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Optimization and Characterization of *Self Nano Emulsifying Drug Delivery System* (SNEDDS) Naringenin Components with *D-Optimal Mixture Design* Method and Anti-Aging Test *In Vivo*

Adriani Taena[1*], Ilham Kuncahyo[2],Titik Sunarni[3]
Department of Pharmacy, Faculty of Pharmacy, Setia budi University, Surakarta, Indonesia
Corresponding Author: Adriani Taena adriani.taena09@gmail.com

A R T I C L E I N F O

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A B S T R A C T

Naringenin (5,7-dihydroxy-2-(4-hydroxyphenyl) chroman-4-one) is one of the flavanone aglycone compounds that has the main activity as an antioxidant. The purpose of this study was to determine the optimum formula and characterization of naringenin SNEDDS and to see the anti-aging activity of the optimum formula of naringenin SNEDDS. The optimum formula of naringenin SNEDDS was obtained by a combination of oil components, surfactants and cosurfactants (miglyol, tween 80 and transcutol) using the D-optimal mixture design method. The characteristics of naringenin SNEDDS are seen based on parameters such as drug loading, emulsification time, % transmittance, particle size analyzer (PSA) and zeta potential. The results of the optimum formula characterization test of naringenin SNEDDS obtained drug loading of 2.004507 mg/5 ml, emulsification time of 13.07 seconds, percent transmittance of 85.1%, particle size of 78.76 nm and zeta potential of -8.14 mV. Based on the results of the characteristics obtained from the optimum formula, the naringenin SNEDDS preparation meets the characteristics of good SNEDDS and has the potential to be developed into a drug preparation.

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INTRODUCTION

Indonesia is a tropical country that gets a lot of exposure to sunlight throughout the year. Sunlight is very important for human survival, excessive exposure will cause adverse effects on the skin, namely from ultraviolet radiation (UVR), which consists of around 95% UVA and 5% UVB (Vinka et al., 2022). Exposure to UV rays over a long period of time can trigger hyperpigmentation, sunburn and skin damage to the point of skin cancer known as malignant melanoma and skin aging (Saffrin & Sureshkumar, 2019). To overcome these skin problems, cosmetic products with antioxidant properties are needed.

Skin aging is a complex biological process resulting from intrinsic aging (from within the body such as genetics) and changes that develop over time. External factors that greatly influence aging include repetitive facial expressions, improper sleeping positions, smoking and other factors. Skin aging can be in the form of skin that looks thin and transparent, appears fine wrinkles, spots that appear due to pigmentation, skin becomes loose, dry with or without itching, lack of sweat, gray hair, hair loss, unwanted hair, thinning nail plates, loss of half-moon nails and influenced by environmental factors. Based on these factors, premature aging factors are often associated with the free radical theory as the cause. Free radicals are atoms or molecules that are highly reactive with unpaired electrons (Winarsi, MS, 2007). Excessive production of free radicals can damage the collagen of the skin cell membrane, causing wrinkles and loss of skin elasticity (Pamela, 2008).

Compounds that can counteract free radicals are antioxidants. As active ingredients, antioxidants are used to protect the skin from damage due to oxidation so that they can prevent premature aging (Masaki, 2010). Antioxidants have a small molecular weight, but are able to inactivate the development of radicals. Antioxidants are also compounds that can inhibit oxidation reactions, by binding free radicals and highly reactive molecules, as a result cell damage will be inhibited. One of the antioxidants found in nature is orange peel.

Naringenin is a pure flavonoid compound known to have anti-inflammatory, antiteratogenic, antifibrogenic, anticancer and secondary antioxidant effects (absorbing UV radiation) (Salehi et al., 2019). According to Saiz *et al*., 2010, 0.02 g of naringenin isolate has antioxidant activity indicated by an IC50 value of 7.9 µg/ml. The strong antioxidant activity of naringenin is influenced by three hydroxyl groups in the compound structure.

There are several methods used to increase the solubility and absorption of naringenin including nanoparticles, Self Nanoemulsifying Drug Delivery System (SNEDDS), Solid Lipid Nanoparticle (SLN) and elastic liposomes (Yen et al., 2009; Khan et al., 2015; Ji et al., 2016; Tsai et al., 2015). In this study, the Self Nanoemulsifying Drug Delivery System (SNEDDS) method was chosen because it is a suitable drug delivery system for drugs that have low solubility properties. Antioxidants as anti-aging can prevent premature aging. The use of cosmetics as anti-aging that are rich in antioxidants can make facial skin more

well-maintained (Winarsi, MS, 2007). This antioxidant can be made in the form of cosmetic preparations either in the form of gel, cream or lotion preparations.

SNEDDS is an isotropic natural or synthetic oil, surfactant and cosurfactant that when it meets the water phase through mild agitation will produce an oil-in-water dispersion with an emulsion drop size ranging from nanometers that occurs spontaneously. SNEEDS is a drug delivery system that is widely developed because it has greater advantages including increasing the bioavailability of drugs with low solubility, stable in reducing the frequency of drug administration, more stable than nanoemulsions/microemulsions physically, can carry active drug substances to their targets without affecting the surrounding conditions and has a smaller drug drop size, resulting in increased surface area in the digestive tract. (Wahyuningsihh et al., 2015; Wang et al. 2010).

Research conducted by Khan et al., 2015 showed the results of the selection of the most optimum components in SNEDDS orally to rats from various variations of oil, surfactants and co-surfactants. In the study of Yuliani et al., 2016, the use of miglyol as the oil phase in nanoemulsion produced a droplet size of 230-280 nm. Among the surfactants screened, Tween 80 showed the maximum oil solubilization capacity, which was 1.47 w% ($p < 0.001$). Co-surfactants were selected based on their ability to maximize the nanoemulsion area with the selected surfactant. Therefore, a ternary phase diagram was made with a mixture of surfactants and various co-surfactants at a ratio of 1:1Smix. Transcutol HP showed the maximum nanoemulsion area due to its better compatibility with the selected surfactant compared to other co-surfactants tested.

Formula determination using Design-Expert software by selecting the D-optimal mixture design. Independent variables were entered along with the upper and lower limits of each variable, namely oil (10-30%), surfactant (50-80%), and co-surfactant (10-30%). The results of the study showed an increase in the oral bioavailability of naringenin SNEDDS development compared to pure suspension, with the peak plasma concentration (Cmax) of NRG in T14 and NRG suspensions being 28345.48 ± 134 & 5745.79 ± 78 ng/ml, respectively.

Topical drug delivery system by direct application to the target is the choice of local drug use because it produces faster effects. Naringenin has very low solubility in water or hydrophobic, but is soluble in ethanol, methanol, DMSO and lipids (Wissing et al., 2001; Muller et al, 2002). The development of this study is the use of different oil components from previous studies using the D-optimal mixture design method and in vivo anti-aging tests. Naringenin formed by SNEDDS is expected to produce small particle sizes, large surface areas, can provide occlusive effects, high drug loading, phase interactions at the interface and its potential to increase the pharmaceutical effects of its solid properties.

LITERATURE REVIEW

Preparation of Self-Nano Emulsifying Drug Delivery System (SNEDDS) Naringenin

SNEDDS was made by mixing the oil phase, surfactant and co-surfactant according to the ratio of each component in each run obtained through the application of expert design 13. Then mixing was carried out using an ultrasonicator and stirred with a stirrer for 5 minutes at 300 rpm. SNEDDS naringenin was made by adding naringenin little by little until saturation conditions were achieved (mixing for 48 hours at a temperature of 26 ± 10 C). the mixture was centrifuged at 500 rpm for 45 minutes. The naringenin supernatant results were stored in vials and protected from exposure to sunlight at room temperature (Khan et al., 2015).

Characterization of the Self-Nano Emulsifying Drug Delivery System (SNEDDS) Naringenin

Drug Loading. Drug loading determination is done by taking 0.1 SNEDDS naringenin then making it up to 10 ml and homogenizing it. The dissolved naringenin content is read using a UV spectrophotometer at maximum wavelength (Salim, 2020).

*Emulsification time. W*Emulsification time is the time required for SNEDDS to spontaneously form an emulsion when diluted into aqueous media. Pipette 2.5 ml of SNEDDS sample then add 250 ml of distilled water at a temperature of 37 ± 1^{o}C, put it in an Erlenmeyer flask then stir at a speed of 100 rpm. The time required to form a homogeneous emulsion dispersion system is then recorded (Kuncahyo, et al. 2021).

Percentage transmittance (%T). The results of the emulsification time determination are used to determine the transmittance percentage. The transmittance percentage is carried out by means of a dispersion system formed in a stirrer at a speed of 500 rpm for five minutes and then viewed with UV spectrophotometry with a wavelength of 650 nm using distilled water as a blank to determine the level of clarity. The use of high wavelengths with the aim of achieving a sample is said to have clarity that resembles a blank if the transmittance results are close to 100% (Kuncahyo et al., 2021).

*Particle Size Analyzer (PSA)***.** The particle size test was carried out by diluting 1 ml of SNEDDS naringenin into 100 ml of distilled water and then homogenizing it using a Microtrac Nanotrac Wave II device with an RI setting of 3.33 until a nanoemulsion system was formed. Determination of particle size with a particle size requirement of 50-1000 nm (Pattewar, 2018).

Zeta Potential. The zeta potential test was conducted to determine the surface charge of particles spread in the dispersing medium with a zeta potential value of -30 mV or greater than +30 mV having a high stability value (Mudock, et al., 2008). Measurement of the zeta potential value was carried out using a Microtrac Nanotrac Wave II tool with an RI setting of 3.33 mV.

Determination of Optimum Formula

The determination of the optimum formula is done by analyzing the results of the characterization test including emulsification time, %

transmittance, particle size, and drug loading using the Design Expert Version 13 application with the D-optimal mixture design method. The characterization test according to each critical parameter is carried out by an analysis test and must show significant. Then in the numerical optimization section we can determine the desired goal and then the solutions section can be selected so that the optimum formula run appears that we can use to create the optimum formula.

Optimum Formula Creation

The combination of oil, surfactant, and Co-Surfactant was homogenized using a magnetic stirrer at a speed of 500 rpm then naringenin was added until saturated or precipitation of naringenin powder occurred. After saturated, the mixture was centrifuged at a speed of 5,000 rpm for 45 minutes then the supernatant was taken. The resulting SNEDDS was stored at room temperature for further testing (Kuncahyo et al., 2021).

Security Test

Safety tests are carried out to prevent side effects of the formula on the skin of test animals. Safety testing is carried out using the Draize method where the rabbit test animal's fur is shaved and the optimum formula is applied to the rabbit's back area. The rabbit's back is covered with sterile gauze and bandages, leave for 24 hours then opened and left for 1 hour and observe the formation of edema and erythema and close the rabbit's back again. The process is repeated in the next 48 and 72 hours and observe the formation of edema and erythema again then make an assessment based on the level of skin irritation reaction.

Anti-aging test

Anti-aging testing can be done in vivo. A total of 1 rabbit is adapted for one week in a cage. The rabbits to be used in the study have their fur shaved on their backs. The rabbit's back is divided into 2 parts. The fur on the rabbit's back is then shaved using a hair clipper carefully. After the rabbit's fur is shaved, measure the percentage of collagen, percentage of moisture, and percentage of elasticity using a Skin Analyzer. The rabbits were given UV-A irradiation treatment. Irradiation was carried out using Exoterra® Daylight Basking Spot containing UV-A rays at a distance of 30 cm with a dose of 63.69 J.cm-2/hour for 6 hours (Budiawan 2018). After the rabbits were induced to wrinkle, the wrinkle parameters were observed using the Skin Analyzer tool on day 0 before applying the cream. On day 0 to day 28, the cream was applied according to the experimental group once a day for 28 days (Duraivel et al. 2014) & Depkes RI, 2020).

Wrinkle parameters were observed before light induction, on day 0 (before cream application), and on day 30 (after cream application). Wrinkle parameters observed included collagen percentage, moisture percentage, and elasticity percentage using the*Skin Analyzer*. Collagen percentage, moisture percentage, and elasticity percentage can be directly viewed on a computer that has the Skin Analyzer application installed to show these percentages.

METHODOLOGY

Research design

The research design to be conducted is an experimental study with variations in the amount of oil components, surfactants and co-surfactants. The parameters of the SNEDDS naringenin test include drug loading, emulsification time, percent transmittance, particle size analyzer (PSA)/particle size and zeta potential using the D-optimal mixture design method. Furthermore, the anti-aging activity test of the optimum formula of SNEDDS naringenin was carried out compared to pure naringenin on rabbit test animals.

Material

The materials used in this study were naringenin isolate, miglyol as oil, tween 80 as surfactant, transcutol as co-surfactant, test animals (rabbits), distilled water, ethanol *p.a*.

Tools

The tools used in this study were analytical scales, analytical scales, micropipettes, Hot plate magnetic stirrer, centrifuge, Particle Size Analyzer (Microtac Nanotrac Wave II), Polydispersity index (Microtac Nanotrac Wave II), Zeta potential (Microtac Nanotrac Wave II), and UV-Vis Spectrophotometer, skin analyzer tool.

RESEARCH RESULT

Making SNEEDS naringenin

The process of making SNEEDS naringenin is carried out by mixing oil, surfactants and cosurfactants that have been weighed based on calculations, then mixing using a magnetic stirrer. The active substance naringenin is added little by little into the oil phase until a saturated mixture is obtained. The suspension is then centrifuged to separate the sediment and then separate the clear part in the vial for further testing (Kuncahyo et al., 2021).

Characterization of naringenin SNEDDS

Characterization of naringenin SNEDDS was then tested for characteristics including drug loading, emulsification time, percent transmittance, particle size analyzer (PSA)/particle size and zeta potential. The results of the characterization of naringenin SNEDDS are presented in the following table.

Table 1. Observation and Measurement Results of the SNEDDS Naringenin Formula

Formula	Composition			DL	WE	% T	PSA	ZP
	A	B	C	(µg/mL)	(second)		(nm)	(mV.)
1	15.6	57,2976	27,1024	764,039	13.02	97.1	27.66	-10.85
2	15.6	57,2976	27,1024	958,493	13.34	98.3	25.38	-11.85
3	15.6	57,2976	27,1024	1750,835	12.89	98.1	24.15	-11.33
4	22,7296	57,144	20,1264	1340,695	18.26	42.7	140.2	-6.01
5	15.6	50	34.4	1208,824	15.57	85.5	78.76	-9.49
6	22,6402	50,1272	27.2327	916,026	26.5	93.2	56.59	-5.18

7	22,7296	57,144	20,1264	673,518	18.12	91.5	147.3	-6.04
8	17.9475	50,6379	22,4145	786,390	11.74	97.9	62.09	-16.13
9	15.6	50	34.4	1212,176	10.88	92.5	81.35	-8,275
10	15.6	50	20	787,508	14.7	46.1	113.1	-5,301
11	26,2441	50	23,7559	1117,185	15.22	77.3	76.3	-6.14
12	18,0276	52,4666	29,5058	1193,178	12.31	69.1	43.69	-9,689
13	22,6402	50,1272	27.2327	901,498	27.07	85.3	49.38	-6.17
14	15.6	64.4	20	1144,006	33.32	97.8	16.38	-10.72
15	26,3259	53,6741	20	1159,652	37.14	53.1	82.56	-5.21
16	20,3336	54,9211	24,7453	758,451	13.43	27.3	29.9	-4.23

Information:

A :Miglyol
B : Tween 80
C :Transcutol
DL :*Drug Loading*
WE : Emulsification Time
%T : %Transmit
PSA :*Particle Size Analyzer*(PSA)
ZP : ZetaPotential

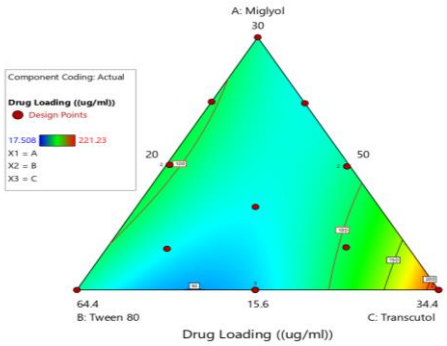

Figure 1. Parameter graph *residual drug loading*

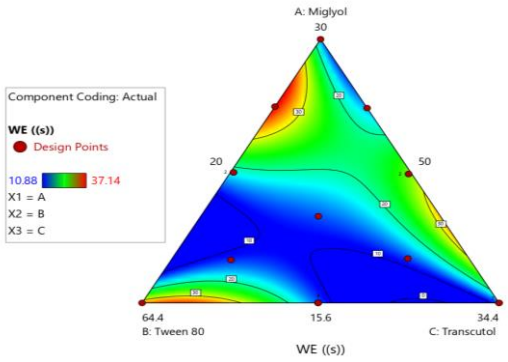

Figure 2. Parameter graph *residual emulsification time*

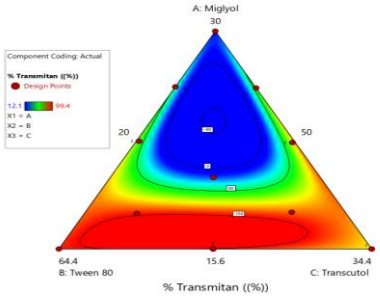

Figure 3. Parameter graph *arameter graph residual % transmitan*

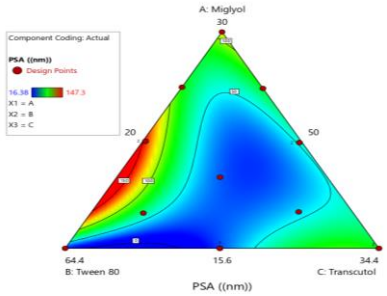

Figure 4. Parameter graph *residual particle size analyzer (PSA)*

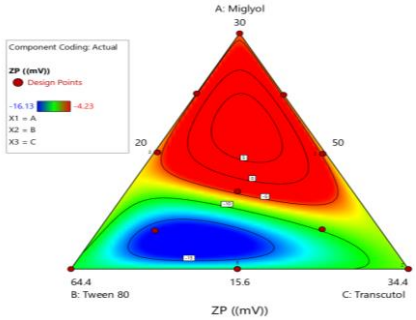

Figure 5. Parameter graph*residual zeta potential*

Determination of the optimum formula

The optimum formula of naringenin SNEDDS was determined through a numerical approach in the Design Expert 13 application. The determination of this optimum formula uses drug loading parameters, emulsification time, particle size analyzer (PSA)/particle size, % transmittance and zeta potential. The determination of the optimum formula of naringenin SNEDDS was determined by filling in the target, lower and upper for each parameter in the design expert application.

Table 2. Criteria for Determining the Optimum Formula

Parameter	Goal	Lower Limit	Upper Limit
Miglyol	*in range*	15.6	30
Tweens 80	*in range*	50	64.4
Transcutol	*in range*	20	34.4
Emulsification Time	*in range*	0	60
% Transmittance	*in range*	0	100
Particle size analyzer	*in range*	50	1000
Drug Loading	*maximize*	17,508	221.23
Zeta potential	*in range*	-30	30

Table 3. Results of Optimum Formula Response Measurements

Number	Miglyol	Tweens 80	Transcutol	DL (µg/mL)	WE (second)	% T	PSA (nm)	ZP (mV.)	Desirability	
1	15,600	50,000	34,400	215,913	13,202	77,495	79,966	-8,880	**0.974**	**Selected**

Information:
DL :*Drug Loading*
WE : Emulsification Time
%T : % Transmittance
PSA:*Particle Size Analyzer*
ZP : Zeta Potential

Optimum formula creation and characterization

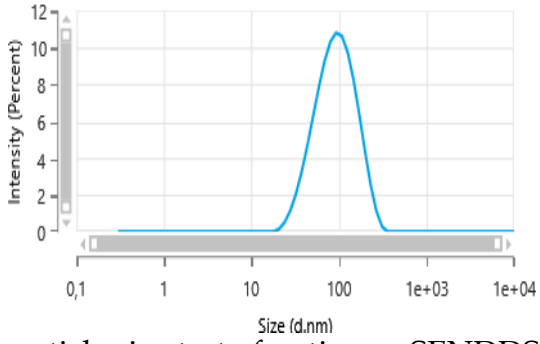

Figure 6. Results of particle size test of optimum SENDDS naringenin formula

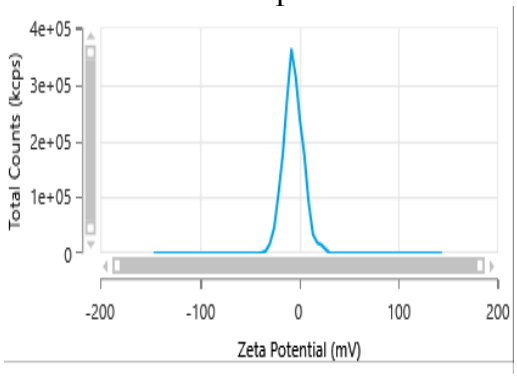

Figure 7. Zeta potential of the optimum formula of SNEDDS naringin

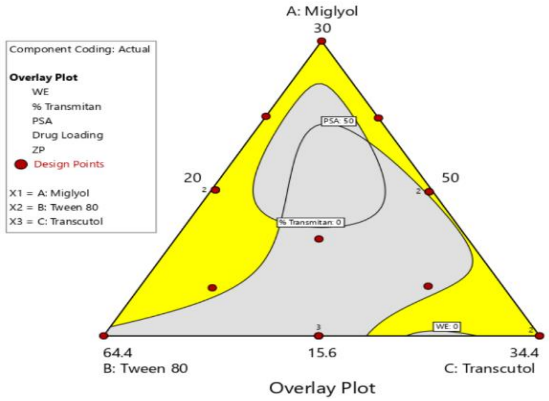

Figure 8. *Overlay plot on optimum area*

Optimum formula verification and statistical analysis

Table 4. Prediction values of critical parameter characterization results of SNEDDS Naringenin

Critical parameters	Theoretical response	Trial response	Sign.	Information
Drug loading	215.913 µg/ml	204.507 µg/ml	0.053	Non-significant
Emulsification time	13.20 seconds	13.07 seconds	0.057	Non-significant
% Transmittance	77,495	85.1%	0.378	Non-significant
Particle size	79.966 nm	78.76 nm	0.072	Non significant
Zeta potential	-8,880 mV	-8.14 mV	0.566	Non-significant

Anti-Aging Test

Safety test of optimum formula of SNEDDS naringenin

Table 5. Safety Test (Irritation)

SNEDDS naringenin	IIPR Value			Information
	24	48	72	
Optimum Formula	0	0	0	Non-Irritant
Pure Naringenin	0	0	0	Non-Irritant

Information :

IIPR :Primary Irritation Index

DISCUSSION

Drug loading

The measurement of SNEDDS naringenin drug loading was measured based on the results of the determination of naringenin isolate levels using a UV-Vis spectrophotometer with a wavelength of 288 nm with operating time measurements at 27-30 minutes. Evaluation of the contribution of miglyol, tween 80 and transcutol with different compositions and emulsification time response variables has the final equation of the model:

Y1= + 89.61(A) + 88.22(B) + 215.91(C) + 77.48(AB) – 255.71(AC) -365.02 (BC).

Information:

A:Miglyol

B: Tween 80

C:Transcutol

Based on the evaluation of the contribution to the equation above, the interaction relationship between coefficients A, B, C, and AB is significant, which is indicated by a positive value. Based on the coefficient value between components and response data, the percentage amount (AB) has only a slight effect on the increase in drug loading, which is indicated by a coefficient value of +77.48. While the percentage (AC) and BC) have a negative effect on drug loading because the more the mixture, the lower the drug loading value.

Figure 1 shows that the concentration of transcutol has a greater effect on increasing the drug loading value compared to miglyol and tween 80. While the increasing concentration of miglyol-transcutol (AC) and the concentration of tween 80-trancutol (BC) have an effect on decreasing the drug loading value and resulting in suboptimal drug loading. Drug loading in this study ranged from 17.508 µg/ml to 221.23 µg/ml.

Emulsification Time

The purpose of the emulsification time test is to determine how quickly the SNEEDS naringenin formula forms an emulsion. (Huda & Wahyuningsih, 2016). The emulsion time is said to be good if the emulsification occurs in less than 1 minute with a clear or transparent visual. The emulsification time of the 16 runs obtained a range of 23.31 seconds - 37.14 seconds.

Evaluation of the contribution of miglyol, tween 80 and transcutol with different compositions and emulsification time response variables has the final equation of the model :

Y2= +14.59(A) + 33.27(B) + 13.20(C) -22.30(AB) + 51.71(AC) -43.04(BC) -148.16(ABC) + 238.80(AB(AB)).

Information:
A:Miglyol
B: Tween 80
C:Transcutol

Based on evaluation of the contribution above components and mixtures of components that have positive values can affect the increase in emulsification time, where the addition of components A, B, C, a mixture of components AC, AB (AB) and BC (BC) results in an increase in emulsification time and is unstable. While the components and mixtures of components that have negative values will have a good effect in reducing emulsification time, where the more mixtures of components AB, BC, ABC and components AC (AC) then the stability of the nanoemulsid components is better with the provision of an emulsification time of <1 minute.

The results of the emulsification time test on each SNEEDS formula were different, but from the 16 runs, the emulsification time formed did not exceed 1 minute. In the contour plot above, the tween 80 and trascutol components have a major effect on reducing the emulsification time where the addition of tween 80 and transcutol components results in a smaller time being produced in forming a nanoemulsion system.

Transmittance

The dilution results from the emulsification time were then tested for % transmittance value to see the level of clarity of each formula. Reading of % transmittance value using UV spectrophotometry at a wavelength of 650 nm with distilled water as a blank. The % transmittance response has a final equation from the model :

Y3= +11.45(A) + 96.79(B) +77.49(C) +62.52(AB) + 164.12(AC) +47.49(BC) -9915.53(A2BC) + 2110.51(AB2C) +1629.26(ABC2)

Information:
A :Miglyol
B : Tween 80
C :Transcutol

Based on the results of the contribution evaluation equation above, the positive value has an effect on increasing the % transmittance value where the addition of components A, B, C, a mixture of components AB, AC, BC, AB2C

and ABC2 increases the % transmittance value, meaning that the clarity of the nanoemulsion is maximized and vice versa. The mixture of tween 80 concentration is more dominant in increasing the % transmittance value because it has a higher concentration value.

In the contour plot above, the red color indicates the high concentration of tween 80 and transcutol which affects the % transmittance value to be higher. A higher concentration of tween can form an o/w nanoemulsion system so that the level of clarity of the nanoemulsion increases.

***Particle size analyzer* (PSA).**

In SNEEDS naringenin preparation, particle size plays an important role because it can determine the rate of drug release which affects the absorption process. The variable in the particle size response has a final equation of the model:

Y4 = +112.69(A) + 16.18(B) +79.97(C) +339.24(AB) -162.26(AC) -84.20(BC) -1403.48(ABC) -743.42(AB(AB)) +45.40(AC(AC)) -314.48(BC(CC)).

Information:

A:Miglyol

B: Tween 80

C:Transcutol

Based on the evaluation of the contribution above, if there is an increase in the concentration of AC, BC, ABC, AB (AB) and BC (CC) shows a positive effect on the particle size where the addition of the concentration of these components can reduce the size of the nanoemulsion particles resulting in a smaller nanoemulsion particle size value. If the particle size is smaller, the surface area is larger so that it can accelerate the release of the drug and facilitate drug absorption.

The contour plot image above shows that the concentration of miglyol, tween 80 and transcutol has a positive effect because it can reduce the particle size, but a larger concentration of tween 80 has a very negative effect in increasing the particle size. The image above shows that the red color indicates a larger particle size which is influenced by the mixture of tween 80 and miglyol concentrations.

Zeta potential.

Zeta potential is the potential difference between the surface layers of particles. The high electrical charge on the surface of the particles can prevent nanoparticle aggregation due to the strong repulsive force between particles. Zeta potential reading on the nano zetasizer tool to calculate zeta potential using the principle of electrophoresis Evaluation of the contribution of miglyol, tween 80 and transcutol with different compositions and variables on the zeta potential response has a final equation of the model:

Y5 = -5.24 (A) -10.75 (B) -8.88 (C) +6.20 (AB) +0.6732 (AC) -6.07 (BC) +1378.65 (A2BC) -828.65 (AB2C) -188.37 (ABC2).

Information :

A:Miglyol

B: Tween 80

C:Transcutol

The evaluation of the above contributions shows a significant relationship between the zeta potential response and the components of miglyol (A), twenn 80 (B) and transcutol (C). Based on the evaluation of the above contributions, components A, B, C, BC, AB2C and ABC2 have a negative effect on decreasing the zeta potential value, while variations in the mixture of components AB, AC and A2BC have an effect on increasing the zeta potential value.

The contour plot above shows that the red and blue colors, namely the miglyol and tween 80 components, have a major effect on increasing the zeta potential value. The addition of miglyol and tween 80 concentrations will result in the large and small zeta potential values, meaning that it can maintain particle aggregation with the repulsive force of the dispersed particles so that the nanoemulsion is stable.

Determination of the optimum formula

The optimum formula obtained was then replication and characterization three times including drug loading, emulsification time, particle size, transmittance percentage and zeta potential. The composition of the optimum formula and the characterization results of the optimum formula of SNEDDS naringenin can be seen in the following table 3.

The filled criteria produce a solution for the optimum formula with the desirability value. The desirability obtained in this study is 0.974. A good desirability value is close to 1. This means that it can be concluded that the optimum formula obtained is quite good because it is close to 1.

Optimum formula creation and characterization

After determining the optimum formula, it was continued with the creation and characterization of the optimum formula based on the specified parameters. Observation and measurement of emulsification time obtained an emulsification time of 13.07 seconds with a clear visual appearance. From these results it can be concluded that the optimum formula of naringenin SNEDDS is in accordance with the emulsification time criteria because it is able to form nanoemulsions at a time speed of <1 minute.

The results of the emulsification time measurement were then continued with the measurement of the transmittance percentage using UV-Vis spectrophotometry, the optimum formula transmittance percentage value was 85.1%. It can be concluded that naringenin SNEDDS has a good level of clarity because it is close to 100%.

The measurement of the optimum drug loading formula of SNEDDS naringenin was carried out by replication and an average absorption of 0.370 was obtained. The absorption results were then calculated using a linear regression equation to obtain a naringenin content of 2044.507 µg/5ml or equal to 2.004507 mg/5ml.

The results of the optimum formula particle size test in this study were 78.76 nm. This indicates that the optimum formula particle size of SNEDDS

naringenin meets the nanoparticle characterization criteria, namely 50-1000 nm. The results of particle measurements can be seen in the following figure.

The zeta potential value is used to predict the stability of the optimum SNEDDS naringenin formula preparation during storage. The requirement for the zeta potential value is in the range of less than -30 mV or more than +30 mV indicating a good level of stability of the preparation. Surfactants are components of the nanoemulsion system that can affect the zeta potential value because the surfactant position is at the interface of two immiscible liquids. The results of the optimum SNEDDS naringenin formula test obtained a zeta potential value of -8.14 mV which indicates that the optimum SNEDDS naringenin formula has a negative surface. Figure 6 & 7.

In the optimum formula on figure 8, overlay plot, the gray area is the optimum area. The overlay plot is a combination of contour plots of critical parameters, namely drug loading, emulsification time, percent transmittance, particle size analyzer, and zeta potential. The red dot in one of the optimum areas is the optimum formula suggested by the expert design application from several optimum formula predictions in the optimum area.

Optimum formula verification and statistical analysis

Verification of the optimum formula is done by comparing the theoretical response value obtained from the expert design application with the experimental response value to determine the significance between the optimum formula test parameters of the experimental results and the optimum formula. Verification is done by testing the normality using SPSS. Normally distributed data is then continued using the t-independent test. Verification test by entering the optimum formula data results into SPSS and then comparing them with the optimum formula characterization test results and testing for normality. The predicted values of the critical parameter characterization results of SNEDDS naringenin can be seen in the following table 4.

Normality test of data distribution assessment to see whether a group of data or variables is normally distributed or not. In the normality test of theoretical responses and experimental responses, the results show that the data is normally distributed so that it is continued with the t-independent test with statistical analysis with a 95% confidence level to see the significance between the two responses. The results of the verification of the optimum formula using the SPSS application show that the parameters of the optimum formula from the test results compared to the predicted values show normally distributed data with a significant value of > 0.05. Furthermore, in the t-independent test, the predicted value compared to the test value also shows significant results. It can be concluded that there is no significant difference between the results of the optimum formula test and the predicted results.

Anti-Aging Test

Safety test of optimum formula of SNEDDS naringenin

Safety test of the optimum formula of SNEDDS naringenin to prevent side effects such as itching and irritation of the skin. The test was carried out in vivo using 1 rabbit for 24, 48 and 72 hours after applying the optimum formula

and then observing the amount of erythema and edema that appeared. The irritation score can be seen in the following table 5.

Based on the observation results, the optimum formula of SNEDDS naringenin did not cause any irritation to the rabbit's back skin so it can be said to be safe for further testing.

Anti-Aging Test

The antiaging activity test was carried out by shaving the fur on the rabbit's back and then reading the percentage of collagen, moisture and elasticity on the rabbit's back skin using a skin analyzer. Parameter observations were carried out 1 day before the calculation of the day of application and irradiation with UV light (calculated as H0) from the first day to the 28th day.

Measurement of collagen parameters was carried out on day 0 before irradiation and application of the optimum formula, showing that the rabbit's back skin was rich in collagen and healthy with a collagen percentage value of 60%. On day 28 after irradiation for 6 hours and daily application, the collagen% result was 25%. In pure naringenin, the collagen% measurement result on day 0 was 60% and on day k2-28 after irradiation and application was 25%.

The results of the elasticity of the rabbit's back skin on day 0 were 50%. After applying the optimum formula on day 28, the results of the elasticity of the rabbit's back skin were 15%. In pure naringenin, the results of the measurement of % elasticity on day 0 were 71% and on day 28 after irradiation and application, the % elasticity of the rabbit's back was 28%.

The results of the rabbit's back skin moisture percentage on day 0 were 65%. After applying the optimum formula on day 28, the rabbit's back skin moisture percentage was 42%. In pure naringenin, the measurement of the rabbit's back moisture percentage on day 0 was 28% and on day 28 after irradiation and application, the moisture value was 18%. Measurement of the percentage of collagen, elasticity and moisture in the optimum formula of SNEDDS naringenin and pure naringenin on day 28 had a smaller value than day 0 because the components were still in the form of a delivery system formula and had not been made into a preparation so that they could not provide a good enough percentage value for the levels of collagen, elasticity and moisture in the rabbit's back skin.

CONCLUSIONS AND RECOMMENDATIONS

First, the combination of Miglyol, tween 80 and transcutol can influence the characterization test of SNEDDS naringenin in the form of drug loading, emulsification time, % transmittance, particle size and zeta potential.

Second, the optimum formula proportion of miglyol, tween 80 and transcutol in naringenin SNEDDS is reviewed from the critical parameters in the form of drug loading of 2.004507 mg/5mL, emulsification time of 1307 seconds, % transmittance of 85.1%, particle size of 78.76 nm and zeta potential of -8.14 mV.

Third, the antioxidant activity in the optimum formula of SNEDDS naringenin has less than perfect or low anti-aging activity compared to pure naringenin because the formula is still in the form of a delivery system formula and has not been made into a dosage formula.

ADVANCED RESEARCH

After conducting research related to the optimization and characterization of naringenin SNEDDS, it is suggested that further research is needed on several topics that have not been continued, such as the need to make an optimum formula into a dosage form, testing the optimum formula using the TLC test, and the need for further research related to the stability of SNEDDS.naringenin.

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