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Optimization of Gold Particle-Vitamin E Nanoemulgel and in Vivo Test

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Abstract*—Gold is a substance that can be used as a collagen forming catalyst, it can help regenerate the skin. Vit. E is a natural antioxidant compound that can be used to help regenerate and brighten the skin. Gold is a metal so it is difficult to apply into the skin. Gold and vit. E are formulated with SNEDDS (Self Nano Emulsifying Drugs Delivery System) technique, in order to reduce the size and dispersing of gold particles homogeneously. SNEDDS formula consists of olive oil, tween 80 (surfactant), propylene glycol (co-surfactant), vit. E and gold particles, which are optimized to obtain the optimum component using the D-Optimal method. Software will obtain 16 run formulas with difference composition of olive oil, tween 80, and propylene glycol. Transmittance value and pH as response. Loading dose capability was also carried out. Verification with statistical analysis using one sample t-test and then evaluated particle size, polydispersity index, and zeta potential. SNEDDS gold-Vit E optimum formula then dispersed into matrix hydrogel (Carbopol). The formula of Nanoemulgel gold was evaluated for physical properties and in vivo tests compared with brand gold cosmetic. The optimum composition of olive oil (9,09%), tween 80 (80,62%), and propylene glycol (10,29%) with loading dose of 2,6 mg gold and 250 µL of vitamin E. Characterization results showed the transmittance value is 97,22±0,06% and pH is 6,72±0,04 which shows no significant difference (p> 0,05) between the observation and software prediction. The results of SNEDDS evaluation showed a particle size of 150,2 nm, PI of -0,52 and potential zeta -24,9 mV. The results of stability test of nanoemulgel showed a semisolid, broken white colour, transparent, odorless, homogeneous. pH value of 6,78±0,06; viscosity of 346,67±15,27 dPas; and spread test of 5,48±0,035 cm. The results of the in vivo test showed that on the 6th day there was a significant skin regeneration when compared to brand product.

Keywords—SNEDDS, gold, vitamin e, skin regenerate, design optimum introduction

I. INTRODUCTION

Indonesia is a country crossed by the equator line, so the intensity of UV exposure becomes greater, causing so many skin health problems, and also cause the formation of free radical compounds. Dark-colored skin is a major problem especially for women. Free radicals can cause cell damage if left too long it will cause tissue damage such as loss of elasticity of collagen and elastin so skin becomes wrinkled, dark spots and can even cause skin cancer.

Gold (Au) is an inert metal that is not easily subjected to oxidation, gold can be used as one of the materials for cosmetics because it tends to be safe if it is in the body (Fatimah, 2012). Gold has a function as an agent to accelerate skin regeneration and skin rejuvenation because it is a catalyst for the formation of collagen which is the main protein in skin tissue. Gold is a metal compound that has limitations when it is used as a cosmetic preparation, which is difficult to apply to skin. To overcome this problem, gold is formulated into a system that can reduce the size and dispersing of gold particles homogeneously. Gold in the size of 1-100 nm has antioxidant properties more effective than natural antioxidants such as vitamin E, vitamin C, flavonoids, and tannin. Nano-sized gold also has the ability to repair damaged skin tissue. According to research conducted by Cao et al., (2016) the levels of gold used in cosmetic preparations that have been circulating in the market are 0,000051-0,082 mg/gram matrix system.

Vitamin E is a one of natural antioxidant compound that can reduce skin damage due to UV light and inhibit carcinogenic photos. In this study, gold particles and vitamin E will be combined in a formula with Self-Nanoemulsifying Drug Delivery System (SNEDDS) technique. SNEDDS is a method of drugs delivering that consist of three component isotropic mixtures, there are: oil phase, surfactant, and co-surfactant. If the mixture is dispersed into a water medium, it will form nanoemulsion oil in water spontaneously and produce nanometer-sized droplets (Makadia et al., 2013).

SNEDDS consists of olive oil because it contains oleic acid (80%) so it has high self-emulsifying ability and has a large drug loading capacity (Nugraheni, 2012). Tween 80 was chosen as surfactant because it has HLB value of 15 and is hydrophilic, besides that the use of tween 80 also produces SNEDDS preparations which have transmittance values above 80% (Diba et al., 2014). The co-surfactant used is propylene glycol because it has the ability to help surfactants in reducing interface tension. The optimum isotropic mixture of oil, surfactant and co-surfactant are unknown, so it is necessary to optimize with D-Optimum method using Design Expert Software. SNEDDS gold-vit e then dispersed into the hydrogel matrix. Hydrogels are selected because they are easier to apply to the skin, spread well on the skin at a concentration of gelling agent (carbopol) 0,5-2% (Hidayanti et al., 2015), and tested for effectiveness when compared to brand cosmetics containing gold.

II. Material and Method

A. Materials

Gold particles (Gold leaf kimpo 24 rust) as active substances, vitamin E (Ever E), olive oil (Bratachem), Tween 80 (Bratachem), Propylene glycol (Bratachem), Carbopol 940 (Bratachem), Glycerin (Bratachem), Triethanolamine (Bratachem), Nipagin (Bratachem), Aquadest, ethanol 70% (Bratachem), and bioaqua 24K essence gold face serum as a comparison product.

B. Marix Hydrogel orientation

Carbopol was dissolved in warm aquadest, stirring in a mortar, while ad some of triethanolamine until a transparent gel formed. Nipagin which has been dissolved in propylene glycol is put into the gel together with glycerin, then added the remaining triethanolamine and stirring until homogeneous. After that, add the remaining aquadest and stir until a homogeneous gel formed.

C. Optimization of SNEDDS Formula

Composition of SNEDDS was done by making 9 SNEDDS formulas with mixing the oil phase: surfactant: co-surfactant ratio of 1: 1: 1 to 1: 9: 1, then the transmittance value was seen by spectrophotometer UV-VIS 650 nm, aquadest as a blanko. High percent transmittance value indicating nanoemultion has been succeeded. The ratio value submited as an upper and lower limit in Design Expert Version 9 Trial Stat Ease software with D-Optimum method, 16 run formulas will appear with variable responses entered into the software there are : percent transmittance and pH value of SNEDDS. Loading dose of gold and vitamin E were done by dissolving amount of gold and vitamin E into SNEDDS. The gold dose used is 0,026 mg/gram, this dose refers to the research conducted by Cao et al. (2016), while the dose of vitamin E used are 250 µL, 500 µL and 1000 µL. The two substances will be homogenized, then will be assessed visually and by looking at the transmittance value. Maximum Gold and vitamin E which can dissolve in SNEDDS and have optimum transmittance are expressed as loading doses (Aprian, 2015).

D. Characterization of Physical Responses of SNEDDS Formula with D-optimum Methods

Formulation of SNEDDS is done by weighing and mixing the three components (Table-2), then gold and vitamin E are added to the flacone disk. The mixture then vortexed for 5 minutes and sonicated for 15 minutes. SNEDDS then incubated at 45ºC on waterbath for 15 minutes. Sixteen of SNEDDS formula was stored at room temperature, then characterized test (Wahyuningsih and Putranti, 2015). Some of 100 µL SNEDDS add with 5,0 mL aquadest into flacon disk, then the solution is vortexed for 30 seconds until homogeneous. The solution was checked for percent transmittance value using UV-Vis spectrophotometer at wavelength (λ = 650 nm) with an aquadest as blanko (Anindhita and Oktaviani, 2016). Gold-Vit E SNEDDS then checking the pH value with weighing of 1,0 gram of Gold-Vit E SNEDDS and dissolved into 9,0 mL aquadest. The solution then checked for pH values using a pH meter (Naibaho et al., 2013). The Optimum SNEDDS formula is done using the Design Expert with D-Optimum method. The concentration of the three components of SNEDDS there are olive oil, tween 80 and propylene glycle from 16 formula will be characterized based on the results of the transmittance value and pH value (Table-2). Statistical results not significantly different will be indicated by p value> 0,05 (Winarti et al., 2016).

E. Particle Size and Potential Zeta Analyzer

The optimum of SNEDDS formula of 100 µL SNEDDS were dissolved with aquadest add 5,0 mL into flacon disk. Then the solution was analyzed using PSA. For potential zeta measurements, it is carried out using the same tool, but the menu analysis settings (Qureshi et al., 2015).

F. Physical Characteristic of Nanoemulgel gold-vit E

Optimum formula of Nanoemulgel Gold-Vit E is stored at room temperature 25±2ºC RH 60% for 30 days then tested for stability at day-0 and day-30, were follows : organoleptics, homogenity, pH, Viscosity, and spread test.

G. In Vivo Test

Wistar male rats aged 2-3 months were divided into 3 groups : positive, negative and test group, each containing 3 rats each group. The hair on the back of the mouse is shaved 2x2 cm and wiped with 70% alcohol. The three groups of rats were irradiated by UV light for 24 hours. The next day, the positive control group was wiped with brand cosmetics product consist of gold (Bioaqua), the test group was wiped with nanoemulgel gold-vit E, and negative control group was no given. The erythema score used was 0-4 which showed no redness (score = 0) until the red light with area expansion (score = 4) (Dervish, 2013). Erythema in rat skin was observed and noted how long it took for each group until the erythema disappeared.

H. Data Analyzer

IBM SPSS Statistic 21 software with one sample t-test method with a confidence level of 95%, where the results can be said not to differ significantly if the prediction results by D-Optimum with the test results have p-value >0,05. Physical stability and effectiveness evaluation in vivo are analyzed using the One Way ANOVA method where the results can be said to be significantly different if it has a p-value <0,05.

III. Results and Discussion

A. Optimum Formula of Matrix Hydrogel

Four formulas have organoleptic properties that are almost the same which have a consistency gel mass, clear and odorless. Spread test is carried out to find out the optimum spread area of hydrogel when applied on to the skin. The hydrogel base is a determinant of the speed of active ingredients released so that it will affect the effectiveness of active efficacy. According to Basha et al. (2011), dispersion is part of psychoreology which can be used as a acceptability parameter. F1 has spread area of 4,2 cm, F2 has spread area of 5,8 cm, F3 has spread area of 7,6 cm and F4 has spread area of 8,8 cm. According to Kaur et al., (2010), optimum spread area for hydrogels is 5-7 cm. F3 with a carbopol concentration of 0,4% w/w is chosen and will be used as matrix hydrogel for SNEDDS gold-Vit E.

B. Optimum Formula of SNEDDS gold-vit E Based on D-optimum methods

The results showed that gold with a dose of 0,26 mg and vitamin E of 250 µL into 10 gram system had the highest transmittance value (94,43%), so it was chosen as the maximum dose that can be loaded into system. Oil contained in SNEDDS will affect the clarity of SNEDDS because the value will decrease. The mathematic equation in the transmittance response (Table-2) shows the single effect or interaction of the three variables (A, B, C) on the transmittance response (Y). This can be caused because the interaction of these two variables has an important role in increasing the clarity of nanoemulsion. Research by Priani et al., (2017) shows that oil phases containing oleic acid will have better clarity when formulated with surfactants. The interaction of surfactant and co-surfactant can reduce surface tension, so nanemulsion formed has nanosize particle (Ana et al., 2016). Mathematics equation of pH response shows a single influence or interaction of the three variables (A, B, C) on the response (Y). The positive effect of increasing pH values is indicated by the components of olive oil, tween 80, and propylene glycol, while the negative effect of decreasing pH is indicated by the interaction between olive oil and tween 80, olive oil and propylene glycol and tween 80 and propylene glycol. Based on the results obtained, it is known that the components of olive oil have the greatest value so that olive oil has a dominant influence on the response to an increase in pH value.

TABLE I. RESULTS OF MATHEMATICS EQUATIONS AND MODEL OF MIXTURE DESIGN FROM THE PHYSICAL RESPONSES THAT DESCRIBES THE INTERACTION BETWEEN OLIVE OIL-TWEEN80-PROPYLENE GLYCOL IN THE MIXTURE

Response of Physical characteristic of *SNEDDS*	**Mathematics Equations of Mixture Design**	**Mathematics Model**	***p-value* [ANOVA] p>0,005**
Transmittance (%)	-43,01684A-12,27590B-492,56706C+1,07852AB +1,80956AC+6,73498BC	*Quadratic*	0,040
pH	26,87093A+0,83482B+22,67544C–0,34288AB–0,51751AC–0,28741BC	*Quadratic*	0,039

C. Optimum composition of oil-surfactant-co surfactant

The optimum formula suggested by Design Expert software has a desirability value of 0,949. Proportion composition of olive oil, tween 80, and propylene glycol are 9,09% : 80,62% : 10,29%. This shows that the formula will produce a formula that has the characteristics closest to the predetermined target limit of 94,9%. After the optimum formula prediction is obtained, then the optimum formula is verified, to compare the observations results with the prediction of Design Expert software. The optimum formula of SNEDDS gold-vit E has met the criteria as a good nanoemulsi dosage form. The transmittance value of the optimum formula of SNEDDS gold-vit E meets the requirements of transmittance value >90% (Winarti et al., 2018). The pH value of the optimum formula of SNEDDS gold-vit E also meets the requirements, which are in the range of 4,5-7 (Tirmiara et al., 2018).

Particle size is one of the important things to consider in SNEDDS formulation. The test results show that the optimum formula of SNEDDS gold-vit E has a particle size of 150,2 nm, this result expected which is in the range of 10-200 nm (Mahmoud et al., 2013). The Polydispersity Index (PI) is a value for particle size measurements used to determine the homogeneity of nanoemulsion particles. According to Pratiwi et al. (2018), the range optimum of PI values is 0,0-1,0. Based on the test results, it is known that the optimum formula of SNEDDS gold-vit E has a PI value of 0,52 which indicates that particle size is uniformly distributed. Potential Zeta is a parameter of electrical charge between particle and regulates the force between charged particles scattered in the solution. The stable value of the potential zeta is more than +30 mV or less than -30 mV because at that range it can provide a significant particle charge to avoid a repulsive force. The higher of potential zeta value, it will prevent the occurrence of flocculation or colloid incorporation from small to large. The results of the potential zeta value in the optimum formula of SNEDDS gold-vit E is -24.9 mV.

D. Physical Caracteristics of Gold-vit e nanoemulgel formula

Nanoemulgel gold-vit E was tested including organoleptic, viscosity, pH, and spread area. The test is carried out before and after storage for 30 days. The requirements for nanoemulgel dosage form are semi-solid, clear, give a cold effect when applied to the skin, and contain active substances dispersed in a colloidal system (Edi et al., 2016), so that gold nanoemulgel meets the organoleptic test requirements.

E. In Vivo Efficacy of nanoemulgel gold-vit e

Observations for 7 days were expressed in the form of scores = 0 which showed no erythema to score = 4 which showed erythema with crust and bright red color with area expansion (Table-3). On the first day, all test groups had erythema 4 values because they had erythema with a diameter> 35,10 mm. After being observed for 7 days, it was found that the three groups experienced a decrease in erythema values, but the difference between the three groups was the speed of the rat skin in regenerating the damaged skin cells (erythema). The nanoemulgel gold group has a better speed in reducing the diameter size of erythema in rat skin. On the 6th day, the nanoemulgel gold group had erythema 1 values while the positive control group had erythema 2 values, besides that on the 7th day the positive control group had an erythema diameter of 21,88 ± 0,56 mm (erythema value = 1), while the test group had erythema diameter of 19,54 ± 0,54 mm (erythema value = 1). Rats given gold nanoemulgel have faster skin cell regeneration rates when compared to brand gold cosmetics. The results of the normality test show that are normally distributed with p-value > 0,05 and the homogeneity test shows homogeneous data with p-value > 0,05. Based on the two results, it shows that the One Way ANOVA test results show data with p-value <0.05, which means there are differences in erythema values from the three test groups.

TABLE II. FORMULAS THAT SUGGESTED BY DESIGN EXPERT SOFTWARE OF SNEDDS AND THE RESULTS OF RESPONSES TO GET AN OPTIMUM COMPOTITION OF SNEDDS

Run	Olive Oil (%)	Tween 80 (%)	Propylene glycol (%)	Respons	
				Transmittance (%)	pH
1.	11,11	79,80	9,09	80,27	6,35
2.	9,50	81,01	9,50	85,51	7,02
3.	9,90	80,20	9,90	87,20	6,21
4.	9,10	81,81	9,09	91,67	6,98
5.	11,11	77,78	11,11	66,07	6,79
6.	10,00	79,50	10,51	92,03	6,32
7.	9,09	79,80	11,11	92,04	6,99
8.	11,11	77,78	11,11	65,12	7,04
9.	9,09	79,80	11,11	90,28	6,88
10.	10,11	80,81	9,09	89,61	6,24
11.	11,11	79,80	9,09	72,91	6,96
12.	9,10	81,81	9,09	90,74	7,12
13.	11,11	78,79	10,10	90,78	6,43
14.	9,09	80,81	10,11	99,36	6,89
15.	11,11	78,79	10,10	91,83	6,09
16.	10,10	78,79	11,11	93,95	6,65

TABLE III. RESULT OF ERYTHEMA SCORE OF WISTAR MALE RAT SKINS AFTER TREATED WITH UV-LIGHT AND PRODUCT

Treatment Group	Erythema Score (Day)						
	1	2	3	4	5	6	7
Bioaqua (+)	4	4	3	3	2	2	1
Without treatment	4	4	4	4	4	3	3
Nanoemulgel gold-vit.E	4	3	3	3	2	1	1

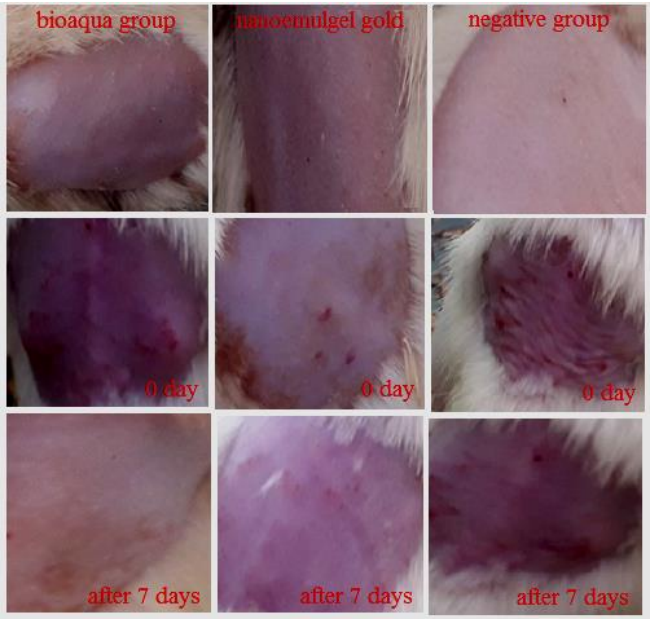

Fig. 1. The image of skin's wistar male rats was treated with bioaqua essence, optimum formula of nanoemulgel gold-vit E, and without treatment. After 7 days the nanoemulgel gold-vit E group experienced an improvement in erythema due to irradiation by UV B light when compared to the positive control group (bioaqua).

IV. CONCLUSIONS

The optimum composition of olive oil (9,09%), tween 80 (80,62%), and propylene glycol (10,29%). The optimum formula nanoemulgel gold-vit E fulfills the criteria of good physical stability which transparent white bone, sorbitan odor, homogeneous, has a pH value of 6,78 ± 0,055, viscosity 346,67 ± 15.27 dPas, and spread area 5,48 ± 0,035 cm. Nanoemulgel gold-vit E have fast skin regeneration ability when compared to brand gold cosmetics that is proven on the 6th day. The test group has erythema value = 1, while the positive control group has an erythema value = 1 but on the 7th day.

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