Optimization of self nanoemulsifying drug delivery system with ethanolic extract of ginger (*zingiber officinale*) and eel bone oil (*anguilla spp.*) extracted by maceration as carrier

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Abstract. SNEDDS is a drug delivery method by mixing oil, surfactant, cosurfactant and drug that can form spontaneous nanoemulsion oil in water in the digestive tract and produce nanometer-sized droplet. Aim of this study was to get optimization for SNEDDS (Self Nanoemulsifying Drug Delivery System) with ethanolic extract of ginger, eel fish bone oil extracted by maceration as carrier, tween 80 as surfactant and PEG 400 as cosurfactant that applicable as a nanoemulsion formula. Optimization formula was measured based on transmittance and emulsification time of mixture of eel fish oil, ginger extract, tween 80, and PEG 400 into 8 different compositions. The Transmittance values were measured using UV/VIS Spectrophotometric at 650 nm and emulsification times were measured using stopwatch by diluting 1 mL of SNEDDS into AGF (Artificial Gastric Fluid), aquadest, and aquabidest. The optimum SNEDDS formula was determined based on the optimization results using Design Expert 7 program with the independent variable was composition of tween 80 and composition of PEG 400 and the dependent variable was transmittance and emulsification time in AGF, aquadest, and aquabidest. Result of Design Expert 7 program optimization formula showed that SNEDDS of ginger extract will be optimized in 4.2 g of tween 80 as surfactant; 0.25 g of PEG 400 as a cosurfactant; 500 mg of ethanolic ginger extract and 0.55 g of maceration extract of eel fish bone oil with transmittance value was 92 ± 0.034 %; emulsification time in AGF was 10 second, in Aquadest was 10 second, and Aquabidest was 9 second. The result of Analysis of Variance indicated not significant differences between treatment groups and optimization by design expert 7 program ((P = 0.1359 (P>0.05)).
1. Introduction
Nanoparticles are currently used widely as an alternative to targeted treatments and carrier agents. Interdependence between the shape, size, and biochemical properties of the surface of the nanoparticles gives effect to biodistribution, cellular internalization, and intracellular trafficking from micro and nanoparticles [4]. SNEDDS (Self Nanoemulsifying Drug Delivery System) can be defined as a multi isotropic component consisting of oil, surfactants, co-surfactants which have the ability to form oil molecules as micro and nano-sized drug delivery through mild stirring with dilution (soluble) in a liquid medium such as gastrointestinal fluid. Inside the gastrointestinal tract, SNEDDS can form oil droplet less than 5 µm which carries drug compounds and increases drug absorption and drug molecular stability [8].

Oil can be absorbed properly as an oil droplet in the form of oil emulsion in water or in the form of digestion. Nanoemulsion is a thermodynamically stable system with a droplet size of less than 100 nm. Nanoemulsion has been widely studied to improve the bioavailability of drugs that are difficult to dissolve in water [11]. The presence of surfactants and cosurfactants is known to have effect on the size of particles formed because some of the materials used as surfactant and cosurfactant have hydrocarbon chain structures with hydrophobic chains. PEG 400 is known to have low toxic levels and has been widely used in various fields such as pharmaceuticals, biomedicine, and dispersing agents [7][14]. The type of cosurfactant and the relative length of the hydrophobic chains affect the area of microemulsion and nanoemulsion [9].

Ginger (Zingiber officinale) has been widely used throughout the world as a food additive that causes a spicy taste and traditional medicine as a deterrent to treating hypertension and some other cardiovascular diseases. Active ingredients found in ginger include volatile compounds and phenol compounds known as gingerol, sesquiterpenoid and shogaol [1].

The eel known as ‘unagi’ in Japan is very expensive because it has a protein content of 16.4% and high vitamin A of 4700IU [5]. Based on the results of the analysis, eel oil obtained SFA as much as 19.87%, MUFA 25.84%, PUFA 13.84%. The main fatty acid composition of eel oil is palmitic acid as much as 13.58%, oleic acid 20.94%, linoleic acid 4.01%, EPA 1.57% and DHA 4.84% [12].

This research was done to get optimization formula for SNEDDS with ethanolic extract of ginger, eel fish bone oil as a carrier, tween 80 as a surfactant and PEG 400 as a cosurfactant that applicable as a nanoemulsion formula.

2. Experimental
2.1 Materials
Materials used in this research includes rotary evaporator (RVO 400 SD Boeco Germany), waterbath (Grant), vortex (Heidolph REAX top), sonicator (Branson 1510), magnetic stirrer (IKA C-MAG HS 7), flacon, analytic pair of scales (KERN ALJ), measuring glass (Pyrex), test tube, UV/Vis spectrophotometric (Perkin Elmer Lambda 25), yellow tip (Kan Jian), micropipet 50 – 200 μl, Particle Size Analyzer (PSA) (Horiba SZ – 100), ethanol p.a (Merck), Tween 80 (Brataco), aquades (General), NaCl, HCL 37% (Merck), Aquabides (Aldrich Yogyakarta).

2.2 Extraction of ginger
Ginger extract is obtained by macerating ginger powder with ethanol at ratio 1: 5 (w/v) for 2 x 24 hours and stir for 30 minutes every 24 hours. The ethanol solution was separated from the sediment by using a flannel cloth to be subsequently evaporated using a rotary evaporator at 70 °C to obtain a thick ginger extract.

2.3 Extraction of eel fish bone oil
Eel fish bone oil was obtained by maceration of eel fish bone with methanol and chloroform in ratio of 1: 2 (v/v) for 3 days. Then methanol and chloroform solution was evaporated at temperature 55 °C using rotary evaporator to obtain oil extract.
2.4 SNEDDS formulation
SNEDDS formulation was done by mixing eel bone oil, tween 80 as surfactant, PEG 400 as cosurfactant, with 8 compositions, vortex for 2 minutes, sonication for 15 minutes and conditioned in a waterbath at 45 °C for 10 minutes. Then they were allowed to stand for 24 hours at room temperature.

2.5 Measurement of transmittance value
Transmittance values were observed using UV/Vis spectrophotometric by diluting 1 mL of SNEDDS without ginger extract into 50 mL of aquadest through transmittance measurement at 650 nm wavelength with aquadest as a blanko to determine the level of clarity of the emulsion. Transmittance values approaching to the aquadest (100%) are estimated to have reached the nanometer size of the emulsion.

The results of the comparison of eel bone oil, tween 80 and PEG 400 were selected based on the largest transmittance value (close to aquadest) then developed into 4 formulas by changing the composition of tween 80 and PEG 400. The four formulas were performed mathematical calculations to determine the upper and lower limits to put into the expert design program 7. In this study, the optimal formula selected was SNEDDS with ratio of oil: surfactant: cosurfactant as much as 1: 7: 1.

2.6 SNEDDS formulation with ginger extract
The addition of ginger extract as a drug compound was given at an optimal formula ratio of 500 mg extract. Then it was homogenized with vortex for 2 minutes, sonication for 15 minutes and conditioned in a waterbath at 45 °C for 10 minutes. Then, the mixing results were allowed to stand for 24 hours at room temperature for later observation if there was sediment formed.

2.7 Emulsification Time measurement
Measurement of emulsification times were measured by diluting 1 mL of samples in artificial gastric fluid (AGF), aquadest, and a quabidest at temperature 37 °C while stirring at magnetic stirrer.

3. Results and Discussion
3.1 Result
Formula composition of oil: surfactant: cosurfactant then obtained into comparison with the highest transmittance value that is a ratio of 1: 7: 1 (96%). Then made into 4 composition groups to determine the upper and lower limits of the formula. The results of measurement of upper and lower limit of oil, surfactant, and cosurfactant are presented in Table 1.

| Oil : Tween 80 : PEG 400 | Oil (%)      | Surfactant (%) | Cosurfactant (%) |
|--------------------------|--------------|----------------|------------------|
| 1 : 7 : 1                | 12.5 (upper limit) | 87.5 (lower limit) | 12.5 (upper limit) |
| 0.75 : 7.25 : 0.75       | 8.57         | 91.43          | 8.57             |
| 0.5 : 7.5 : 0.5          | 5.88         | 94.12          | 5.88             |
| 0.25 : 7.75 : 0.25       | 3.13 (lower limit) | 96.87 (upper limit) | 3.13 (lower limit) |

The four composition groups with the upper limit and lower limit, then made into 8 compositions to measure transmittance value and the emulsification time in AGF, aquadest and aquabidest. The results of measurement of transmittance value (%) are presented in table 2.
Table 2. Transmittance value of 8 runs of SNEDDS without ginger extract

| Run | Transmittance |
|-----|---------------|
| 1   | 92.16         |
| 2   | 91.94         |
| 3   | 92.5          |
| 4   | 93.26         |
| 5   | 92            |
| 6   | 91.68         |
| 7   | 92.04         |
| 8   | 92.39         |

Figure 1. Pseudoternary phase diagram of transmittance value for oil, surfactant and cosurfactant compositions

The eight run compositions then measured to obtain emulsification time into AGF, aquadest, and aquabidest. The results of measurement of emulsification time (in seconds) presented in Table 4.

Table 3. Results of the experiment run 8 emulsification time

| Run | AGF | Aquadest | Aquabidest |
|-----|-----|----------|------------|
| 1   | 8   | 35       | 7          |
| 2   | 6   | 21       | 8          |
| 3   | 6   | 32       | 9          |
| 4   | 10  | 33       | 9          |
| 5   | 13  | 23       | 9          |
| 6   | 12  | 41       | 10         |
| 7   | 8   | 36       | 8          |
| 8   | 7   | 34       | 9          |
Transmittance and emulsification time data were then analyzed using design expert program 7 to obtain optimal formula data for oil, surfactant and cosurfactant composition. The result of optimization formula based on design expert program 7 was presented in figure 5.
The results of the optimal formula were then obtained into 5 g of SNEDDS with ginger extract and eel bone oil as carrier with calculations using the upper and lower limit of oil, surfactant and cosurfactant. So that the optimal formula was obtained with the composition of 0.54 g of eel bone oil, 4.2 g of surfactant (tween 80), 0.3 g of cosurfactant (PEG 400) and 500 mg of ginger extract. The final optimal formula then verified by transmittance and emulsification time measurement to observe if there are differences between programme and verification result. The results of measurements of transmittance value, emulsification time of SNEDDS of ginger extract with eel bone oil as carrier were 92% of transmittance value, 10 second of AGF emulsification time, 10 second of aquadest and 9 second of aquabidest. The result of Analysis of Variance indicated not significant differences between treatment groups and optimization by design expert 7 program ((P = 0.1359 (P>0.05)).

3.2 Discussion
The results of measurements of transmittance value and emulsification time of SNEDDS of ginger extract with eel bone oil as carrier were 92% of transmittance value, 10 second of AGF emulsification time, 10 second of aquadest and 9 second of aquabidest.

Transmittance value of 92% indicated that SNEDDS transmittance was close to the aquadest transmittance value of 100%. The higher of transmittance value showed that the particle size was getting closer to the nanometer. SNEDDS is one of the drug formulations that can produce nanometer droplet size and increase solubility in liquid media and oral absorption of a type of lipophilic drug with oil phase as carrier. SNEDDS particle size is generally less than 100 nm and can increase oral bioavailability for drug compounds that have low solubility in water [15]. Measurement of emulsification time to determine the length of time needed by the system to be digested in some liquids such as aquadest, aquabidest, and the digestive tract which in this case is AGF (Artificial Gastric Fluid) at 36 °C, the faster an emulsified system, the faster the absorption in digestive tract, this
showed that the SNEDDS system emulsification time below 1 minute was only 10 seconds in AGF media, 10 second in aquadest, and 9 second in aquabidest can be fully emulsified.

Tween 80 used in TiO$_2$ nanoparticles is known to have antifungal and antibacterial abilities both gram-positive and gram-negative bacteria [10]. Surfactants can increase the solubility of hydrophobic compounds in water. Tween 80 is known to have a low level of toxicity [2][3].

Tween 80 was chosen as surfactant because it has several advantages such as being able to transfer hydrophobic upconversion nanoparticles into the liquid phase, low prices, biocompatible and has the ability to prevent absorption of non-specific proteins due to its unique molecular structure. Tween coated was formed from hydrophobic interactions between the tails of fatty acids in tween and the oleic acid layer on the surface of the nanoparticles. Tween that coated the nanoparticles maintains the shape of the nanoparticles, has good solubility in water, low toxicity and has the ability in drugs delivery [6][13][15]. Some cosurfactants such as PEG 400 can change the shape of nanoparticles, size, and other surface properties at different levels depending on the molecular structure, nature of head group, hydrophobic tail length and ion counter type. Termination of the growth of nanoparticles is controlled by diffusion lanes and surfactant attachments on the molecular surface [14].

4. Conclusion

Optimization formula showed that SNEDDS of ginger extract will be optimized in 4.2 g of tween 80 as surfactant; 0.25 g of PEG 400 as cosurfactant; 500 mg of ethanolic ginger extract and 0.54 g of maceration extract of eel bone with transmittance value was 92 ± 0.034 %, emulsification time in AGF was 10 second, in aquadest was 10 second, and aquabidest was 9 second. The result of Analysis of Variance indicated not significant differences between treatment groups and optimization result by “design expert 7 program” (P = 0.1359 (P>0.05)).

Acknowledgments

This study was supported by the Directorate General for Strengthening Research and Development, Community Service, Ministry of Research and Higher Education of the Republic of Indonesia.

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