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An Experimental Design of SNEDDS Template Loaded with Bovine Serum Albumin and Optimization Using D-Optimal

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ABSTRACT

Self Nanoemulsifying Drug Delivery System (SNEDDS) is not only limited to develop Biopharmaceutics Classification System (BCS) class II drugs, but also BCS class III and IV. Protein is classified into BCS class III because of its good solubility and its poor permeability. It needs suitable composition to load hydrophilic substance into lipophilic carrier such as SNEDDS. The objective of the study was to optimize SNEDDS template for Bovine Serum Albumin (BSA) protein using D-optimal mixture design. In this study, BSA was used and loaded into a SNEDDS template. Mixture of D-optimal design was used for optimization. The optimized template contained minimum amount of surfactant and maximum amount of oil that shows enhanced transmittance and emulsification rate. Sixteen formulas consisting of oil phase (X1, Mygliol 812), surfactant (X2, Chremophore EL 40), and co-surfactant (X3, Span 20) were evaluated for transmittance and emulsification time. The optimum SNEDDS template was then loaded with 1mg/ml BSA. Characterization results of the optimum template showed 55,4 nm of the size, -0,9 mV of zeta potential, spheric morphology, 34,24±0,53 second of emulsifying time, 94,20±0,82% of transmittance, and 87±0,4 ^{0}C of cloud point. The template was also resistant to 100, 200 and 1000 times dilution using water as medium, Simulated Gastric Fluid (SGF), and Simulated Intestinal Fluid (SIF).

Keywords: SNEDDS, BSA, D-optimal design, highly water-soluble compound

INTRODUCTION

SNEDDS is a mixture of oil, co-surfactant, surfactant, and water free co-solvent. It is a transparent form that will be emulsified with the presence of water and be agitated by the peristaltic motion in gastrointestine[1,2,3]. Previous studies showed evidence that SNEDDS is superior than traditional lipid solution because the surfactant component in SNEDDS may increase bioavailability of the carried drug. Beneficial features of SNEDDS are consistency of drug absorption, protection of drug from gastrointestine environment, improvement of bioavailability, and higher efficiency of drug entrapment[4]. According to Albeit Pouton SEDDS is not only used to develop BCS class II drugs but also can be used for BCS class III and IV[5]. BCS is classified based on the solubility and permeability profiles of substance, and protein falls into the third class as its high solubility and its low permeability. Since protein is easily degraded in gastrointestinal environment[6], the use of SNEDDS could protect it from degradation[7]. Moreover, the delivery system may ameliorate protein's gastrointestinal permeability due to its high surfactant content, and may ease protein's absorption due to nano sized droplets of emulsion formed by small agitation in gastric fluid[8,9,2]. In this study, BSA was used as a model that is loaded into SNEDDS system by dissolved in glycerine. This method is simple and easier than the former study[10] that used solid dispersion technique using SoyPC to change BSA into a hydrophobic compound to facilitate incorporation to the oil phase. The objective of the present study was to optimize SNEDDS template for BSA protein using D-optimal mixture design. This optimization approach has distinct advantages in terms of efficiency of time, money and efforts. Formulation optimization was based on ease of emulsification and the clarity of emulsion that is confirmed by its transmittance. Selected formula was used for BSA template and then the physicochemical properties such as particle size, zeta potential, particle morphology, emulsification time, transmittance, cloud point, stability in SIF and SGF, and its robustness to dilution were investigated in detail.

MATERIALS AND METHODS

BSA (Bovine Serum Albumin), Span 20, and Span 85 were obtained from Sigma Aldrich (Germany). Mygliol 812 was purchased from Cremer Oleo GmbH & Co.KG. Chremophor EL 40 was a gift from Shanghai Terppon China. Tween 20, Tween 80, Oleic Acid, and Propylene Glycol were from Bratachem Indonesia, and all other chemicals were of analytical grade.

Preliminary Screening of Ternary Liquid Formulae

Efficiency of different combination of oil, surfactant, and co-surfactant in forming nano emulsion upon dilution were

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Table 1: Independent variables and level used for optimization

Factors	Levels (%w/w)	
	Low	High
X1 (Mygliol 812)	20	40
X2 (Chremophor EL 40)	30	60
X3 (Span 20)	20	30

Table 2: The formulations of mixture design

Run	X1 (Mygliol 812 % w/w)	X2 (Chremophor EL 40 % w/w)	X3 (Span 20 %w/w)
1.	40	30	30
2.	30	50	20
3.	25	50	25
4.	40	30	30
5.	20	50	30
6.	20	55	25
7.	35	40	25
8.	40	35	25
9.	30	45	25
10.	20	60	20
11.	20	50	30
12.	30	50	20
13.	40	40	20
14.	40	40	20
15.	20	60	20
16.	30	40	30

evaluated at the ratio 1:3:1. All mixtures of the selected oils, surfactants, and co-surfactants were homogenized by stirring for 15 minutes. Each formed isotropic mixture was diluted 200x using simulated gastric fluid without pepsin (SGF, pH 1.2). The clarity of the formed aqueous dispersion (% transmittance) was measured by UV-VIS spectrophotometer (Hitachi U-2900). Measurement was replicated 2 times[11]. Combination of oil, surfactant, and co-surfactant with % transmittance of > 80% was chosen for making ternary diagrams.

Construction of Ternary Phase Diagrams

Ternary-phase diagrams show an area where the formula can form self-nanoemulsion. Thirty six formulas with various concentration of oil, surfactant, and co-surfactant ranging from 10%-80% were prepared to form mixture in total concentration of 100%. Oil phase was mixed with surfactant and co-surfactant using magnetic stirrer for 15 minutes. Each formed system was diluted 200 times with SGF pH 1.2[11] and was measured for its % transmittance with UV-VIS spectrophotometer. Aqueous dispersion with transmittance > 80% was chosen as self-nanoemulsion system because a former study showed that the transmittance value of 80 % indicated particle size of < 100 nm, which is a requirement of a SNEDDS[12,13].

Optimization of Snedds Template Using D-Optimal Mixture Design

The result of ternary diagram evaluation was used to determine level of independent variable that will be used in optimization using D-optimal mixture design. Combination of oil, surfactant, and co-surfactant was chosen for optimization from the widest area of nano-emulsion that can be formed in ternary diagram. As many as 16 formulas (Table 2) of D-optimal mixture design were used in this study to describe the correlation of 3 independent variables (Table 1), i.e. oil surfactant, and co-surfactant versus chosen response, emulsification time and % transmittance. Design-Expert software was used to construct a model and candidate points such as factorial points (high and low level from the constraints on each factor), centre of edges (points midway between adjacent factorial points), constrain plane centroids, axial check points, and an overall centre point. A statistically significant result was measured by a p-value < 0.05. The polynomial equation (special cubic model) generated by this experimental design (using Design expert software version 7.1.5) is as follows:

$$Yi = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3$$

Where, Yi is the dependent variable, b_0 is the intercept, b_1 to b_{123} are regression coefficients and X_1, X_2 dan X_3 are the independent variable.

Verification of Optimum Formula

Verification of optimum formula from optimization using D-optimal mixture design was used to conform between predicted value and observasion value. Verification was conducted using OpenStat software and was recognized as statistically significant different if p-value is less than 0.05.

Preparation of BSA SNEDDS

Optimum SNEDDS template consisted of oil, surfactant, and co-surfactant was stirred using magnetic stirrer for 15 minutes. After homogeneity was reached, 6.67% v/v of BSA in glycerin (15mg/mL) or equal to 1 mg BSA/mL were added to SNEDDS template. Then it was stirred using magnetic stirring for 15 minutes.

Evaluation parameters of BSA SNEDDS

Table 3: Percentage of transmittance from system consist of oil:surfactant:co-surfactant 1:3:1 after diluted 200x using SGF pH 1.2

S. No	Surfactants	co-surfactants	Oils		
			Mygliol 812	Oleic acid	Span 85
1.	Tween 80	PG	94,43±0,37%	18,60±0,53%	46,03±0,25%
		Span 20	23,20±0,21%	24,50±0,50%	9,53±0,45%
2.	Tween 20	PG	35,00±0,30%	7,67±0,35%	9,63±0,40%
		Span 20	58,63±0,40%	13,60±0,46%	39,47±0,50%
3.	Cremophor EL 40	PG	44,63±0,55%	28,93±0,40%	58,03±0,25%
		Span 20	88,72±0,00%	28,67±0,49%	89,53±0,50%

Table 4: Composition of oils: surfactants: co-surfactants for constructing ternary diagram

Formula	Oils	Surfactants	Co-surfactants
F1	Mygliol 812	Tween 80	PG
F2	Mygliol 812	Cremophor EL 40	Span 20
F3	Span 85	Cremophor EL 40	Span 20

Table 5: Regression results of the measured responses

Model	Coefficient	% Transmittance	Emulsification Time
Special Cubic	SD	1.15	0.09
	R^2	0.96	0.96
	Adjusted R^2	0.94	0.94
	PRESS	54.17	0.23

Droplet size analysis

The droplet size of SNEDDS was measured by Horiba Scientific (SZ-100) Particle Size Analyzer at 25^{0}C at a fixed angle of 90^0. The formulation was dispersed 100x in SGF pH 1.2 under gentle stirring in a glass beaker. Then a 1 mL aliquot was withdrawn and added into a sample cell for droplet size measurement[14].

Zeta Potential Measurement

Zeta potential was measured using Particle Size Analyzer with temperature of the holder 24.9^{0}C, medium viscosity 0.897 mPa.s, conductivity 25.847 mS/cm, average electrophoretic mobility -0.000006 cm^2/Vs, and electrode voltage 1.3 V.

Transmission Electron Microscopy (TEM)

Morphology and structure of the nanoemulsion were studied using transmission electron microscopy (TEM JOEL-JEM 1400) after the sample was diluted with SGF pH 1.2 (1:1000). Samples were stained with 1% phosphotungistic acid solution for 30s and were dropped on a copper grid.

Self-Emulsification Time Determination

The formulation was assessed visually to determine the emulsification time using a magnetic stirrer-beaker assembly. A 100µL volume of SNEDDS BSA was added into 100 mL of SGF pH 1.2 at 37±1^{0}C under 100 rpm continuous stirring. Self-emulsification time was taken as the time for a pre-concentrate to form a homogenous mixture upon dilution[15].

Percentage of Transmittance

SNEDDS BSA was diluted 100x using SGF then the presence of turbidity was visually observed. Percentage of transmittance was measured by UV-Vis spectrophotometer at 650 nm.

Cloud Point Determination

Cloud point temperature (Tc) was determined by visual observation of SNEDDS BSA formula that was diluted 100x using distilled water. Observation was conducted at visual dispersion system with a gradual increase of temperature at 25-90^{0}C. The temperature at which the turbidity appeared was determined as Tc. Re-heating was conducted to ensure the measurement reproducibility[16].

Effects of Dilution Media

SNEDDS BSA formula was tested its resistance toward dilution using several media (distilled water, SGF pH 1.2, and SIF pH 6.8) with a series of dilutions (100, 200, and 1000x) for mimicking physiology process that happen after the formula was orally administered. SNEDDS BSA which had formed nano emulsion at some kind of media was stored for 24 hours and was observed visually if precipitation or phase separation incurred[16].

Stability in SGF and SIF Incubation

An amount of 100 µL BSA SNEDDS was added with distilled water, SGF pH 1.2, and SIF pH 6.8 until the volume reaching 5 mL. Nanoemulsion system was heated and kept at 37^{0}C for 4 hours. The presence of precipitation was observed every hour. Comparatively, other observation as also conducted at the room temperature (25±2^{0}C).

Thermodynamic Stability Studies of BSA SNEDDS

Thermodynamic stability was evaluated by observing the phase separation of SNEDDS-BSA that was diluted using aqueous medium (1:50) and centrifuged at 4000 rpm for 30 minutes. The presence of phase separation and drug precipitation in formula were observed visually.

RESULTS AND DISCUSSION

Preliminary Screening of Ternary Liquid

Before constructing ternary diagram which consisted of oil, surfactant, and co-surfactant, preliminary study was conducted to select oil, surfactant, and co-surfactant combination of those components that can form wide area of nano-emulsion in ternary diagram. Oil compounds used were Mygliol 812, Oleic acid, and Span 85, while Tween 80 (HLB 15.0), Tween 20 (HLB 16.7), and cremophor EL 40 (HLB 13.5) were used as surfactant. Co-surfactant used in this study were Propylene glycol and Span 20. Efficiency of nano-emulsion formation was affected by some variables such as HLB value of surfactant, lipid-surfactant afinity, and viscoelasticity of emulsion base[18]. As shown in Table 3, the highest transmittance was produced by combination Tween 80-propylene glycol-Mygliol 812; Cremophor EL 40-Span 20-Mygliol 812 and Cremophor EL 40-Span 20-Span 85. These three combinations of oil, surfactant, and co-surfactant were used to construct ternary phase diagrams. Although HLB value of Tween 80 is higher than HLB value of Chremphor EL 40, the later could form nano-emulsion with Mygliol 812 and Span 85 while Tween 80 could only produce nano-emulsion with Mygliol 812. It could be attributed by difference of lipid-surfactant affinity that caused improvement of surfactant adsoption in certain oil droplet[18]. Compared to Tween 80, Tween 20 did not form nano-emulsion with some oils and co-surfactant used in this study. This was showed by lower % transmittance (<80%). It was because Tween 80 was better than Tween 20 in forming smaller particles, hence it can improve in vivo absorption[19,20]. Mygliol 812 is medium chain triglyceride with the HLB value 15.36[21], while the HLB of Span 85 is 1.8. It was reported that lipid compound with

Table 6: Three solutions of optimum formula

S.No	Mygliol 812	Chremophor EL 40	Span 20	% Transmittance	Emulsification Time	Desirability
1.	20.00	56.21	23.79	97.92	22.21	0.889
2.	32.34	40.41	27.25	94.73	22.45	0.803
3.	32.92	39.58	27.50	94.42	21.91	0.802

Table 7: The predicted and observed values of Y1 and Y2 of F3

Variables	Values	Response	Observed values	Predicted values	p-value
X1	32.92	Y1	94.10±0.3	94.42	0.294
X2	39.58	Y2	22.89±2.3	21.91	0.543
X3	27.50				

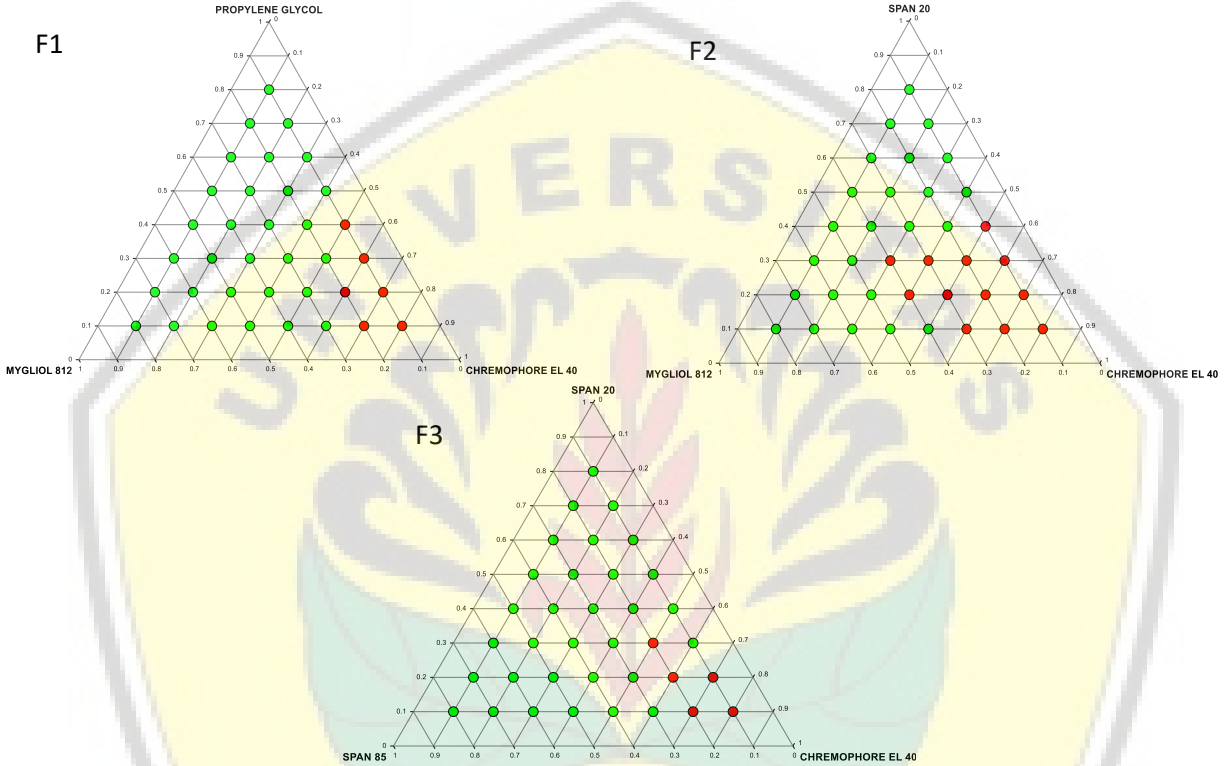


Figure 1: Ternary Phase Diagram F1, F2 and F3

higher polarity is easier to form nano-emulsion[22] and oils with higher HLB are better to form SNEDDS than lower HLB values[17].

Three ternary diagrams from preliminary study were constructed by evaluating transmittance value. Chosen component of oil, surfactant, and co-surfactant was shown in Table 4.

Construction of Ternary Phase Diagrams

Based on preliminary study, three ternary phase diagrams were constructed in order to know the correlation between phase behavior and the composition. In addition, it also helped to determine the range of component concentrations that were able to form nano-emulsion. Red dots in the ternary diagram show the area of nano emulsion (Figure 1). The best of nano emulsion was determined based on transmittance measurement.

From the results depicted in Figure 1 it can be seen that the F2 formula had the largest area of nanoemulsion compared to the others. It could be attributed to the high HLB of lipid content (Mygliol 812) and its high lipid-surfactant affinity. Results also deduced that increasing oil content was attributed to the increase of emulsion particles size[23]. On the other hand, increased surfactant concentration would increase the clarity of emulsion produced[24]. Ternary phase diagram can also be used to describe co-surfactant effect on nano-emulsion area. SNEDDS would be easily formed by right combination of high HLB surfactant (Chremophor EL 40) and low HLB co-surfactant (Span 20)[25,26]. The function of co-surfactant was to decrease surface tension because it can penetrate into monolayer surfactant giving extra fluidity so that can bother liquid crystal phase formed when the surfactant film was too rigid[27]. Finally, from the results observed during construction of ternary phase diagrams discusssed earlier, ternary phase diagram (F2) was chosen for optimization using D-optimal mixture design. F2 ternary phase diagram had the widest area of nano-emulsion and the highest concentration of oil which can form nano-emulsion. The range of oil concentration (Mygliol 812) that will be optimized was 20-40%, surfactant (Cremophor EL 40) was 30-60%, and co-surfactant (Span 20) was 20-30%.

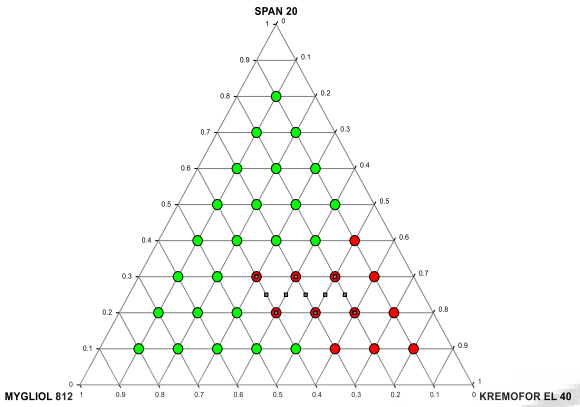


Figure 2: Position of run formula D-optimal in F2 Ternary Phase Diagram

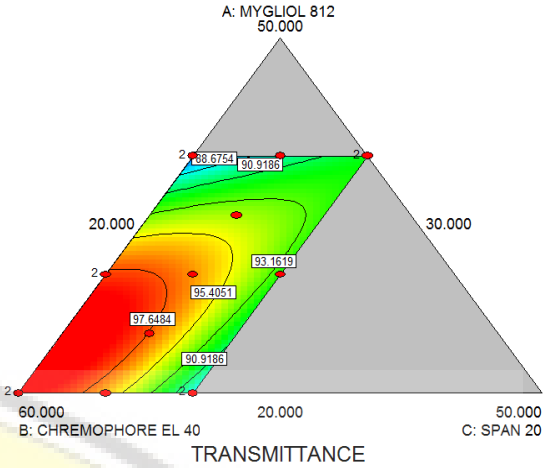


Figure 3: 2D contour plots for the effect of variables on % transmitance

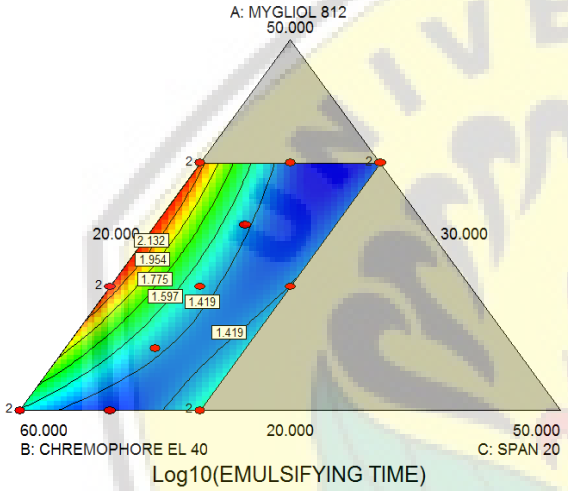


Figure 4: 2D contour plots for the effect of variables on emulsification time

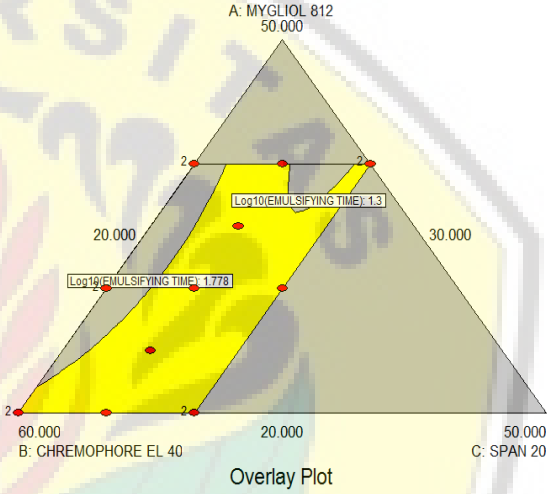


Figure 5: Overlay plot effect 2 responses i.e.% transmittance and emulsification time

Optimization of SNEDDS Template Using D-Optimal Mixture Design

Optimization by D-optimal mixture design for three factor i.e. oil (Mygliol 812), surfactant (Cremophor EL 40), and co-surfactant (Span 20) using 16 formulas. The formulas were figured as black square in ternary diagram (Figure 2). Mygliol 812 (X1), Chremophor EL 40 (X2), and Span 20 (X3) were chosen as variable formula, while % transmittance (Y1) and emulsification time (Y2) were the response variable. Response variable and independent variable were connected by polynomial equation with statistical analysis. Special cubic model was the most suitable and was selected base on several statistical parameters including the standard deviation (SD), the multiple correlation coefficient (R^2), adjusted multiple correlation coefficient (adjusted R^2) and the predicted residual sum of square (PRESS). As illustrated in Table 5, p-value of ≤ 0.05 for all factors in analysis of variance (ANOVA) indicated significant effect of the corresponding responding factors on Y1 and Y2.

Final equation in coded factor for % transmittance and emulsification time are given below:

% Transmittance (Y1) = +63.46*X1+99.06*X2+10.58*X3+48.27*X1*X2+207.01*X1*X3+87.33*X2*X3-89.87*X1*X2*X3

Emulsification Time (Y2) = +1.44*X1+1.58*X2+7.57*X3+3.20*X1*X2-9.49*X1*X3-8.97*X2*X3-10.98*X1*X2*X3

Where Y1 = % transmitance, Y2 = Emulsification Time, X1 = quantity of Mygliol 812, X2 = quantity of Chremophor EL 40, X3 = quantity of Span 20. Equation above represents effect variables (X1, X2, X3) quantitatively and its interactions to response variable (Y). Coefficient of X1, X2, and X3 are related to effect of variable toward response. Positive sign shows a sinergistic effect, while negative sign indicates an antagonistic effect. The highest value of coefficient shows that the variable has more impact on the response, while coefficient with more than one factor shows an interaction. Figure 3 and 4 show the contour diagrams illustrating the effect of various ratios of X1, X2, and X3 on the % transmittance and emulsification time.

The purpose of optimization was to determine variable level that produced desired spesification. Response for % transmittance was maximized with the lower limits 80%

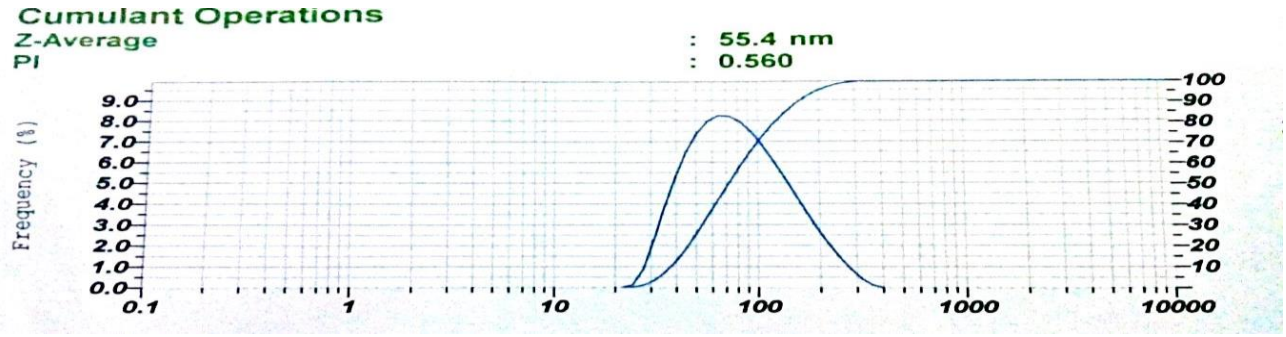
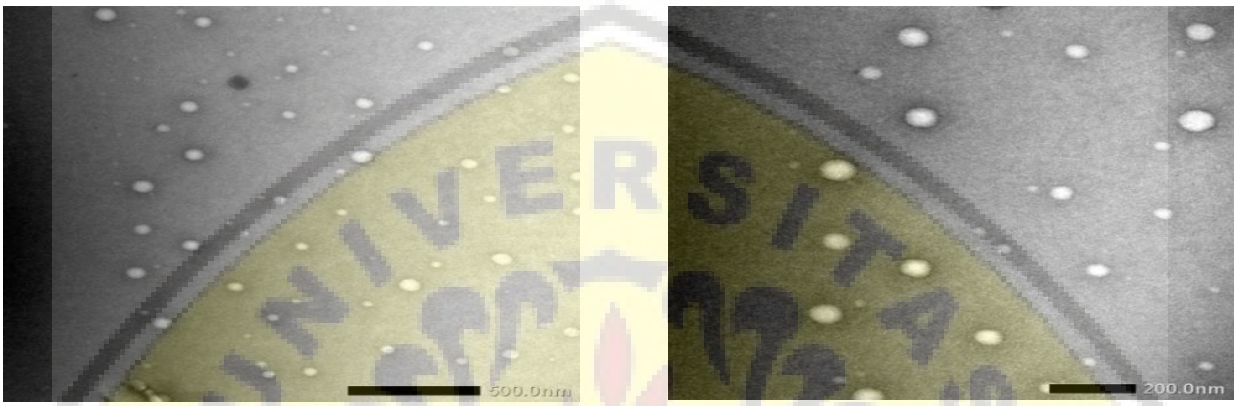


Figure 6: The result of particle size measurement using particle size analyzer



A B

Figure 7: TEM image of BSA SNEDDS (A) 12.000 magnification, (B) 20.000 magnification

and upper limits 100%, while emulsification time was minimized with the lower limits 20 seconds and upper limits 60 seconds. Results of response testing were analyzed using Design Expert to produce overlay plot of two responses. Figure 5 shows optimum area that meets respond criteria. Based on optimization result, there were 3 solutions given by design expert which have desirability > 0.8 (Tabel. 6). Chosen optimum formula for verification was formula 3 considering the highest oil and less surfactant composition then solution formula no 2. Formula that would be verified was prepared with 3 times of replication.

Verification of Optimum Formula

The optimum formulation, F3, was then prepared for further verificatio according to the above values (Table 6) of the factors and subjected to previous test (emulsification time and percentage of transmittance). Non-statistically significant results between predicted and observed values of Y1 (p=0.294) and Y2 (p=0.543) of the optimum formulation were shown in Table 7. However, a good agreement between the model prediction and experimental obseration was sufficient to establish a valid model.

Preparation of BSA SNEDDS

The verified optimum formula of SNEDDS template was loaded with BSA dissolved in glycerin 15mg/mL. BSA solution (6.67% v/v) was added into the SNEDDS template, equal to 1 mg BSA per 1 mL SNEDDS.

Evaluation Parameters of BSA SNEDDS

Droplet Size Analysis

Droplet size is an important factor in self-emulsification. The smaller is better in order to absorption and drug release[28]. Result of the particle measurement using photon correlation spectroscopy shows the size of SNEDDS BSA was 55.4 nm (less than 100 nm) (Figure 6). Theoritically, the size of nano-emulsion is depends on the ratio of surfactant and co-surfactant. Higher the ratio, the smaller nano-emulsion was produsedd[29]. Particle size of nano-emulsion also depends on oil and surfactant composition[30]. Oil can increase SNEDDS ability to carry drug but it will increase SNEDDS size, thus the ratio used is always lower than surfactant[31].

Zeta Potential Measurement

Zeta potential depend on the type of surfactant being used. In this study, Cremophor EL 40, a nonionic surfactant, was used. Nonionic surfactant has several advantages such as less toxic and insensitive toward pH change and electrolyte. In theory, nano-emulsion is stable towards particle deflocculation, if the charge is in between -10 up and -30 mV. The BSA SNEDDS has zeta potential value -0.9 mV. Generally, negatively charges of the droplet was obtained because of the presence of free fatty acid.

Transmission Electron Microscopy

The morphology of diluted SNEDDS was examined by using a TEM (Figure 7). The nanoemulsion droplets appear clearly in spherical shape.

Some droplet sizes are measured using TEM, as this equipment is capable to produce point-to-point resolution. The droplet size is in agreement with the results obtained from droplet size analysis using PSA.

Self-Emulsification Time Determination

Determination of emulsification time was conducted to describe the effortless of emulsion formation from SNEDDS inside the body. Emulsification time is an important parameter to assess efficiency of self-

emulsification of SNEDDS formula[32,33]. Emulsification time testing requires low energy to stimulate the peristaltic of the digestive tract. SNEDDS BSA formula showed short emulsification time in SGF pH 1.2 (34.24±0.53 second). Emulsification time was mediated by surfactant and co-surfactant by form a layer between oil and water.

Percentage of Transmittance

The BSA SNEDDS formula in SGF pH 1.2 had clear appearance with percentage of transmittance of 94.20±0.82. Since good nano-emulsion requires to have clear appearance with transmittance > 90% [34], it can be said that the F3 of this current study could produce a nano-emulsion in aqueous medium. This result corresponded to the analysis result of particle size, where the droplet size was less than 100 nm or have been became nano-emulsion.

Cloud Point Determination

Cloud point is temperature at which a nano-emulsion system is turning from clear into turbid appearance. Cloud point is an important factor of SNEDDS formula containing non-ionic surfactant. It is also responsible for successful formation of a stable nano-emulsion. When the temperature is above the cloud point, there will be irreversible phase separation, so that the turbidity can affect the drug absorption. Cloud point of SNEDDS should be above 37^0C to avoid phase separation in gastrointestinal tract and it was 87 ± 1.2^0C. Cloudiness was reversible after minutes. Therefore, it suggests a stable nano-emulsion of BSA can be formed at physiological temperature in vivo.

Effect of Dilution Media

Formulation of BSA SNEDDS was then subjected to dilution with different media. Increased dilution and change in diluents had no effect on the appearance and stability of nano-emulsion formed. It indicated that formulation of BSA SNEDDS was robust to dilution with different diluents. Thus the formula can maintain its performance in vivo.

Stability in SGF and SIF Incubation

To know the stability of nano-emulsion of BSA SNEDDS as in gastrointestinal tract, observation was conducted for 4 hours to describe the retain time of the dosage form in the gastrointestinal tract at 37°C inside 3 different media, they are distilled water, SGF pH 1.2, dan SIF pH 6.8. Visual observation of nano-emulsion stability showed that nano-emulsion was stable in those three media as there was no presence of clod and precipitate. The presence of them are the sign of nano-emulsion breaking so that the oil was no longer capsulated by surfactant and co-surfactant. BSA SNEDDS formula was stable in acid pH and electrolyte in the gastrointestinal tract after forming nano-emulsion. Chremophor EL 40 as non-ionic surfactant was not easily affected by acid condition and electrolyte so it kept active as surface layer between water and oil.

Thermodynamic Stability Studies of BSA SNEDDS

Formulation of BSA SNEDDS diluted in aqueous medium was subjected to centrifugation test. No phase separation of the formula was observed on centrifugation test. BSA in optimized SNEDDS template was found to be thermodinamically stable.

CONCLUSION

This study used D-optimal mixture experimental design to optimize SNEDDS template for BSA. As many as 16 formulas were prepared to get optimum formula with transmittan >90% and emulsification time <60 seconds. Optimization result showed SNEDDS with composition of oil 32.92 %; surfactant 39.58 %; and co-surfactant 27.50 % was the optimum formula, in which after being verified, no significant difference was found between the predicted and observed result. Characteristic of SNEDDS template that was optimized and loaded with BSA showed rapid formation of nanoemulsion in SGF pH 1.2, stable when diluted into several media and centrifuge testing. This study provides evidence for future use of SNEDDS template as a carrier in protein-based therapy.

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