Optimization SNEDDS (Self-Nano Emulsifying Drug Delivery System) of ZnO that dispersed into Hydrogel Matrix as UV-Protective

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Abstract. World Health Organization in 2015, estimated the case of non-melanoma cancer increasing due to the depletion of the ozone layer. The case of skin cancer in Indonesia reaches 6-8%, so the skin needs effective protection. ZnO is a sunscreen with SPF [Sun Protecting Factor] value of 50 and sun exposure reduction to the skin, especially UV A [320-400 nm] and UV B [290-320 nm]. ZnO is difficult to dissolve in water and hard to dispersed in the hydrogel matrix, so that it is formulated a nanoemulsion preparation into SNEDDS. SNEDDS consists of olive oil, tween 80 [surfactant], and propylene glycol [cosurfactant]. Optimization of the SNEDDS ZnO with the D-Optimal method was using Design Expert Software producing 16 formulas that tested for physical stability response including transmittance values [%] and pH values. The formula that has the highest response value with the desirability value approaching one was the optimum formula, then analyzed done with PSA. The optimum SNEDDS ZnO was dispersed in the hydrogel matrix of carbopol, then tested for physical stability response including transmittance value [%] and pH values. The optimization shows that the optimum composition of olive oil : tween 80 : propylene glycol of 1:9:1. The result of t-test of SNEDDS ZnO optimum formula were not significantly different (p-value > 0.05) with Design Expert prediction. Optimum formula can load 2 mg ZnO with transmittance value is 92%, SPF value 26, PSA 150 nm, and polydispers size of droplets.

1. Introduction
Indonesia is a tropical country with high intensity of sun exposure. Sunlight contains ultraviolet (UV) that harmful for unprotected skin because it could cause skin cancer, eye damage as cataract and melanoma, skin aging, hyperpigmentation, erythema, and immune system damage [9]. UV light consisted of UV A (320-400 nm), UV B (290-320 nm), and UV C (100-290). World Health Organization (WHO) in 2015, estimate non-melanoma cancer cases is increasing to 300,000 cases and melanoma is increasing to 4,500 cases due to depletion of the ozone layer. In Indonesia, skin cancer cases reach 6-8% of all types of cancer [15]. Skin needs an effective protector to reduce the UV light exposure to the skin.

A high SPF sunscreen have an ability to protect skin from UV exposure [7]. SPF or light protection factor have range value of 2-60. Dermatologist recommend the use of sunscreen with the SPF of 15-30 [3]. One of sunscreen compound that contains SPF more than 30 are zinc oxide (ZnO) and titanium oxide (TiO2). ZnO is a material that could absorb sun spectrum with better light quantum than TiO2.
Cosmetics (2015) explains that ZnO have SPF value of 50 so it could protect skin from UV A and UV B effect. ZnO is difficult to dissolve in the water and have cloudy white colored material, those make it difficult to dispersed in hydrogels. ZnO could also cause skin irritation if the concentration is too large, therefore it is formulated in the form of Nano emulsion with the SNEDDS (Self-Nanoemulsifying Drug Delivery System) technique.

SNEDDS is composed of olive oil because it has unsaturated fatty acids consisting of oleic acid (80%), so it has high self-emulsifying ability and a large drug loading capacity. The surfactant used is tween 80, where the greater the surfactant concentration the particle size decreases. In addition, tween 80 is hydrophilic with 15 HLB values and the resulting SNEDDS preparations have transmittance percentages above 80% [9]. Propylene glycol is chosen as co-surfactant because it can reduce the interfacial tension of oil in water and help surfactant work [14].

Optimization of the SNEDDS formula from ZnO in this study was conducted using a D-Optimal mixture design (D-Optimal mixture design). The main advantage of using the D-Optimal method is that it can reduce the number of experimental treatments [2]. In addition, the costs used are lower, because the number of experimental treatments is relatively small [21].

The manufacture of ZnO SNEDDS preparations which are dispersed in the hydrogel matrix is intended to improve the physical properties of turbid hydrogels when added with ZnO. Cosmetic preparations on the market containing ZnO are in the form of creams and emulsions combined with other active substances to provide a synergistic effect. The requirements for hydrogel preparations are semi-solid, clear, stable, translucent, and contain active substances which are dispersed in a colloidal system [1]. Therefore, made in the preparation of ZnO SNEDDS hydrogel to be easily applied to the skin, has a clear and elegant appearance, after dry leaving the film invisibility, easily washed with water, good drug release and good dispersion ability [18].

## 2. Experimental

### 2.1. Materials

Mortar, stamfer, sudip, stirring rod, drop pipette, thermometer, porcelain cup, hot plate, flacon, object glass, pH meter (Eutech), analytical scale (OhausPA413), vortex mixer (Maxi Mix II Thermolyne), sonicator (Branson 1510), UV / Vis spectrophotometer (GenesysTM), particle size analyzer (HORIBA SZ100), gel pot, Eppendorf, incubator shaker and glassware (Pyrex).

ZnO, Olive Oil, Tween 80 (Brataco) for surfactants, Propylene Glycol (Brataco), co-surfactant, Carbopol 940 for suspending agents, Glycerin (Brataco), TEA, Nipagin, Aquabidest (IKA), Aquadest, and 70% Ethanol. Materials used by pharmaceutical grade.

### 2.2 Research Implementation

#### 2.2.1 The making of Carbopol hydrogel matrix

A number of Carbopol were developed with warm water until the dispersion was all assisted by stirring, then added some TEA and stirred until a transparent gel period formed. Added glycerin and nipagin which have been dissolved in propylene glycol and put it until homogeneous. Finally, the addition of residual TEA and aquadest stirred at a constant speed until homogeneous, as in Table 1 [12].

| Material           | F1  | F2  | F3  | F4  |
|--------------------|-----|-----|-----|-----|
| Carbopol           | 0.10 g | 0.08 g | 0.07 g | 0.06 g |
| Glycerin           | 2.00 g | 2.00 g | 2.00 g | 2.00 g |
| Propylene Glycol   | 1.00 g | 1.00 g | 1.00 g | 1.00 g |
| Nipagin            | 0.01 g | 0.01 g | 0.01 g | 0.01 g |
| TEA                | 0.75 g | 0.75 g | 0.75 g | 0.75 g |
| Aquadest           | Ad 20.00 mL | Ad 20.00 mL | Ad 20.00 mL | Ad 20.00 mL |
2.2.2 SNEDDS Formula Component Consideration. The design of the SNEDDS formula was carried out using the Design Expert D-Optimal software method. The main components in the manufacture of SNEDDS were oil (Olive oil), surfactants (Tween 80), and co-surfactants (Propylene Glycol). Prior orientation has been made so that the lower and upper limits are set (Table 2).

| Material             | Lower Limit | Upper Limit |
|----------------------|-------------|-------------|
| Olive Oil (Oil)      | 9.09 %      | 11.11 %     |
| Tween 80 (Surfactant)| 77.77 %     | 81.81 %     |
| Propylene Glycol (Co-Surfactant) | 9.09 % | 11.11 % |

2.2.3 Loading Dose Determination. The determination of the ZnO dose (loading dose) was done by dissolving a number of ZnO into the SNEDDS formula based on the Optimal D-method. The ZnO dose series used was 10 mg, 8 mg, 5 mg, 3 mg and 2 mg in each gram of the system [11]. The highest ZnO content in cosmetics found on the market was 10%. ZnO homogenized into the SNEDDS formula using aid vortex for 3 minutes, sonication for 5 minutes, and incubated with a water bath at 45 °C for 15 minutes. ZnO observations that dissolved in SNEDDS were carried out visually (Table 3). Perfectly dissolved ZnO was determined as maximum loading dose every 1,00 grams of SNEDDS [1]. SNEDDS was stored at room temperature during the characterization process [13].

2.2.4 SNEDDS Characteristics (Response Test). SNEDDS characterization results from the formulation was using the D-Optimization method based on the physical response of transmittance values (%) and pH, then inserted into the response variable in the Expert Design software to obtain the optimal formula composition.

Transmittance Test: A total of 1,0 mL of SNEDDS was dissolved up to 50,0 mL with aquabidest at room temperature. The solution was vertexing for 3 minutes until homogeneous. The transmittance percentage was measured using a UV / Vis spectrophotometer at λ650 nm with blank aquabidest [16]. pH test: a number of hydrogens dissolved in 9 parts of aquabidest and measured using a pH meter [13].

2.2.5. Verification and Optimal Formula Determination. The optimal SNEDDS formula was analyzed using S-Optimal Design Expert Method. The three variable components used were oil, surfactant, and co-surfactant evaluated by the transmittance response (%) and the pH value. ANOVA statistical analysis with p-value 0.05 [16]. Then it was characterized and verified the results of optimal formula testing using an analysis of one sample t-test with IBM SPSS Statistics 22 software.

2.2.6. SNEDDS Formula Evaluation and Hydrogel SNEDDS ZnO Physical Characteristics

2.2.6.1. Particle size. The optimal SNEDDS formula was evaluated by measuring particle size distribution using Particle Size Analyzer (PSA) in the Nanotechnology section of the Mathematics and Natural Sciences Faculty, Indonesian Islamic University, Yogyakarta.

2.2.6.2. Organoleptic Test

Organoleptic test was done by observing visually using five senses to describe the shape, color, and smell of the gel [1].

2.2.6.3. Disperse Test. Disperse test was done by weighing 0,5 grams of gel and mixing with a 150,0 grams load for 1 minute [16].

2.2.6.4. Viscosity test. Viscosity test was carried out with gel samples inserted into the glass speaker and placed under the spindle hanger, then the spindle lowered to the immersed limit into the gel
preparation. Rotor signaled and observed of the red needle on the scale, then read the term shown by the needle [16].

2.2.6.5. Determination of SPF values by In Vitro. A total of 100 mg of SNEDDS ZnO hydrogel was diluted using Aquadest up to 10 mL, then take 1.0 mL and put in 10 mL plus aquadest to the limit. The filtered solution and the absorbance measurements were carried out using a UV/Vis spectrophotometer in the range of 290-320 nm, every 5 nm interval. The blank used was aquadest. The absorbance results obtained in each dose were recorded and calculated by the SPF value [17].

Table 3. Oil, Surfactant, and Co-Surfactant D-Optimization Formula Design.

| Run | Olive oil (%) | Tween 80 (%) | Propylene Glycol (%) |
|-----|---------------|--------------|----------------------|
| 1   | 10.105        | 80.805       | 9.090                |
| 2   | 10.100        | 78.790       | 11.110               |
| 3   | 9.090         | 79.800       | 11.110               |
| 4   | 9.090         | 80.805       | 10.105               |
| 5   | 11.110        | 79.800       | 9.090                |
| 6   | 11.110        | 78.790       | 10.100               |
| 7   | 9.497         | 81.005       | 9.497                |
| 8   | 11.110        | 77.780       | 11.110               |
| 9   | 11.110        | 77.780       | 11.110               |
| 10  | 10.000        | 79.495       | 9.090                |
| 11  | 11.110        | 79.800       | 9.090                |
| 12  | 9.090         | 81.810       | 9.100                |
| 13  | 11.110        | 78.790       | 10.100               |
| 14  | 9.900         | 80.200       | 9.900                |
| 15  | 9.090         | 79.800       | 11.110               |
| 16  | 9.090         | 81.810       | 9.100                |

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2.3 Statistical Analysis
Optimal formula verification done with one sample t-test analysis using IBM SPSS Statistic 22 software. SNEDDS optimal Formula characteristics result includes transmittance presentation and SPF value analyzed using IBM SPSS Statistic 22 software in trust value of 95% where the result was not significantly different when the result of D-Optimal method prediction and the result of the observation have p-value >0.05 [5].

Optimal formula determination was using Design Expert Version 9 Trial – Stat Ease software. The main component of SNEDDS were oil, surfactant, and co-surfactant varied with D-Optimal method in Design Expert Version 9 Trial – Stat Ease.

3. Results and Discussion
The making of hydrogel matrix was using Carbopol 940 as a gelling agent because it has a clear appearance. The acid properties possessed by Carbopol was neutralized by adding Triethanolamine (TEA) to a concentration of 1%. In addition, in the manufacture of hydrogels the addition of nipagin as a preservative was carried out and the addition of glycerin and propylene glycol as humectants and emollients. Determination of the optimum formula was carried out by pH test and viscosity of hydrogel preparations. The pH test was carried out to see the acidity level of the hydrogel preparation to avoid skin irritation, while the viscosity test was carried out to see the level of viscosity. The results of pH evaluation (Table 4) showed formula 1 and formula 2 was unqualified, while formula 3 and 4 was qualified to the topical preparations in the range of 4.50 to 6.50 [13]. The viscosity test was showing significant differences in each formula because the amount of Carbopol affects the viscosity of hydrogel preparations, so that only formula 4 qualified to the viscosity requirements of gel preparations in the range of 20,00-40,00 dPas [16]. From those two parameter tests, it concluded that formula 4 qualified to the requirements of the hydrogel preparation basis.

| Formula | pH  | Viscosity (dPas) |
|---------|-----|-----------------|
| Formula 1 | 6,60 | 1,00            |
| Formula 2 | 6,54 | 10,00           |
| Formula 3 | 6,48 | 18,00           |
| Formula 4 | 6,48 | 23,00           |

Optimization of the optimum formula was done with the D-Optimal method using Design Expert 9 Trial software to determine the upper and lower limit values that were possible to produce SNEDDS formulas according to the desired criteria. Three components in determining the formula composition of SNEDDS were olive oil (A), surfactant tween 80 (B), and co-surfactant propylene glycol (C) determined as independent variables with transmittance values (%) and pH as response variables. The free variables that have been done in the previous orientation have the lower upper and upper limits of 9.09-11.11 (%), 77.77-81.81% (B), and 9.09-11.11% (C) The use of oil and co-surfactant to produce a good SNEDDS formula was <20% and surfactant reaches 60% [3]. The results obtained from D-Optimal were 16 run formulas with different compositions where the total of each component is 100%.

Loading dose determination done with ZnO variation of 10 mg, 8mg, 5 mg, 3 mg and 2 mg in every gram of SNEDDS. The aim of loading dose determination was to know the maximal value of ZnO that could dissolve in the SNEDDS system. The result of ZnO loading dose test that could
dissolve perfectly if the visual observation turns into clear, homogeneity and there no deposits on the SNEDDS system.

The SNEDDS physical characteristics used in optimal formula determination was transmittance value (%) and pH. Transmittance test result from 16 run test formula was about 54.752% to 95.021%. The higher transmittance value signed the smaller particle size so that the solution become clear. SNEDDS Transmittance test result using Design Expert software shown that the data was normally distributed because every result point placed near and followed the diagonal line as predicted by the software so can be continued to the ANOVA test stage. Transmittance test result was modelized in the quadratic form because p value <0.05 of 0.0025 that shown a meaningful difference to the different use of the composition.

Response test using the pH value of hydrogel SNEDDS ZnO preparation was used to determine the pH of the hydrogel SNEDDS ZnO preparation to avoid skin irritation. The pH test results (Figure 1) of 16 run formulas were between 6.816 and 7.678. According to SNI 16-43399-1996 the pH for sunscreen preparation ranged from 4.50 to 8.00. The result of Hydrogel SNEDDS ZnO pH test shown that the data normally distributed because the plot points still on the diagonal line as predicted in the software, so could be continued to the ANOVA test stage. The pH test result modelized as quadratic form with p-value <0.05 of 0.0043 that shown a meaningful difference in the use of composition formulation

Optimal formula for SNEDDS determination was obtained based on the determined limits so that the desirability value approaching to 1 [4] shown the formula that produced was more in line with the optimal formula desired. The optimal formula suggested by Design Expert software was one solution with desirability value of 0.838. Optimal formula verification was done to compare the results of the observation with the results of the Design Expert software response using the one sample t-test analysis (Table 5).

Table 5. Verification Result of SNEDDS Optimal Formula based on D-Optimal Method using Software.

| Response                  | Prediction | CI           | Observation ±SE | p-value |
|---------------------------|------------|--------------|-----------------|---------|
| Transmittance value (%)   | 92.59      | 82.05-103.14 | 4.66-7.99       | 0.05    |
| pH                        | 7.37       | 7.19-7.56    | 0.082-0.16      | 0.07    |

Each response shown low average standard error (SE), where the position near to zero was imaging the average result appeared almost the same to the result in every test. Statistical analysis of transmittance value response (%) and each pH shown un-significance difference (p-value>0.05), so it could be concluded that optimum formula was suggested by verified Design Expert software. The test result obtained was lower than the prediction of transmittance value (%) 92 to pH 7.30.

Optimal formula evaluation was done using particle measurement determination to ensure the formula in the nano-emulsion range of 20-200 nm [6]. The test result shown that SNEDDS optimal formula have the expected nano-emulsion particle size of 150, with polydisperse index (PI) of 0.53. PI value was used to image the nano-emulsion particle homogeneity where the SNEDDS ZnO optimal formula have same particle size distribution in the range of 0.0-1.0.

The physical characteristics test of Hydrogel SNEDDS ZnO preparation was carried out by organoleptic test, dispersion, viscosity, and SPF test. Organoleptic test results of Hydrogel SNEDDS ZnO were transparent bone white, typical tween 80 odor, and semi-liquid. These results were in accordance with the requirements of the gel preparation which it was semi-solid in shape with clear color [1]. The dispersion test of the Hydrogel SNEDDS ZnO preparation showed an increase along with a decrease in viscosity caused by temperature and humidity during storage.
Table 6. Disperse Test and Hydrogel SNEDDS ZnO Preparation Viscosity.

| Test Parameter | Week-1 | Week-2 | Week-3 | Week-4 |
|----------------|--------|--------|--------|--------|
| Disperse       | 5.35   | 5.60   | 6.05   | 6.35   |
| Viscosity      | 38.00  | 35.00  | 32.00  | 27.00  |

Figure 1. Normality Graphic of Residual Value SNEDDS Transmittance Test. Detecting normal residual values is done by looking at the resulting plot points whether or not they are around the diagonal line.

Figure 2. Normality Graphic of Hydrogel SNEDDS ZnO pH Residual Value. Detecting normal residual values is done by looking at the resulting plot points whether or not they are around the diagonal line.
Table 7. SPF Value Test Result Using UV/Vis Spectrophotometer [10]

| Wavelength | EE x I | Absorbance |
|------------|--------|------------|
| 290        | 0.015  | 0.430      |
| 295        | 0.0817 | 0.405      |
| 300        | 0.2874 | 0.381      |
| 305        | 0.3278 | 0.357      |
| 310        | 0.1864 | 0.320      |
| 315        | 0.0837 | 0.298      |
| 320        | 0.018  | 0.267      |
| Total      | 1      | 2.458      |

SPF testing was carried out in vitro using UV / Vis spectrophotometer and obtained SPF 26 results. These results prove that with small doses of ZnO was still able to provide SPF 15-30 which was able to provide moderate protection to the skin. SPF calculations were performed using the method of Mansur et al. (1986), as shown in Table 7.

4. Conclusion

ZnO SNEDDS with the dose of 2 mg/g SNEEDS produce a qualified particle size to the nano-emulsion requirement of 150 nm. After it was disperse hydrogel matrix, it was obtained a qualified preparation of organoleptic test requirement, dispersion, and viscosity with SPF value of 26 (moderate protection).

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