OPTIMIZATION OF OLIVE OIL, TWEEN 80, AND PROPYLENE GLYCOL OF SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEM OF ZINC OXIDE BY D-OPTIMAL METHOD

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Received November 19, 2018; Accepted April 17, 2020

ABSTRACT

The incidence of skin cancer in Indonesia reaches 6-8%, so the skin needs effective protection. Zinc Oxide (ZnO) is a sunscreen with Sun Protecting Factor (SPF) 50 which is able to reduce exposure to Ultraviolet rays. ZnO is difficult to dissolve in water making an obstacle if dispersed in a hydro gel matrix, so it is formulated into a Self-Nanoemulsifying Drug Delivery System (SNEDDS) preparation. SNEDDS formula was made using tween 80 as surfactant, propylene glycol as a co-surfactant, and olive oil. The optimum proportion of the three components was optimized with the D-Optimal method using Design Expert Stat-Ease 9 Trial software. Software obtained 16 formulas which were tested for physical stability response: transmittance value (%) and pH value. SNEDDS optimum formula was compared with the D-Optimal prediction formula using the student's t-test statistical analysis (p>0.05), the loading dose of ZnO, Particle Size Analysis, and Zeta Potential. The optimum proportion of propylene glycol, tween 80, and olive oil making up SNEDDS were 9.9%: 81%: 9.1% respectively. The result of the percent transmittance response was 92.30% and the pH value was 7.20. Software prediction results: transmittance value was 92.59% and pH value was 7.37. Statistical analysis of one sample t-test showed no difference between observations and D-Optimal predictions. SNEDDS was able to load 2.0 mg ZnO/gram SNEDDS with a particle size of 150.2 nm; polydispersity index of 0.54 and zeta potential of -28.50 mV. The SPF value of SNEDDS ZnO was 16.

Keywords: D-Optimal; SNEDDS; UV protective; ZnO.

INTRODUCTION

Indonesia is a tropical country that is crossed by the equator line. This situation causes the region of Indonesia to be always exposed to sunlight with high intensity, where the sunlight contains ultraviolet (UV). UV light A (320-400 nm) can penetrate the deeper layers of the skin to the dermis and could cause aging, pigmentation, erythema, tanning, and DNA damage due to the presence of reactive oxygen compounds or ROS (Reactive Oxygen Species). UV B (290-320 nm) could penetrate into top surface layer of the skin and cause DNA damage. Whereas UV C (100-290 nm) could be filtered by the atmosphere and it could not penetrate to the surface of the earth (Cefali et al., 2016; Kulkarni et al., 2014). Human’s skin, when getting too much exposure of UV rays for a long period, will be susceptible to cancer, sunburn, eye damage such as cataract and melanoma, premature skin aging, pigmentation, erythema, and also immune system damage (Lolo et al., 2017; Kockler et al., 2012).

World Health Organization (WHO) in 2015 estimated that the incidence of non-melanoma cancer increased by 300.000 cases due to ozone depletion. The incidence of skin cancer in Indonesia reaches 6-8% (Suharyanto and Prasetyo, 2004). Therefore, skin needs...
Optimization of Olive oil...
Table 1. Upper and lower limit value of SNEDDS formula entered in DX software

| Materials          | Lower limit (%) | Upper limit (%) |
|--------------------|-----------------|-----------------|
| Olive oil          | 9.09            | 11.11           |
| Tween 80           | 77.77           | 81.81           |
| Propylene Glycol   | 9.09            | 11.11           |

Optimization of SNEDDS formula using D-Optimal method

Software Design Expert Stat-Ease 9 Trial would design 16 formulas after determining the upper and lower limits of the components of the SNEDDS formula. All ingredients were weighed (Table 2), then ZnO with the prescribed dose was dissolved into the SNEDDS formula into flacon disk. The mixture was homogeneous with vortex for 3 minutes and sonicated for 10 minutes. This sonication method was carried out to help reduce the size of the emulsion droplets. The SNEDDS ZnO mixture was then incubated using water bath at temperature of 45°C for 15 minutes until homogeneous. SNEDDS was stored at room temperature during the characterization process (Savale, 2015; Ke et al., 2015).

Physical responses of SNEDDS ZnO

Transmittance Test (%): amount of 1.0 mL SNEDDS was dissolved with redistilled water ad 50 mL at room temperature, then vortex for 3 minutes until homogeneous. Percent transmittance was measured using Spectrophotometer UV-VIS at maximum wavelength of $\lambda$ 650 nm with the redistilled water as a blank (Winarti et al., 2016). Mixture formula with high percent transmittance value was chosen as optimum loading dose of ZnO into SNEDDS.

Optimum formula of SNEDDS ZnO

The optimum formula of SNEDDS was determined using Design Expert Stat-Ease 9 Trial software with D-Optimal method. The SNEDDS formula consist of oil, surfactants and co-surfactants were evaluated by percent transmittance value (%) and pH values. ANOVA statistical analysis with p-value ≤ 0.05 (Winarti et al., 2016; Gohel et al., 2016) for analysis and included into software, then characterization of optimum formula with software prediction was verified using one sample t-test analysis with IBM SPSS Statistics 22 software. The particle size distribution for the optimum formula of SNEDDS ZnO was also checked using a particle size analyzer (PSA) in the Nanotechnology Department of the Faculty of Mathematics and Natural Sciences, Islamic University of Indonesia, Yogyakarta. Amount of 1.0 mL SNEDDS ZnO was dissolved with redistilled water ad 50 mL then vortexed for 3 minutes until homogeneous. The emulsion formed was taken by 3.0 mL and was put into a cuvette for analysis. Replication was carried out 3 times for each test (Jevana and Sreelaksmi, 2011). The optimum SNEDDS ZnO was observed under SEM (Scanning Microscope Electron) to determine the distribution of ZnO nano particles into SNEDDS.

RESULTS AND DISCUSSION

Optimization of composition of olive oil: tween 80: propylene glycol was carried out to obtain the optimal proportion that was able to form colloidal dispersion so as to reduce the size of ZnO particles. The upper and lower limits of the concentration of each component were determined based on a preliminary test in
order to obtain the optimum percentage range with the D-Optimal method using the Design Expert Stat-Ease 9 Trial software according to the desired criteria. Three components in determining the composition of the SNEDDS formula consisting of olive oil (A), tween 80 (B) surfactant, and propylene glycol (C) co-surfactant were determined as independent variables with percent transmittance values (%) and pH value as response variables. The independent variables that had been carried out in the previous orientation had lower limits and upper limits range of 9.09-11.11% (A), 77.77-81.81% (B), and 9.09-11.11% (C). The use of oil and co-surfactants to produce a good SNEDDS formula was less than 20% and surfactants reached 60% (Cerpnjak et al., 2013). The results obtained from D-Optimal were 16 run formulas with different compositions of oil; surfactant; co-surfactant where the total of each component was 100% and total weight of SNEDDS formula was 5.0 grams.

The loading dose was carried out to determine the maximum amount of ZnO that could be dispersed into SNEDDS. ZnO loading dose test results that could be completely dispersed was at a dose of 2.0 mg/g SNEDDS. ZnO was perfectly dispersed when it has clear visual appearance, homogeneous, and there are no deposits in SNEDDS. Propylene glycol is a co-surfactant that is often used in cosmetic preparations where the use of co-surfactants could reduce the flexibility of surface tension so that it has enough flexibility to form nanoemulsions with large compositions (Senapati et al., 2016).

Physical characteristics parameters include the percent transmittance value, where the higher value of percent transmittance which is close to the water transmittance value (100%) indicates a smaller particle size (Ahmad et al., 2014). The results of percent transmittance value from 16 run formulas ranged from 54.75% to 95.02%. The higher transmittance value indicated the smaller particle size, so the solution appearance was clear (Table 2). Clear visual appearance with transmittance value of more than 80% could be categorized as nanoemulsion (Fratter, 2016; Winarti et al., 2016). The pH value determines the chemical stability of the preparation and the suitability of the formula for the pH of the skin as to not to cause allergic reactions. The range of pH value from 16 run formulas were 4.0-7.5 indicating that SNEDDS ZnO pH was suitable for the human skin (Gurning, 2016).

Experimental designs were often used in research designs because they provide maximum information with only requiring a small amount of experimentation. D-Optimal is a method that was used to optimize the proportion of components formula. The amount of olive oil (A), surfactant: tween 80 (B), and co-surfactant: propylene glycol (C) were chosen as the independent factor. The mixture profile was determined by D-Optimal based on the Bolton equation, where Y was the response, ABC were the proportion of the components, and α was the coefficient:

\[ Y = \alpha_1 (A) + \alpha_2 (B) + \alpha_3 (C) + \alpha_{12} (A) (B) + \alpha_{13} (A) (C) + \alpha_{23} (B) (C) + \alpha_{123} (A) (B) (C) \ldots (1) \]

Table 2. Results of formula analysis with D-Optimal method using DX Stat-Ease 9 Trial software that produced 16 run formulas and response parameters of physical properties: percent transmittance and pH value

| Run | Olive Oil (%) | Tween 80 (%) | Propylene glycol (%) | Transmittance (%) | pH |
|-----|--------------|--------------|----------------------|-------------------|----|
| 1   | 10.11        | 80.81        | 9.09                 | 68.11             | 7.34 |
| 2   | 10.10        | 78.79        | 11.11                | 66.59             | 7.33 |
| 3   | 9.09         | 79.80        | 11.11                | 95.02             | 7.27 |
| 4   | 9.09         | 80.81        | 10.11                | 94.28             | 7.53 |
| 5   | 11.11        | 79.80        | 9.09                 | 69.05             | 7.42 |
| 6   | 11.11        | 78.79        | 10.10                | 62.28             | 7.40 |
Table 3. Results of software analysis into mathematics model based on physical properties and ANOVA statistical analysis

| SNEDDS response of physical properties | Mathematics Equation | Mathematics Model | p-value [ANOVA] |
|----------------------------------------|----------------------|-------------------|-----------------|
| Percent transmittance (%)              | -3652.73(A)-111.78(B)-4513.94(C)+52.39(A)(B)+523.42(A)(C)+523.42(\((A)(B)(C)\) | Cubic            | 0.053           |
| pH                                     | 27.56(A)+0.70(B)+14.70(C)-0.35(A)(B)-0.45(A)(B)+0.18(B)(C) | quadratic        | 0.077           |
Figure 1. Results of Contour plot diagram showed that the optimum pH response parameter area was in the range of 6.82-7.68 with red color area.

Figure 2. Results of Contour plot diagram showed that optimum percent transmittance response parameter area was almost 95.02% with red color area.

Table 4. Results of observation value of SNEDDS ZnO optimum formula compared with Software Prediction value

| Response of physical properties | D-opt prediction | Observation results | Sig-value |
|--------------------------------|------------------|---------------------|-----------|
| Transmittance (%)              | 92.59            | 92.30               | 0.053     |
| pH                             | 7.37             | 7.20                | 0.077     |

The software predicted value which was obtained from the D-Optimal method shows that the confidence level of prediction interval of 95% if compared with the response value of the observations for the optimal formula using statistical analysis. The criteria that were given were maximized for percent transmittance value and target for pH value. Based on the test results as presented in table 4, the response percentage of transmittance (clarity)
and pH value from the observation of the optimal formula did not differ significantly from the predicted values given by the Design Expert Stat-Ease 9 Trial software (p-value>0.05).

**Measurement of particle size and zeta potential of SNEDDS ZnO optimum formula**

The nanoemulsion droplet size of ZnO into SNEDDS was 150.2 nm with a polydispersity index value of 0.54. The recommended PI value requirements indicate that the particles in the SNEDDS formula are stable and reduce the possibility of deposition because brown motion of ZnO particles are rapidly. PI values that meet the observed PI value was quite good and still met the good PI standard value.

PI in particle measurement was used to describe the homogeneity of nanoemulsion particles which have a range of 0.0 to 1.0 (Pratiwi et al., 2016). The small particle size could increase the surface area of the particle so it could increase absorption of the drug when it was applied on skin surface area. Nano size in the droplets would reduce the time of emulsification (Bandyopadhyay et al., 2012).

Low polydispersity index value shows a narrow particle size distribution meaning that the particle size in the SNEDDS was uniform (Avachat and Patel, 2014). Uniform particle size could increase homogeneity of ZnO when dispersed into SNEDDS and would be also absorbed faster with relatively the same speed (Balakumar et al., 2013). The potential zeta value obtained from SNEDDS ZnO was -28.5 mV. SNEDDS with the potential zeta value in range of more than +30 mV and less than -30 mV would produce relatively stable preparations.

This negative potential zeta value indicates that the SNEDDS formula has a negative charge and it was sufficient to counteract the repulsive force so that it would produce a stable preparation (Dash et al., 2015). The range of potential zeta values to maintain stability was less than -30 mV or more than +30.

**Sun protective factor (SPF) value and scanning electron microscope (SEM) of SNEDDS ZnO optimum formula**

SEM results at 100 times magnification showed that ZnO powder had an irregular surface shape and formed an aggregate, whereas on SNEDDS ZnO showed smaller particle sizes and uneven agglomerated in SNEDDS. The smaller the particle size would increase the specific surface area of the particle, thereby increasing the particle distribution (Figure 3). The SNEDDS formula (olive oil: tween 80: propylene glycol) has an SPF value of 5.0 while SNEDDS ZnO has an SPF value of 16. Research Idaho (2008) stated that sunscreen was recommended at least 15 minutes that a person has natural resistance to sunlight for 30 minutes (Moore, 1982).
Figure 3. Results of SEM analysis using carbon coating. ZnO powders (a); SNEDDS ZnO (b) at 100 times magnification showed that ZnO powder had an irregular surface shape and formed an aggregate, whereas on SNEDDS ZnO showed smaller particle sizes and uneven agglomerated in SNEDDS

CONCLUSION
The optimum proportion of composition based on D-Optimal method with physical characteristics of transmittance and pH value was olive oil: tween 80: propylene glycol of 9.1%: 81%: 9.9% where the interaction of olive oil and propylene glycol would increase the transmittance value but reducing the pH value. SNEDDS ZnO optimum formula result of the percent transmittance value was 92.30% and pH value of 7.2. D-Optimal prediction value for percent transmittance value was 92.59% and pH value of 7.37. The results of the one sample t-test statistical analysis showed that there was no difference between the observations and D-Optimal predictions value. SNEDDS optimum formula was capable load of 10 mg ZnO particle with SPF value of 16. The particle size of SNEDDS ZnO was 150.2 nm; polydispersity index of 0.54; zeta potential of -28.50 mV; and SPF value of 16. As further studies, SNEDDS ZnO would be dispersed into the hydrogel for topical preparations that is used as UV Protection.

ACKNOWLEDGEMENT
Mandiri Aktif Research Grant from LPPM (Lembaga Penelitian dan Pengabdian Masyarakat) Universitas Sebelas Maret 2018.

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