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Optimization of Nanoemulsion Formula from Asiaticoside Active Compounds Using Design Expert ® *13*

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INTRODUCTION

Asiaticoside is a triterpenoid glycoside compound obtained from gotu kola *(Centella asiatica* L. Urban) which can be used forproduce collagen in the skin so that the skin will feel more supple, elastic, tighten the skin, make the skin texture smoother and scavenge free radicals. This asiatica can be used as an ingredient for wound healing and reducing redness of acne scars (Nagai *et al.*, 2018). However, asiaticoside has poor absorption and excellent solubility (Ozdemir *et al.*, 2016). Thus, nanoemulsion preparations will improve the absorption of asiaticoside whichbadbecauseNanoemulsions can improve the properties of active compounds that have poor absorption with very small particle sizes ranging from 1-100 nm , aiming to increase the permeability of active substances and improve absorption (Zhang *et al* .,2016).

Nanoemulsion preparations are very attractive when applied in cosmetic products. In addition to its good effectiveness, side effects such as skin irritation and low toxicity, nanoemulsions can also be applied easily through the skin and mucous membranes (Yang *et al.*, 2019).Optimization can be interpreted as an approach to get the best combination of a product or process characteristics under certain conditions, it can also be interpreted as choosing the best elements or materials from several available options (Amalia *et al.*, 2017). In determining the optimal formula using a *design expert* 13 who can help design variations of the formula in the preformulation and analysis of experimental results (Singh *et al.*, 2007).

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,MicroscopeOptilab (Thinky), *planetary centrifugal mixer* (Thinky) andPSA (*particle size analyzer*), *zeta potentialAnalyzers* “SHIMADZU” and “HORIBA” .

The materials used in this study include: asiatocoside compounds (Markherb) , aquadest (Novalindo), olive oil (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, olive oil 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	Olive oil 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	

TableII . Optimized Amount of Nanoemulsion Base

No	Tween 80	Lecithin	Olive Oil	Stirring Time	Stirring Speed	Particle Size (nm)
1	15%	1%	1%	10 minutes	40 0	14,850
2	15%	1%	1%	15 minutes	40 0	14,030
3	15%	1%	3%	10 minutes	40 0	24,810
4	15%	1%	3%	15 minutes	40 0	16,570
5	15%	3%	1%	10 minutes	40 0	26,470
6	15%	3%	1%	15 minutes	40 0	10.070
7	15%	3%	3%	10 minutes	40 0	19,390
8	15%	3%	3%	15 minutes	40 0	12,460
9	15%	1%	1%	10 minutes	20 00	18,100
10	15%	1%	1%	15 minutes	20 00	58,970
11	15%	1%	3%	10 minutes	20 00	75,520
12	15%	1%	3%	15 minutes	20 00	42,740
13	15%	3%	1%	10 minutes	20 00	29,590
14	15%	3%	1%	15 minutes	20 00	25,960
15	15%	3%	3%	10 minutes	20 00	54,460
16	15%	3%	3%	15 minutes	20 00	49,920
17	35%	1%	1%	10 minutes	40 0	56,440
18	35%	1%	1%	15 minutes	40 0	47,910
19	35%	1%	3%	10 minutes	40 0	17,940
20	35%	1%	3%	15 minutes	40 0	19,460
21	35%	3%	1%	10 minutes	40 0	15,960
22	35%	3%	1%	15 minutes	40 0	24,920
23	35%	3%	3%	10 minutes	40 0	37,780
24	35%	3%	3%	15 minutes	40 0	27,830
25	35%	1%	1%	10 minutes	20 00	18.1
26	35%	1%	1%	15 minutes	20 00	11.5
27	35%	1%	3%	10 minutes	20 00	11,720
28	35%	1%	3%	15 minutes	20 00	22,630
29	35%	3%	1%	10 minutes	20 00	38,620
30	35%	3%	1%	15 minutes	20 00	17,860
31	35%	3%	3%	10 minutes	20 00	37,430
32	35%	3%	3%	15 minutes	20 00	12,460

Preparation of Nanoemulsion Base Formulation

The working procedure begins by mixing tween 80 with lecithin then stirred with *a planetary centrifugal mixer* for 10-15 minutes at a speed of 400-2000 rpm , then the oil phase is added (olive oil) and stirred again for 10-15 minutes at 400-2000 rpm to form a mixture . surfactants and oils. After that, add distilled water little by little over the *planetary centrifugal mixer* until the volume of the preparation is 100 ml then stirred again for 10-15 minutes at 400-2000 rpm (Damayanti*et al.,* 2019).

Evaluation of the Nanodispersion Preparation Base

1. **Organoleptic Examination**

This examination was carried out to see the physical appearance of the nanodispersion formulaincludes 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 AndOptilab)**

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.

Making the optimal nanoemulsion formula with active ingredients

Based on the base optimization that has been done, one optimal base formula is selectedas base formulananoemulsion. The nanoemulsionbase formula was added with an active substance with a concentration of 3% w/v (Damayanti *et al* ., 2019).

First, lecithin and tween 80 were put in *a magnetic centrifugal mixer* at a temperature of 75ºC at a speed of 400 - 20 00 rpm for 10-15 minutes (according to the basic formula).Asiaticoside is dissolved first in the oil phase *(olive oil).*Then the oil phase which already contains asiaticoside is mixed in a *planetary centrifugal mixer* . Then aquadesare added little by little to the limit (Damayanti *et al.,* 2019).

Evaluation of nanoemulsion base formula with active substance.

1. Organoleptic Examination

This examination was carried out to see the physical appearance of the nanodispersion formulaincludes 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. Calculation of % Transmittan

The transmittance calculation was carried out using UV-Vis Spectrophotometry at a wavelength of 650 nm. The transmittance value close to 100% indicates a nanodispersion-sized mixture (Makula*et al.,* 2017) .

4. Freeze *and thaw*

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) .

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 polydispersity 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, olive oil 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 (1-24) and (27-32) showed the desired size results, but in formulas 25-26 it was not visible or blurry, so it could not be measured using an optilab microscope. This happens because the possible particle size is already nanometers, so the formula must be checked using *a particle size analyzer* (PSA). This results in the desired particle size.

Table III. The results of the examination of the globule size of 32 nanoemulsion base formulas.

No	Formula	Globule Size (nm)	No	Formula	Globule size (nm)
1	1	14,850	17	17	56,440
2	2	14,030	18	18	47,910
3	3	24,810	19	19	17,940
4	4	16,570	20	20	19,460
5	5	26,470	21	21	15,960
6	6	10.070	22	22	24,920
7	7	19,390	23	23	37,780
8	8	12,460	24	24	27,830
9	9	18,100	25	25	18.1
10	10	58,970	26	26	11.5
11	11	75,520	27	27	11,720
12	12	42,740	28	28	22,630
13	13	29,590	29	29	38,620
14	14	25,960	30	30	17,860
15	15	54,460	31	31	37,430
16	16	49,920	32	32	12,460

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 equation of design expert 13 on the particle size response is as follows:

$$Y= 27224.36 – 2823.14 X_1 + 413.76 X_2 + 2175.64 X_3 – 2719.27 X_4 + 3066.24 X_5 + 1921.26 X_1 X_2 – 3116.86 X_1 X_3 + 131.98 X_1 X_4 – 9650.01 X_1 X_5 -2105.11 X_2 X_3 – 2105.11 X_2 X_4 + 2881.89 X_2 X_5 – 2761.98 X_3 X_4 + 4473.76 X_3 X_5 – 172.39 X_4 X_5 + 1446.24 X_1 X_2 X_3 – 1018.86 X_1 X_2 X_4 + 4155.64 X_1 X_2 X_5 + 2591.77 X_1 X_3 X_4 + 157.51 X_1 X_3 X_5 – 1369.89 X_1 X_4 X_5 + 1916.36 X_2 X_3 X_4 – 2851.89 X_2 X_3 X_5 – 1441.98 X_2 X_4 X_5 - 2188.86 X_3 X_4 X_5 - 4507.39 X_1 X_2 X_3 X_4 – 5225.64 X_1 X_2 X_3 X_5 – 2139.48 X_1 X_2 X_4 X_5 + 3421.14 X_1 X_3 X_4 X_5 + 2693.23 X_2 X_3 X_4 X_5 – 1619.27 X_1 X_2 X_3 X_4 X_5$$

Information :

Y = particle size response (anova) intercept

X_1 = tween 80 X_2 = olive oil X_3 = lecithin

X_4 = stirring time X_5 = stirring speed

$X_1 X_2$ = the interaction of the two factors $X_1 X_2 X_3$ = the interaction of the three factors

$X_1 X_2 X_3 X_4$ = interaction of the four factors $X_1 X_2 X_3 X_4 X_5$ = interaction of the 5 factors

Based on the results of the equation, the coefficient values from the highest response to the lowest response are +3066.24 (stirring speed), +2175.64 (lecithin), +413.76 (olive oil), -2719.27 (stirring time), and - 2823.14 (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.

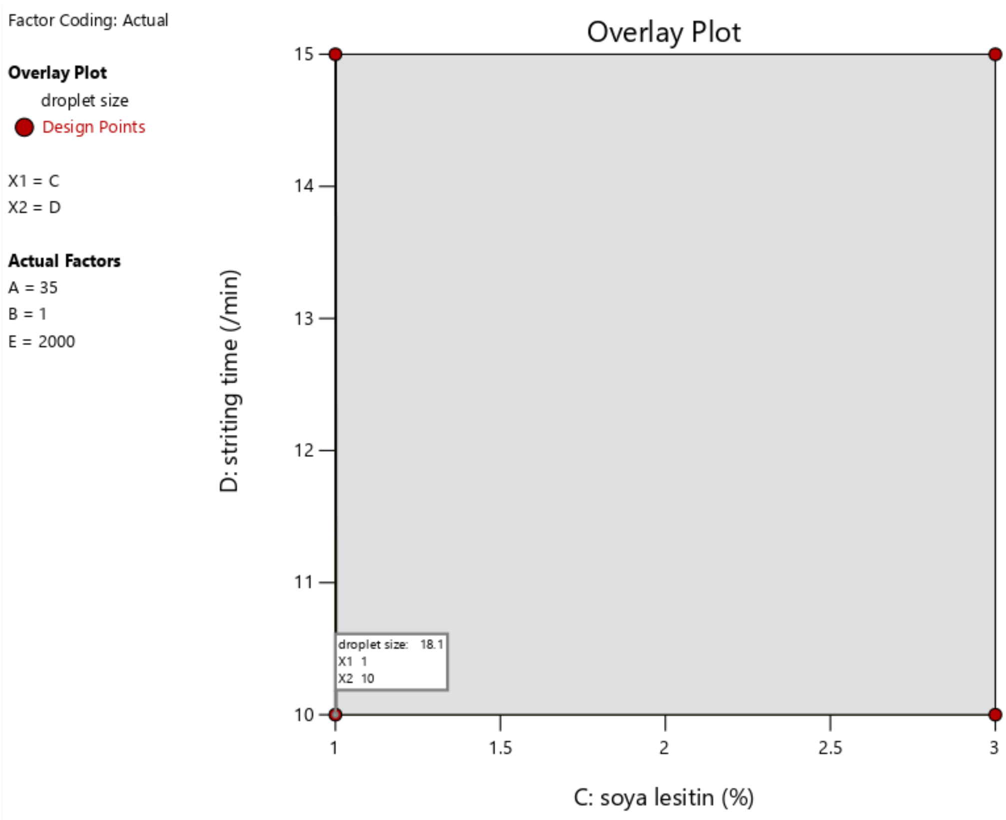
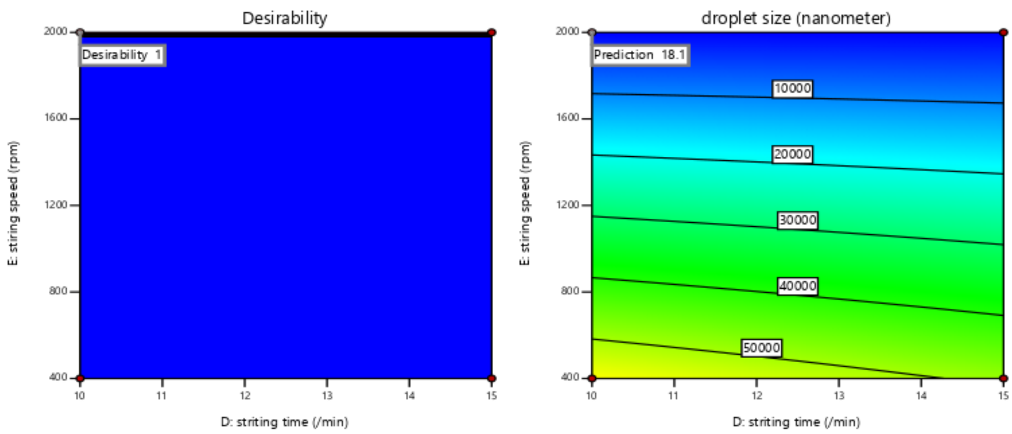


Image 1. Overlay plot of the optimal formula for the nanoemulsion base.

The optimal formula is obtained from the analysis of the design expert 13 program by making an overlay plot. In Figure 1 it can be seen that the area that has its own box in the picture is the optimal area or formula that has been predicted by the design expert 13.



(a) **(b)**

Figure 2. (a) results of desirability between concentrations of tween 80 and lecithin, figure (b) cotour plot of tween 80 and lecithin levels on particle size response and prediction of optimal formula size.

In Figure 2 (a) it can be seen that the estimated optimal base formula is at tween 80 with a concentration of (30-35)% and 1% lecithin will make desirability close to 1000. The desirabilitu value closer to 1 will indicate the program's ability to produce the desired product. more perfect. The purpose of optimization is not to obtain a desirability value of 1, but to find the best conditions that meet all objective functions. Figure (b) is the determination of the optimal formula where the estimated concentration of tween 80 is between (30-35)% . While the concentration of lecithin at a low level is 1%. It gives a particle size response relationship between tween 80 and lecithin which predicted a particle size of about 18.1 nm.

CONCLUSIONS AND RECOMMENDATIONS

Conclusion

Based on the research that has been done, the following conclusions can be drawn:

1. The results of the optimal nanoemulsion formula from the active compound asiati k oside were formed from the composition of the tween 80 formula between (30-35%), 1% lecithin, agitating speed of 1500 rpm and a stirring time of 15 minutes.
2. Characterization and stability of nanoemulsion of the active compound asiati k oside according to the requirements of nanoemulsion technology.

Suggestion

It is recommended in further research to be able to conduct testing using TEM tools.

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