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Optimization of Nanoemulsion Formula From Madecoside Active Compounds Using Design Expert ® *13*

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INTRODUCTION

Madecoside is the bioactive component in the gotu kola herb (*Centella asiatica* L. Urban) who working strengthen skin cells and increase repair, stimulate cell blood and cell immune, andas antibiotics experience. Madecoside also play a role important in repair damage cell with synthesize collagen (Selfitri, 2008). The active substance madecoside is widely used in the pharmaceutical industry, food, and in the cosmetic world. However, this active substance has very poor absorption pharmacokinetic properties, so a drug delivery system is needed that can improve the absorption properties of madecoside. So that one of the preparations that can improve the absorption of madecoside is nanoemulsion (Tan *et al.*, 2021; Zhang *et al* ., 2016) . Nanoemulsions can improve the solubility of active compounds that have poor solubility with very small particle sizes ranging from 1-100 nm which aim to increase the stability of the active substance and improve absorption (Zhang *et al.* , 2016). Optimization could interpreted as approach for get combination best from something product or process characteristics in lower condition certain elements, can also be interpreted as choosing the best elements or materials from some choices which available (Singh *et al*., 2008). In design formula optimal use *design expert* ® 13 could help designing variation formulas, use *design expert*also can formulate drugs conventional like tablet and also capsule.

RESEARCH METHODS

Tools and Materials

The tools used in this research include: 100 ml Infusion bottle, Erlemeyer, Dropper Pipette, beaker glass, measuring cup, Aluminum Voil, Funnel, Analytical Scales (Shimizu®, Jepang), Object Glass, Cover glass, Vis cometer, pH meter ATC, Microscope Optilab (Thinky), *planetary centrifugal mixer* (Thinky) and PSA (*particle size analyzer*), *zeta potential Analyzers* "SHIMADZU" and "HORIBA" .
The materials used in this study include: asiatocoside (Markherb) , aquadest (Novalindo), VCO (Shakar Soya Products), Lesetin (Sakar Soya Products) and Tween 80 (Barataco).

WORK PROCEDURES

Determination of Nanoemulsion Base Formula

This study uses a *design expert method* with five factors and two levels. The factors in this study were lecithin concentration, tween 80 concentration, VCO concentration , stirring time and stirring speed. The level used consists of high and low levels. The determination of the nanoemulsion base formulation can be seen in the following tables I and II .

Table I. Determination of nanoemulsion base formula by design expert 13 .

No	Factor	Level		
		Low	Tall	Utility
1	Tween concentration 80	15%	35%	Co-surfactant
2	Lecithin Concentration	1%	3%	Phospholipids
3	VCO concentration	1%	3%	Oil phase
4	W of stirring time	10 minutes	15 minutes	Set the stirring speed and time
5	Stirring speed (rpm)	400 rpm	20 00 rpm	

Tablel II . Optimized Amount of Nanoemulsion Base

No	Tween 80	Lecithin	VCO	Stirring Time	Stirring Speed
1	15%	1%	1%	10 minutes	40 0
2	15%	1%	1%	15 minutes	40 0
3	15%	1%	3%	10 minutes	40 0
4	15%	1%	3%	15 minutes	40 0
5	15%	3%	1%	10 minutes	40 0
6	15%	3%	1%	15 minutes	40 0
7	15%	3%	3%	10 minutes	40 0
8	15%	3%	3%	15 minutes	40 0
9	15%	1%	1%	10 minutes	20 00
10	15%	1%	1%	15 minutes	20 00
11	15%	1%	3%	10 minutes	20 00
12	15%	1%	3%	15 minutes	20 00
13	15%	3%	1%	10 minutes	20 00
14	15%	3%	1%	15 minutes	20 00
15	15%	3%	3%	10 minutes	20 00
16	15%	3%	3%	15 minutes	20 00
17	35%	1%	1%	10 minutes	40 0
18	35%	1%	1%	15 minutes	40 0
19	35%	1%	3%	10 minutes	40 0
20	35%	1%	3%	15 minutes	40 0
21	35%	3%	1%	10 minutes	40 0
22	35%	3%	1%	15 minutes	40 0
23	35%	3%	3%	10 minutes	40 0
24	35%	3%	3%	15 minutes	40 0
25	35%	1%	1%	10 minutes	20 00
26	35%	1%	1%	15 minutes	20 00
27	35%	1%	3%	10 minutes	20 00
28	35%	1%	3%	15 minutes	20 00
29	35%	3%	1%	10 minutes	20 00
30	35%	3%	1%	15 minutes	20 00
31	35%	3%	3%	10 minutes	20 00
32	35%	3%	3%	15 minutes	20 00

Preparation of Nanoemulsion Base Formulation

The working procedure begins by mixing tween 80 with lecithin then stirred with a *magnetic stirrer* for 10-15 minutes at 750-2000 rpm with a temperature of 75 C then added the oil phase (*Virgin Coconut Oil*) stirred again with a *magnetic stirrer* for 10-15 minutes at 750-2000 rpm with temperature of 75 C so that a mixture of surfactant and oil is formed after this added aquadest a little for the sake of a little on *magnetic stirrer* until aquadest exhausted (Damayanti *et al* ., 2019).

Evaluation of Nanoemulsion Preparation Base Formula

1. Organoleptic Examination

This examination was carried out to see the physical appearance of the nanodispersion formula includes observations of color, odor, shape and homogeneity of the preparation (Damayanti *et al.*, 2019).

2. pH check

The pH measurement was carried out using a calibrated pH-meter. Measurements were carried out at room temperature. The pH of the preparation must be at pH 5-7 which is the pH of the skin (Damayanti *et al.*, 2019).

3. **Globule Size Examination (Microscope And Optilab)**

Base globule size was measured using an optilab viewer microscope that had been calibrated beforehand. If the results seen in the optilab look blurry or blurry, it means that the size can be categorized as nano, it can be continued on examining the particle size with PSA *(Siza Particle Analyzer).*

4. Design expert

Analysis of the 32 basis formula was used to obtain the optimal base formula using design expert 13. The value of each response was from the data from the particle size test of nanoemulsion base using design expert 13.

Evaluation of Optimal Formulations and 6 Selected Formulas on the Base of Nanoemulsion Preparations

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1. pH check

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2. Turbidity Calculation

Turbidity was determined by measuring the turbidity of the base using UV-Vis spectrophotometry at 502 nm. The results obtained are then calculated using the equation to determine the percent turbidity.

3. Freeze *and thaw*

Bahan sediaan USUC quercetin (quercetin, tween 80, lesitin, almond oil dan aquadest)

To see changes in the stability of the nanodispersion preparation base. The preparations were stored at 25ºC for 24 hours and -5ºC for 24 hours. This cycle was repeated three times and the changes were recorded (Kale & Deore, 2016) .

4. Viscosity testing

Viscosity measurements were carried out with a *Brookfield Viscometer* , using spindle number 3 with a speed of 100 rpm. The results obtained are then recorded and repeated three times (Damayanti *et al.*, 2019).

5. Specific Gravity

Specific gravity was determined using a 10 ml pycnometer by cleaning the pycnometer by rinsing with distilled water, then dried. Then the empty pycnometer was weighed (w_0). Then the pycnometer was filled with aquadest, weighed and recorded the result (w_1). Dry again and then the pycnometer filled with the nanodispersion preparation was weighed and recorded the results (w2). Do three repetitions (Martin & Camarata, 2008).

Preparation of Nanoemulsion Serum Preparation

Based on the optimization of the base that has been done, one optimal base formula and six formulas were selected as the base formula for the nanoemulsion preparation. The seven nanoemulsion base formulas were added with an active substance with a concentration of 3% w/v (Damayanti, 2019).

procedure :

Madekoside is dissolved first in the oil phase (*Virgin Coconut Oil*).

Lecithin and tween 80 were placed on a *magnetic stirrer* at 75ºC at a speed of 750-1500 rpm for 10-15 minutes (according to the basic formula).

Then the oil phase which already contains madecoside is mixed in *magnetic stirrer* again then aquades are added little by little to the limit (Damayanti, 2019).

Characterization and evaluation of optimal nanoemulsion preparations

1. Evaluation of Nanoemulsion

a. Organoleptic examination

Organoleptic was carried out to see the physical appearance of an optimal nanoemulsion serum formula by observing the color, odor, shape and homogeneity of the serum made.

b. Morphological examination of vesicle shape

1) Franz . Diffusion Cell

Franz diffusion cell device is a tool used to determine or see the penetration of the active substance. The working principle of the Franz diffusion cell device is to place a semi-permeable membrane between the donor and receptor compartments, then the levels of compounds that enter the receptor fluid are measured using HPLC *(high performance liquid chromatography).*

2) Examination of globule size , zeta potential and polydispersion index with a *particle size analyzer* (PSA)

The examination of globule size with PSA , zeta potential and polydispersion index was carried out in the drug, food and cosmetic testing laboratory of the Islamic University of Indonesia (UII).

RESULTS AND DISCUSSION

This study uses a pure experimental design with a *factorial design method* five factors and two levels, namely high and low so that 32 basic formulas are obtained to be optimized. The factors that will be optimized in this study are the parameters that determine the formation of the nanoemulsion globule size, namely lecithin content, tween 80 content, VCO content, mixing speed and stirring time. The selection of stirring speed and stirring time is adjusted to the tool used, namely the *planetary centrifugal mixer* , stirring speed and stirring time can determine whether or not a homogeneous base can be formed and has the desired physical stability.

After making 32 nanoemulsion bases, an evaluation was carried out including organoleptic examination where this evaluation was to see different characteristics, namely shape, color, odor and homogeneity which had been observed for 4 weeks. Measurement of the average diameter of the base globules using a microscope and optilab with a magnification of 40x . The results of measuring the diameter of the globules in formulas (1-26) and (29-32) show the desired size results, but in formula 27-28 it is not visible or blurry, so the results of the optilab microscope measurements are not legible. This is because formulas 27 and 28 are probably already nanometer in size, so these formulas must be checked using a particle size analyzer (PSA). so that the desired particle size is obtained.

Table III. Result of examination of globule size 32 nanoemulsion base formula

No	Formula	Globule Size (nm)	No	Formula	Globule size (nm)
1	1	18.590	17	17	22.060
2	2	18.460	18	18	17.320
3	3	26.840	19	19	6.530
4	4	29.660	20	20	23.340
5	5	26.470	21	21	20.520
6	6	23.070	22	22	29.200
7	7	13.560	23	23	17.540
8	8	19.560	24	24	15.380
9	9	24.090	25	25	15.840
10	10	24.030	26	26	24.170
11	11	7.410	27	27	11.7
12	12	10.480	28	28	11.6
13	13	17.540	29	29	30.550
14	14	23.560	30	30	38.340
15	15	23.750	31	31	23.530
16	16	13.930	32	32	12.980

From the results of the examination the size of the globules that affect the levels of lecithin (phospholipids), levels of tween 80 (co-surfactant) and the stirring speed (rpm) used. There are various conditions where each formula has different results, from different levels of concentration and also the speed that affects each formula.

Design expert is one of the methods used in experiments to determine by simulation the effects of several factors and their significant interaction responses (Bolton & Bon, 2003). The difference in levels of each formula causes differences in the physical response produced. The size of the globules in the nanoemulsion preparations is 0-40 nm. The globule size in the nanoemulsion preparation is 0-40 nm. The equation of design expert 13 on the particle size response is as follows:

Y=19322.56 – 739.94 X_1 +2519.94 X2 – 4040.56 X3 + 895.62 X4 – 1183.69 X5 + 2402.44 X1X2 – 2126.81 X1X3 + 614.38 X1X4 + 780.06 X1X5 – 273.19 X2X3 – 735.63 X2X4 + 2363.69 X2X5 – 510.00 X3X4 – 2585.56 X3X4 – 596.87 X4X5 + 293.06X1X2X3 – 304.38 X1X2X4 + 884.94 X1X2X5 – 487.50 X1X3X4 – 293 ,06 X1X3X5 – 216.87 X1X4X5 – 1716.25 X2X3X4 + 2424.31 X2X3X5 – 383.13 X2X4X5 – 1951.25 X3X4X5 – 933.75 X1X2X3X4 – 1493.19 X1X2X3X5 – 385 36.004 X1X3X4 – 1493.19 X1X2X5 – 385 + 36.004 X1X ,00 X2X3X4X5 + 1493.75 X1X2X3X4X5

Information :

Y = particle size response (anova) intercept

X_1 = tween 80 X_2 = VCO X_3 = lecithin

X_4 = stirring time X_5 = stirring speed

X_1X_2 = the interaction of the two factors $X_1X_2X_3$ = the interaction of the three factors

$X_1X_2X_3X_4$ = interaction of the four factors $X_1X_2X_3X_4X_5$ = interaction of the 5 factors

Based on the results of the equation, the coefficient values from the highest response to the lowest response are +2519.94 (VCO), +895.62 (stirring

time), – 4040.56 (lecithin), – 1183.69 (stirring speed), – 739.94 (tween 80).

The meaning of the positive response is the critical factors that affect the formation of globule size so that the nanoemulsion is formed. It can be seen that the positive response value of lecithin affects the formation of the particle size of the nanoemulsion preparation. While the stirring time gave a negative response to the formation of the globule size of the nanoemulsion base, so that the stirring time, both at low and high levels, did not have a significant effect on the formation of globule size in nanoemulsion preparations.

CONCLUSIONS

Based on the research that has been done, it can be concluded that the optimal nanoemulsion formula of the active compound madecoside from the composition of the formula tween 80, lecithin, VCO with a stirring speed of 1500 rpm and a stirring time of 15 minutes.

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