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Research Article

Optimization of Naringenin Self-Nano Emulsifying Drug Delivery System (SNEDDS) Formula with D-optimal Mixture Design Method

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ABSTRACT

This study aimed to optimize and formulate the poorly water-soluble naringenin in the Self-Nano Emulsifying Drug Delivery System (SNEDDS) using D-optimal mixture design. D-optimal mixture design was used to optimize SNEDDS loading naringenin by selecting SNEDDS composition as an independent factor and SNEDDS characterization as a response. SNEDDS in the optimal formula were characterized, including transmittance, particle size, emulsification time, and drug loading. Triacetin, Tween 80, and transcutol p were respectively the selected oil, surfactant, and co-surfactant phases for their greatest ability to dissolve naringenin. The optimization results showed that the optimal formula was that using 10% triacetin, 70% of Tween 80, and 20% of transcutol p. SNEDDS loading naringenin produced nanoemulsion with 88.74±2.27 % of transmittance, 14.8 nm of particle size, 51.13 ± 4.53 mg/L of drug loading, and 18.58 ± 0.62 second of emulsification time. This study concludes that the D-optimal mixture design can be used to optimize and prepare the SNEDDS loading poorly-water soluble naringenin.

INTRODUCTION

Naringenin is a citrus flavanone that is abundant in fruits such as grapes, grapefruit, blood oranges, lemons, pamelos and tamarinds. Naringenin has been reported to have several effects on biological systems such as antioxidant, anti-inflammatory, anticancer, antifibrogenic and antiatherogenic activities. However, other studies have found that administration of this drug is hampered by its extreme water insolubility and low bioavailability (Khan *et al*., 2015). A recent study revealed that single doses of naringenin at 150, 300, 600 and 900 mg were safe and well-tolerated in humans. At these doses, the metabolite naringenin appears in the circulation and disappears after 24 hours (Rebello *et al*., 2020).

Recently, lipid-based formulations have emerged as the best and effective solution for delivering low solubility drugs, among others. The Self-Nano Emulsifying Drug Delivery System (SNEDDS) has proven to be a promising technology to increase the systemic bioavailability of drugs with low water solubility or phytoconstituents. SNEDDS is an isotropic mixture of an active substance, oil, surfactant and usually one or more hydrophilic cosolvents or coemulsifiers which come into contact with an aqueous medium by light shaking, forming a fine oil-in-water nanoemulsion with droplet sizes ranging from 20 to 200 nm (Avachat & Patel, 2015). SNEDDS is based on the phenomenon of self-emulsification and contains more surfactant or hydrophilic cosurfactant, and contains lower fat. SNEDDS can be defined as an isotropic mixture of oils, surfactants, cosurfactants and drugs. When this mixture is dissolved in an aqueous solution *in vivo*, a fine and optically clear

O/W nanoemulsion is formed, assisted by light shaking by motility movements in the stomach and intestines (Kumar *et al*., 2019). SNEDDS has advantages such as the ability to accelerate the dissolution time of lipophilic compounds, reduce the first pass effect and increase absorption (Kyatanwar *et al*., 2010). SNEDDS also has the disadvantage that the simple method of degrading the active ingredient does not work sometimes because the formulation depends on digestion before the active ingredient is released. The chemical instability of the drug and the high concentration of surfactant in the formulation (about 30-60%) can also irritate the gastrointestinal tract (Daga *et al*., 2012)

The maximum solubility of the drug in the oil phase is very important to keep the drug in the dissolved form and prevent the deposition of the drug during dissolution in the intestinal lumen. The higher the solubility of the drug in oil, it is certain that the smaller the amount of oil used in the formulation and consequently the smaller the amount of surfactant and cosurfactant needed to emulsify the drug in oil droplets. It has been observed that the solubility of naringenin is higher in semi-synthetic carrier oils than in natural oils. (Khan *et al*., 2015).

Nonionic surfactants are generally considered safer than ionic surfactants and are generally acceptable for oral administration (Nazzal *et al*., 2002). Surfactant is the main component that can determine the self-emulsification mechanism and droplet size. Co-surfactant works as a solubilizing agent for drugs in oil and is able to help surfactants to stabilize oil dispersion. Failure to select the appropriate oil, surfactant and co-surfactant can lead to errors in the SNEDDS formulation (Kuncahyo *et al*., 2019).

The use of Tween 80 is widely used in research on oil/water (o/w) type SNEDDS formulations to increase the solubility of medicinal ingredients that have poor water solubility such as mefenamic acid (Syukri *et al*., 2020), atorvastatin (Hashem *et al*., 2015), insulin (Winarti *et al*., 2018), and pitavastatin (Kuncahyo *et al*., 2019). Also, one study (Priani *et al*., 2017) with the drug model glimepiride using surfactant tween 80 and Transcutol as co-surfactants showed that the SNEDDS preparation was proven to increase the dissolution of the active substance.

The formula is determined using Design Expert software by selecting the D-optimal mixture design. The independent variables were included along with the upper and lower limits of each variable, namely oil (10 – 30%), surfactant (50 – 80%) and co-surfactant (10 – 30%) (Syukri *et al*., 2020). D – optimal mixture design is used for formula optimization with different concentrations of components used. The D – optimal Mixture Design method has advantages compared to other optimization programs, namely it can automatically display the number of formulations in accordance with predetermined limits. D – optimal Mixture Design will provide variations in the concentration of each ingredient with a predetermined number of formulas making it easier for researchers to make formulas (Wolfe *et al*., 2017). The critical parameters that were observed to see the success of the SNEDDS formula were the emulsification time, drug loading, percent of transmittance and particle size parameters.

METHODS

Materials and Instrument

The tools used are analytical balance (Mettler Toledo ML 204T/00), measuring cup (Pyrex), volumetric flask (Pyrex), beaker glass (Pyrex), micropipette (Dragon LAB NESCO), cuvette, hot plate magnetic stirrer SH-3, ultraviolet-visible (UV-Vis) spectrophotometer Biobase bk-d580, Particle Sized Analyzer (PSA) Horiba PZ-100, ultrasonicator RS19000, 10 mL glass vial Pyrex, type 2 USP dissolution tool 1 chamber, centrifuge. The materials used were naringenin, 96% ethanol (Bratachem), triacetin (Bratachem), tween 80 (Bratachem), transcutol P, distilled water, and methanol PA 0.1 N HCl (Merck).

Preparation of Naringenin SNEDDS Using D-optimal Mixture Design

The SNEDDS formula was made according to the proportions obtained from the software design expert and then adjusted for the manufacture of 10 ml SNEDDS. Parameters ranges for D-optimal mixture design in preparing naringenin SNEDDS were prepared using Design-Expert Version 12.0. Naringenin SNEDDS contains naringenin and excipients: tween 80, triacetin ,and transcutol p. Three independent factors were studied: Comparison of the composition of triacetin = 10 - 30%, tween 80 = 50 - 70 % and transcutol P = 20 - 40 %. Also, four responses were examined: emulsification time, drug loading, the transmittance percentage, and particles size.

Making SNEDDS Naringenin

Each component of SNEDDS, namely triacetin, tween 80 and transcutol p were

pipetted according to the calculations in the formula table in D – optimal mixture design for 10 ml of preparation, then mixed using an ultrasonicator for 10 minutes then stirred with a magnetic stirrer at 500 rpm to form an isotropic mixture (one phase) are homogeneous. The SNEDDS formed was added with naringenin little by little until the saturated condition was indicated by the presence of turbidity in the SNEDDS. SNEDDS naringenin was saturated and then centrifuged at 5000 rpm for 45 minutes. The results of the SNEDDS naringenin supernatant were stored in vials and protected from sun exposure and stored at room temperature. The results of the supernatant were tested for characteristics including emulsification time, drug loading, percent transmittance and measurement of globule size.

Characterization of SNEDDS naringenin

Emulsification Time

Measuring the emulsification time of SNEDDS naringenin by measuring 100 ml of distilled water in a glass beaker then mixing it using a magnetic stirrer at 500 rpm then taking 1 ml of SNEDDS with a micropipette and putting it in distilled water while observing the color change in the emulsion and counting the time until a stable emulsification time is reached with using a stopwatch.

Drug loading

To determine drug loading, a UV spectrophotometer was used with a p.a methanol blank. First, 1 mL of SNEDDS naringenin was pipetted into a 10 mL volumetric flask using a micropipette, then the volume was added with methanol p.a until the boundary marks were shaken slowly until the solution became clear. Second, read the absorption of the solution with a UV spectrophotometer carefully according to the maximum wavelength of naringenin 299 nm. Naringenin drug levels were calculated using a linear regression equation on the calibration standard curve.

The transmittance percentage

The transmittance percentage was determined using a UV Visible spectrophotometer with distilled water as a blank. A total of 1 mL of SNEDDS was dissolved in 100 mL of distilled water and then stirred and observed until homogeneous then read the transmittance of the naringenin nanoemulsion using a UV-Vis spectrophotometer at a wavelength of 650 nm (Sari & Herdiana, 2016).

Particle size

Emulsion droplet size was measured by phorocorrelation spectroscopy using a Zetasizer to measure sizes between 10 and 5000 nm. Light scattering was monitored at 25°C and at a 90 ^{0} angle, using external standardization with polystyrene grains. The nanometric size range of the particles is maintained even up to 100 times diluted with water which proves the compatibility of the system with excess water (Seema & Kumar, 2014).

Determination of Optimum Formula

The optimum formula was selected based on the test results of each characteristic. The conditions that must be met from each characteristic are emulsification time or nanoemulsion formation time < 1 minute; The higher the levels of active substance that can be contained in SNEDDS, the formula is considered optimal. The concentration of Naringenin as a free radical scavenger in the FRAP (Ferric Reducing Antioxidant Power) test is 60 g/mL (Martinez *et al*., 2016) so that naringenin levels are expected to be close to this figure; when the percentage transmittance value is close to 100%, it means that the level of clarity of SNEDDS is close to that of distilled water and has a nanoemulsion droplet size <100 nm.

RESULTS AND DISCUSSION

Formulation of Naringenin SNEDDS

The SNEDDS formula consists of three components, namely triacetin, tween 80 and transcutol P, then the SNEDDS formula is determined using the D-optimal mixture design software on Design Expert 12. The three components are included in the mixture component with a quadratic model and four responses are included, namely emulsification time, drug loading, globule size and the transmittance percentage to obtain 16 run formulas. Based on the design of the formula, 10 mL of SNEDDS preparation was made by mixing the three constituent ingredients into a vial and then putting it in an ultrasonicator for 10 minutes which functions to mix and reduce particle size. These waves surround the sample so that cavitation bubbles are generated which cause nano-diameter particles. The homogeneous SNEDDS mixture was then mixed using a magnetic stirrer at room temperature and the speed was kept constant, then naringenin was added until a slightly cloudy SNEDDS was obtained and there was undissolved naringenin, which indicates that the addition of naringenin is saturated.

The saturated SNEDDS preparation was then centrifuged for 45 minutes at 5000 rpm to obtain a clear supernatant free of impurities. The clear supernatant was then tested for SNEDDS characteristics including emulsification time, drug loading, percent transmittance and globule size. In this study, the results of the SNEDDS supernatant were thick, yellow and clear. The results of observations and characterization of the critical parameters of SNEDDS are presented in Table 1.

Characterization of SNEDDS Naringenin

Emulsification time

Emulsification time is the time required for the preconcentrate to form a homogeneous mixture when diluted. The observations were made by visually observing the loss of SNEDDS and looking at the final appearance of the nanoemulsion. SNEDDS should be completely dispersed and rapidly when diluted with water with light shaking. The selection of oil, surfactant and co-surfactant is very important for the spontaneous emulsification process, since the faster the emulsification time, the higher the absorption of the drug in the gastrointestinal tract. The emulsification time test is conducted using distilled water because it is neutral so it does not affect other components in SNEDDS. The results are used in the formula prediction in determining the optimum formula.

The emulsification time of SNEDDS naringenin when approached with software with D-Optimal mixture design, obtained the following equation:

Y = 6629.20 (A) – 2627.25 (B) + 9501.54 (C) – 17289.65 (AB) + 12704.51 (AC) – 11852.52 (BC)

Description:

Y = emulsification time (second)
A = triacetin
B = tween 80
C = transcutol P

The equation shows that each component has an effect on the emulsification time where the addition of triacetin and transcutol P will increase the emulsification time, while the addition of tween 80 will decrease the emulsification time. The regression coefficient for transcutol P 9501.54 showed the highest value than triacetin and tween 80, which indicated that transcutol P had the greatest effect on emulsification time, namely the greater the composition of transcutol P, the greater the emulsification time. The effect of a co-surfactant depends on its chain length, where only appropriate chain lengths are suitable for good microemulsion formations. Transcutol (medium chain) is the most suitable co-surfactant in combination with the surfactant (Abdul Sisak *et al*., 2017).

Table 1. The results of observations and measurements of the SNEDDS formula

Formula	Composition			Emulsification time (sec)	Drug Loading (µm/mL)	Transmittance (%)	Particle size (nm)
	A	B	C				
1	10	60.07	29.92	30.39 ± 0.095	23.84 ± 0.052	30.22±0.025	25.70±0.100
2	10	50	40	58.15 ± 0.254	25.08 ± 0.010	25.61±0.028	32.23± 0.152
3	10	55.21	34.78	32.26 ± 0.902	29.46 ± 0.173	39.33±0.020	27.23± 0.251
4	30	50	20	32.30 ± 0.041	34.46 ± 0.015	25.90±0.173	332.63± 0.25
5	10	70	20	15.17 ± 0.047	67.73 ± 0.073	90.66±0.292	23.33± 0.416
6	14.88	50	35.11	40.66 ± 0.336	61.93 ± 0.041	44.18±0.160	19.70± 0.200
7	16.68	56.71	26.60	50.35 ± 0.186	64.65 ± 0.015	46.78±0.080	20.13± 0.351
8	19.98	60.01	20	38.31 ± 0.216	26.93 ± 0.058	93.69±0.020	11.40± 0.400
9	20.00	50.13	29.85	80.07 ± 0.457	20.15 ± 0.050	89.39±0.083	44.70± 0.264
10	10	60.07	29.92	55.87 ± 0.272	19.32 ± 0.020	76.21±0.104	12.93± 0.416
11	23.68	53.78	22.52	40.40 ± 0.060	8.40 ± 0.0404	81.34±0.392	282.43± 0.41
12	19.98	60.01	20	46.29 ± 0.095	31.95 ± 0.015	79.74±0.072	11.53± 0.351
13	10	70	20	20.52 ± 0.424	42.90 ± 0.020	84.83±0.040	11.46± 0.550
14	16.68	56.71	26.60	49.90 ± 0.403	56.36 ± 0.025	90.27±0.068	11.86± 0.802
15	20.00	50.13	29.85	78.12 ± 0.112	17.55 ± 0.050	92.12±0.062	42.53± 0.550
16	13.51	63.23	23.24	48.20 ± 0.229	50.58 ± 0.070	89.84±0.040	11.30± 0.700

Drug Loading

Drug loading is used to calculate the levels of naringenin in the SNEDDS. Determination of drug loading is used to determine the ability of SNEDDS to dissolve the drug until the SNEDDS is completely saturated and to determine the drug content of the SNEDDS formula *(Pratiwi et al.*, 2018). Measurement of drug loading on naringenin SNEDDS with the aim of introducing the active substance of naringenin to the maximum into the SNEDDS system, therefore the addition of naringenin is done gradually until a saturated solution is obtained with a slightly cloudy SNEDDS visual appearance and there is an insoluble active substance.

Determination of naringenin levels was calculated using the linear regression equation y = 0.0735x + 0.3698 where y is the measured sample absorption and x is the value of naringenin levels in ppm. The results of the calculation of naringenin levels are then entered into the software and obtained the following equation:

Y = 37794.64 (A) + 2208.39 (B) - 6935.47 (C) – 68.191 (AB) – 90536.95 (AC) + 6894.46 (BC)

description:

Y = *drug loading* (ppm)

A = triacetin

B = tween 80

C = transcutol p

This equation shows that there is a linear relationship between triacetin, tween 80 and the increase in drug loading of naringenin. Triacetin as an oil phase that functions to dissolve naringenin has the main influence in the drug loading process. This equation is also depicted in a three-component contour plot, where there is an interaction between transcutol P, tween 80 and triacetin where there is a red plot in the maximum area of transcutol P and minimal area of tween 80 and triacetin. Enhancers like transcutol p were included in the formulation to facilitate drug diffusion (Leichner *et al*., 2019).

The three-component contour plot, where there is an interaction between transcutol P, tween 80 and triacetin where there is a red plot in the maximal area of transcutol P and the minimum area of tween 80 and triacetin this illustrates that the greater the value of transcutol P and the smaller the value of tween 80 and triacetin, the drug loading will be maximal can be seen in Figure 1.

The transmittance percentage

The transmittance percentage was observed by looking at the level of clarity of the nanoemulsion, the transmittance percentage of the SNEDDS was stated to meet nano-size consistency if the value was close to the aquadest percent transmittance, namely 100% (Seema & Kumar, 2014). SNEDDS with a transmittance value above 80% will produce clear and transparent nanoemulsions. This relates to the size of the SNEDDS droplets which are dispersed into water with a high transmittance value or close to the transmittance value of 100% aquadest so that it looks optically clear.

The results of determining the percentage of transmittance of SNEDDS naringenin which are entered into the software produce the following equation:

Y = - 2201.42 (A) + 208.06 (B) – 311.57 (C) + 2447.13 (AB) + 5364.44 (AC) – 377.76 (BC)

description :

Y = Percent transmittance (%)

A = triacetin

B = tween 80

C = transcutol P

The equation can be concluded that triacetin and transcutol p have a negative effect on the transmittance of nanoemulsions, since the more triacetin and transcutol P, the lower the transmittance value. Tween 80 positively affects the clarity of nanoemulsions, because the greater the amount of tween 80 will give the maximum transmittance percentage. In line with Khan *et al*. (2015) research, there was a significant difference in % transmittance value between the two groups of surfactants (tween 80 and cremophor EL). Formulations containing tween 80 as surfactant showed >90% value and the formula was found to have the highest percentage transmittance.

In the 3-component contour plot image, it can also be observed where there is a red plot in the maximum area of tween 80 which indicates that the greater the value of tween 80 will give the maximum transmittance value. Meanwhile, the blue color in the maximum area of triacetin and transcutol P indicates the greater the value of triacetin and transcutol P, and the transmittance percentage value will be minimal which can be seen in Figure 2.

***Particle size*.**

The average globule size in this study is in the nanometer range, which is <200 nm. The size of the globule produced greatly affects the bioavailability of the drug, because the smaller the size of the globule, the larger the surface area, which accelerates the release of the drug and makes it easier to absorb in the digestive tract. The greater the amount of the oil phase will further increase the size of the globules because the amount of surfactant and cosurfactant is not able to reduce the surface tension in the nanoemulsion system. This can be observed in the equation obtained from the program as follows:

$Y = 331.241 (A) + 16.72 (B) + 36.90 (C) – 649.253 (AB) – 541.763 (AC) + 19042.2 (A^2BC) + 2225.52 (AB^2C) – 19670.6 (ABC^2)$

Description:

Y = particle size (nm)
A = triacetin
B = tween 80
C = transcutol p

The contour plot is marked with a blue plot on the tween 80 and transcutol P sides, indicating that the greater the number of tween 80 and transcutol P, then the lower the globule size has been described in Figure 3.

Determination of the Optimum Formula

The optimization of formulas in pharmaceutical preparations including SNEDDS preparations is to determine the variable level of stable and strong products with high quality characteristics that can be produced. Optimization was done by entering the upper and lower limit values for triacetin oil, surfactant tween 80 and cosurfactant transcutol P using Expert Design software and inputting parameters and criteria, which can be seen in Table 2.

Optimization with D-optimal mixture design is then used to determine the optimal formula with predetermined criteria. The optimal formula obtained was then remade with three replications and characterized including emulsification time, drug loading, globule size, percent transmittance, zeta potential and *in vitro* release test using the dissolution method. The composition of the optimum formula and the results of the characterization of the optimum formula for SNEDDS naringenin can be seen in Table 3.

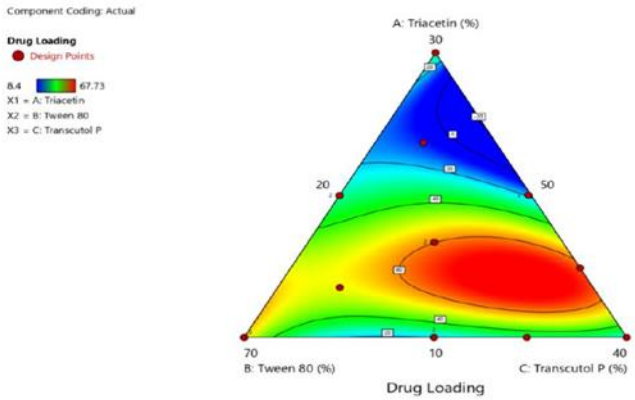


Figure 1. Contour plot of drug loading SNEDDS naringenin.

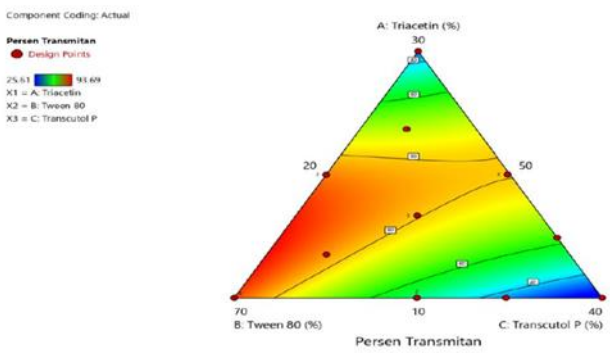


Figure 2. Contour plot of transmittance percentage SNEDDS naringenin.

Table 2. Optimum formula criteria

Parameter	Criteria	Lower limit	Upper limit
Triacetin	*In range*	10	30
Tween 80	*In range*	50	70
Transcutol P	*In range*	20	40
Emulsification time	*In target*	15.17	80.07
Drug loading	*maximize*	8.4	67.73
Particle size	*Minimize*	11.3	332.6
Percent transmittance	*Maximize*	25.61	93.69

Table 3. Optimal formula result and predicted value

No	Triacetin	Tween 80	Transcutol P	Emulsification time (second)	Drug Loading (mg/L)	Particle size (nm)	% Transmittance	Desirability
1	10.16	69.842	20.00	18.58 ± 0.62	51.13 ± 4.53	14.8	88.74 ± 2.27	0.942

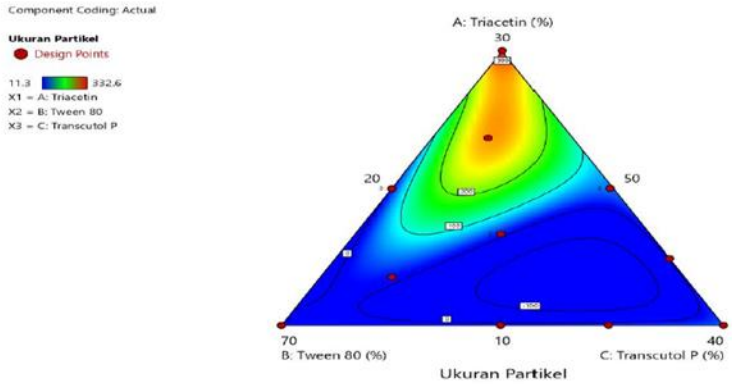


Figure 3. Contour plot of particle size SNEDDS naringenin

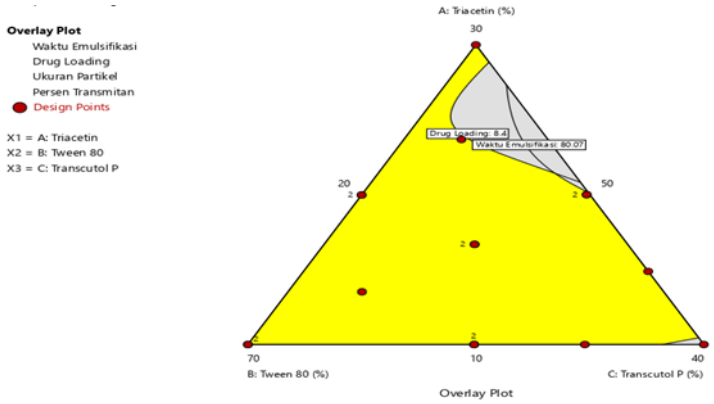


Figure 4. Contour plot of particle size SNEDDS naringenin

Observation and measurement of emulsification time on the optimum formula obtained emulsification time of 18.58 ± 0.62 seconds with a clear and homogeneous visual appearance. From the results of measurements and visual observations of nanoemulsions, it can be concluded that the optimum formula nanoemulsion belongs to grade A (the system forms nanoemulsions quickly in <1 minute with a clear appearance) (Priani *et al*., 2017).

After visual observations, the percent transmittance was measured to determine the clarity level of the nanoemulsion. In measurements using UV-Vis spectrophotometry, the percentage of transmittance of the optimum formula was 88.74% ± 2.275, which can be concluded that the nanoemulsion has a clarity

level that is close to good clarity because it is >80% (Syukri *et al*., 2020).

In the measurement of drug loading, the optimum formula with three replications obtained absorption of 0.753, 0.778 and 0.712. Additionally, after being calculated using a linear regression equation, it obtained naringenin levels of 52.13 mg/L; 55.53 mg/L and 46.55 mg/L and the average value of the optimum drug loading formula was 51.41 ± 4.53 mg/L.

The measurement of the globule size of the optimum formula in this study obtained a globule size of 14.8 nm with a polydispersity index of 0.366. This shows that the globule size in the optimum formula has met the criteria for nanoparticle characterization, namely 10-200 nm and the polydispersity index value describes the uniformity of the size of the nanoemulsion which is polydispersed in which the particle size produced is uniform but has various shapes (Pratiwi *et al*., 2018)

In the overlay plot the optimum formula shows the optimum area which is shown in the grey area. This overlay plot is a combination of contour plots on four critical parameters, namely emulsification time, drug loading, the transmittance percentage and particle size. The red dot in one of the optimum areas is the optimum formula suggested by the Design Expert results from several predictions of the optimum formula in the optimum area, which can be seen in Figure 4.

CONCLUSIONS

Based on the research that was conducted, the following conclusions can be drawn: The optimal proportions of triacetin, tween 80 and transcutol P components in the manufacture of naringenin SNEDDS using the D – optimal mixture design method obtained a ratio of triacetin, tween 80, and transcutol P of 10.16 : 69.84 : 20. The results of testing the critical parameters on the optimum formula obtained the value of emulsification time 18.58 ± 0.62 seconds, drug loading 51.13 ± 4.53 mg/L, transmittance percentage 88.74% ± 2.275, and globule size 14.8 nm .

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