

The simultant optimization for all responses carried out with desirability function. It is calculated by combining the individual desirabilities using geometric mean. The desirability function assigns numbers between 0 and 1, with 0 representing a completely undesirable value and 1 representing an ideal response value.

To maximize the overall desirability, criteria of the controllable factors and responses was set, with Y_1 was set to be minimized while Y_2 and Y_3 were set to be maximized. Fig. 7 shows the overlay plot for the desirability function. The optimized formulation is achieved at X_1 = 18.6 mg, X_2 = 51.4%, and X_3 = 30% with the corresponding desirability (D) value of 0.746. The predicted response of Y_1 = 75.45 nm, Y_2 = 82.2%, and Y_3 = 31.80 mg/mL.

In order to verify the validity of the optimum parameters and predicted responses calculated, three batches of the optimized formulations were prepared. All of the responses were evaluated for each optimized formulation. The comparison of the experimental and predicted results and its are showed in Table 5.

The experimental value of C_{45} of the optimum formulation was 78.20 ± 1.66%, the droplet size was 77.34 ± 9.41 nm, and PGV-0 solubility was 30.57 ± 1.48 mg/mL. All of the experimental responses are not significantly different with the predicted responses (p = 0.05). It can be seen that the experimental values are in very close agreement with the predicted values, indicating the success of the SLD combined with a desirability function for the evaluation and optimization of PGV-0 loaded SNEDDS formulations.

CONCLUSION

The Simplex Lattice Design with desirability function is effective in optimizing PGV-0 loaded SNEDDS and in understanding the effects of the formulation factors on dependent variables.

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