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CHARACTERIZATION AND PHYSICAL STABILITY EVALUATION OF SNAKEHEAD FISH (*OPHIOCEPHALUS STRIATUS*) POWDER NANOEMULSION

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ABSTRACT: Snakehead fish powder containing albumin has macromolecule size which is difficult to penetrate into membrane cell. So, It was formulated into nanoemulsion utilizing the best comparison of surfactant, co-surfactant and oil. Nanoemulsion of snakehead fish powder was characterized by particle size analyzer. The snakehead fish were extracted by atomizer (-40 °C, 1atm) to gain powder which was formulated into nanoemulsion by spontaneous emulsification method using the best comparison of oleic acid, propylene glycol and Tween 80 (1:10). Propylene glycol was added into Tween 80 and oleic acid and stirred until homogeneous mixture. Then water, containing 0.125% of snakehead fish powder, was added drop by drop until becoming a transparent solution by sonication. It was characterized by UV-Vis Spectrophotometry and Particle Size Analyzer. The results of this research showed that snakehead fish nanoemulsion generated clear, stable, and translucent formula having the transmittance value of 100%. The characterization results described nanoemulsion had the average particle size of 147.63 nm. Meanwhile, the average of polydispersity index and zeta potential values were 0.234 and 13.38 mV. This means that nanoemulsion was stable having a uniform particle size, pH 5.5, and the viscosity value of 136.7 cP. The results of the evaluation and preparation stability test showed a good level of stability with the viscosity and pH by one way ANOVA analysis which did not change significantly.

INTRODUCTION: In Indonesia, one of freshwater fish species having main function for human health is snakehead fish (*Ophiocephalus striatus*). It contains high protein such as albumin, amino acids and unsaturated fatty acids which are often used by people to accelerate wound healing with formation of new tissues [1]. Besides that, unsaturated fatty acids in snakehead fish can regulate prostaglandin synthesis having important role in inflammation process and accelerating wound healing.

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Prostaglandin is one of tissue products emerging inflammation reaction which can activate macrophage system strongly [2].

According to Tungadi (2011), snakehead fish powder had been formulated into macro emulsion cream for accelerating wound healing of post-operation *in vitro*. In general, this cream was physically unstable so that the emulsion system was easily broken by adding energy of oil, water phase and storage temperature. This problem can be solved by reducing particle size of active compounds and stabilizer use. The reduction of particle size or droplet oil-water can be done by making nanoemulsion with the comparison of surfactant, co-surfactant, and oil appropriately [3, 4]. The snakehead fish powder can be formulated into emulsion dosage form because the contents of the

fish consists of polar (water soluble protein) and nonpolar (fatty acid soluble oil) parts. Nanoemulsion can be used as transdermal drug delivery system which has to pay attention to penetration of drug active compounds because it has to be able to pass through the skin barrier *i.e.* stratum corneum.

Meanwhile, Tungadi, R. (2016) stated that snakehead fish cream (negative control) was difficult to penetrate through the stratum corneum. By testing on rabbits *in vivo* it can be seen that open wounds longer recover by using snakehead fish cream than penetrant enhancers such as propylene glycol (treatment group). This means that propylene glycol can accelerate the diffusion rate of albumin into stratum corneum which the amount of albumin around 50% compared to without penetrant enhancer about 5-7%. Therefore, snakehead fish powder was formulated into nanoemulsion [5].

Nanoemulsion can increase the diffusion rate of active compounds because it reduces the particle size or droplets from oil and water phase in the emulsion system. Smaller particle size can enhance extensive contact with the membrane cell particles and facilitate carrier particles for penetration into membrane cells.

So, the amount of active compounds are easy to penetrate systemic circulation which will increase bioavailability of active compounds. This means that it does not need a penetrant enhancer to accelerate the diffusion rate of active compounds into membrane cells.

The formulation of snakehead fish powder used a spontaneous emulsification method with comparison of surfactant, co-surfactant and oil appropriately such as tween 80, propylene glycol and oleic acid. The characterization of snakehead fish powder nanoemulsion had important roles in showing stability measurements such as particle size, zeta potential, and polydispersity index by particle size analyzer. This can prove that snakehead fish powder, formulated into nanoemulsion utilizing spontaneous emulsification method, can reduce particle size of snakehead fish powder.

MATERIALS AND METHODS: Snakehead fish powder of pharmaceutical grade was gained by PT. Royal Medical Pharmaceutical, Indonesia, and was certified containing protein 85.6%, albumin 30.2%, omega-3 2.03%, omega-6 2.11% and omega-9 0.92% and polyunsaturated total 5.1% respectively. Basis of nanoemulsion consisted of tween 80 27.5% (surfactant), propylene glycol 22.5% (Co-surfactant), and oleic acid 5% (oil). All of them were bought from PT. Intraco Chemical. propyl paraben 0.02%, methyl paraben 0.18% and BHT 0.1% were purchased from PT. Sentana Chemical. The UV-Vis Spectrophotometry was from Perkin Elmer (USA). The Delsa^{TM} Nano having particle size of 1 nm – 0.7 μm (UK). In addition, a pH meter (Systronics model EQMK), a sonicator (Specta Lab, model UC 40) and a hot air oven (Memmert) were utilized in this study.

Optimization of nanoemulsion basis: The optimizations of nanoemulsion basis were made in different comparisons of surfactant, co-surfactant and oil. There were five formulas with the comparison of different concentrations between surfactant (tween 80), co-surfactant (propylene glycol) and oil (oleic acid) such as F1 (1:6), F2 (1:7), F3 (1:8), F4 (1:9), and F5 (1:10). Tween 80 and propylene glycol were mixed together utilizing magnetic stirrer for 30 minutes 250 rpm (the first mixture). After that, oleic acid was added to the first mixture while stirring and adding water drop by drop containing snakehead fish powder 0.1% then done sonication for 10 minutes. The same procedure was made for all formulas with different comparisons of tween 80, propylene glycol, and oleic acid.

Formulation of snakehead fish powder nanoemulsion: After optimization of nanoemulsion basis, getting the best formula of five formulas was continued by formulating snakehead fish powder using spontaneous emulsification method. The best formula was translucent and clear nanoemulsion which continued by transmittance measurement using UV-Vis Spectrophotometry.

The particle size measurement of snakehead fish powder nanoemulsion: Particle size, potential zeta, and polydispersity index by particle size analyzer (PSA) were measured from snakehead fish

powder by putting nanoemulsion in the cuvette of PSA. Then this was measured by particle size analyzer which is shown in graph [6].

The evaluation of snakehead fish powder nanoemulsion: The evaluation of snakehead fish powder nanoemulsion involved organoleptic, pH, viscosity, and stability tests including centrifugation and freeze-thaw methods [7, 8]. All data were statistically analyzed by utilizing one-way Anova.

RESULTS AND DISCUSSION:

Optimization of nanoemulsion basis: The optimization of nanoemulsion basis indicates formula 5 with the comparison of oleic acid, tween 80 and propylene glycol (1:10). This has the best performance including viscosity, clarity, and stability (**Table 1.**).

TABLE 1: THE OPTIMIZATION RESULT OF NANOEMULSION BASIS

	Formula %				
Materials	**F1 1:6**	**F2 1:7**	**F3 1:8**	**F4 1:9**	**F5 1:10**
Oleic acid	5	5	5	5	5
Tween 80	18	20	23	25	27.5
Propylene glycol	12	15	17	20	22.5
Distilled water	100	100	100	100	100
Observation	Cloudy	Cloudy	Cloudy	Cloudy	Cloudy

Formula 5 was physically stable utilizing centrifugation method (3800 rpm; 5 hours). The formula did not segregate. Otherwise, formulas 1 to 4 gave cloudy appearance and showed segregation after centrifugation. Tween 80 as nonionic surfactant has high hydrophilic and lipophilic balance (15) so that it can be stable in an emulsion system with oil in water [9]. This surfactant has pivotal roles in nanoemulsion basis because it has a large surface area for reducing interfacial and surface tension causing the surfactant to be absorbed on interface phase. Regarding this, it can decrease the surface free energy by ruining globule and resulting small globule [10]. The most surfactants are not able to reduce interfacial tension in emulsion so that it needs to add co-surfactant (Propylene glycol) which can increase the solubility of nonpolar groups [11]. Besides that, it can intensify flexibility of surfactant film and fluidity of emulsion phase [12].

Formulation of snakehead fish powder nanoemulsion: The particle size of snakehead fish powder, containing albumin, protein, and amino acids, used in formulation, was 30 μm [13]. It means that the particle size of powder has important roles in accelerating drug diffusion to penetrate into membrane cell. Therefore, the snakehead fish powder was formulated into nanoemulsion to reduce particle size of powder using a spontaneous emulsification method. Due to small particle size from emulsion, the powder can accelerate active compounds such as albumin, protein, and amino acids to penetrate membrane cells. It causes the increase of the contact area between the carrier particles and the membrane cell [16]. Regarding this, the carrier particles are easy to release active compounds into systemic circulation system causing the increase of bioavailability of snakehead fish nanoemulsion and the acceleration of wound healing processes.

Characterization and Evaluation:

The particle size measurement of snakehead fish powder nanoemulsion: The characterization of snakehead fish nanoemulsion utilized UV-Vis Spectrophotometer and particle size analyzer. The best formula of snakehead fish nanoemulsion, transparent emulsion, measured transmittance on the wavelength 650 nm and resulted in 100%. After that, it was continued by particle size analyzer to know particle size, zeta potential and polydispersity index of snakehead fish nanoemulsion. The results of characterization show that the particle size of snakehead fish nanoemulsion was average 147.63 nm of three replications (**Fig. 1, 2, 3**).

It proves that snakehead fish nanoemulsion meets the criteria of nanostructures which are the particle size range of nanoemulsion 1 – 100 nm or 2 – 500 nm [14]. Meanwhile, zeta potential value of snakehead fish nanoemulsion was 13.38 mV. It means that snakehead fish nanoemulsion has a good degree of stability because zeta potential

value describes the potential of the charge of the particles. Nanoparticles with the value of potential zeta above or below ±30 mV show that colloidal system is physically stable; so that the magnitude of the charge particle can prevent particle aggregation based on electrostatic repulsion [15, 16]. Besides that, polydispersity index of snakehead fish nanoemulsion give good results of three replications *i.e.* 0.205, 0.215, and 0.284 respectively (**Table 2**).

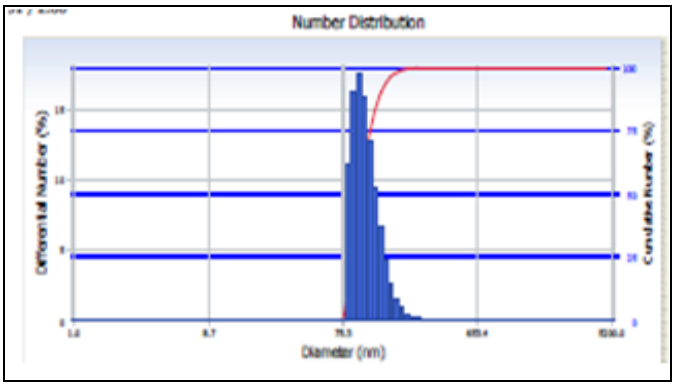

FIG. 1: THE DISTRIBUTION GRAPH OF PARTICLE SIZE OF SNAKEHEAD FISH NANOEMULSION (REPETITION 1)

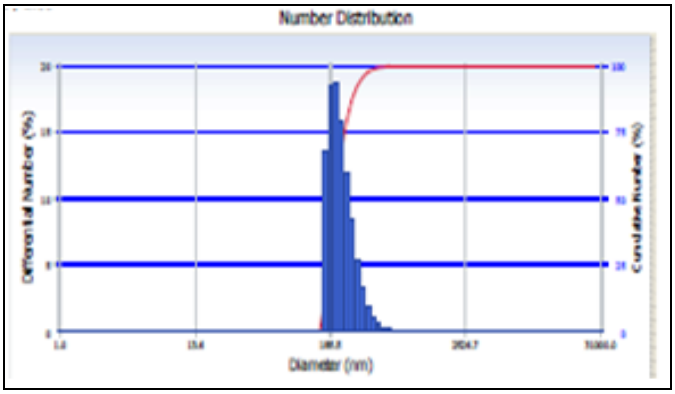

FIG. 2: THE DISTRIBUTION GRAPH OF PARTICLE SIZE OF SNAKEHEAD FISH NANOEMULSION (REPETITION 2)

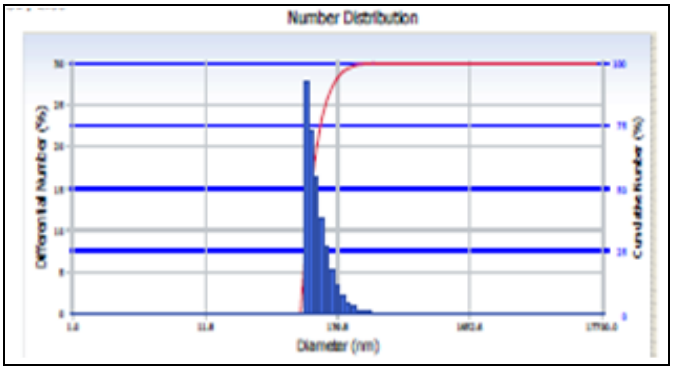

FIG. 3: THE DISTRIBUTION GRAPH OF PARTICLE SIZE OF SNAKEHEAD FISH NANOEMULSION (REPETITION 3)

TABLE 2: THE CHARACTERISATION RESULT OF SNAKEHEAD FISH NANOEMULSION

Sample	Particle size(nm)	Zeta potential mV	Polydispersity index
Snakehead fish nanoemulsion	111 ± 0.2	13.38	0.205
	233 ± 0.5		0.215
	98.6 ± 0.9		0.284

The average of polydispersity index was 0.234. This means that snakehead fish nanoemulsion has uniform particle size and homogeneous dispersion due to the value of polydispersity index below 0.25 [17].

The evaluation of snakehead fish powder nanoemulsion: Based on freeze thaw method, snakehead fish nanoemulsion presented good results which were physically stable at extreme temperatures (4^o and 40^o C) for 7 cycles (28 days). Regarding this, observation results showed that the viscosity value of snakehead fish nanoemulsion was around 136.7 cP and the pH value about 5.3 – 5.5 in different cycles. All data are presented in **Table 3.**

TABLE 3: THE EVALUATION RESULTS OF FREEZE-THAW METHOD

Treatment	Temperature(^{o}C)	Viscosity(cP)	pH
Cycle 1	40, 4	136.7	5.5
Cycle 2	40, 4	136.7	5.5
Cycle 3	40, 4	136.7	5.4
Cycle 4	40, 4	135.6	5.4
Cycle 5	40, 4	135.6	5.3
Cycle 6	40, 4	135	5.2
Cycle 7	40, 4	134.5	5.2

Meanwhile, the statistical analysis of freeze thaw method particularly pH (**Table 4**) and viscosity (**Table 5**) data showed that P value is greater than 1 which mean there is no real difference between pH and viscosity of the stability test.

It means that there were no significant changes (one-way Anova) involving organoleptic tests i.e. clear, homogeneous, and slightly viscous. Meanwhile, centrifugation methods described snakehead fish nanoemulsion was stable which marked no segregation between oil and water phase for 5 hours, 3800 rpm. This means that formulas of snakehead fish nanoemulsion can be saved for 2 years [12]. Based on the data can be concluded that snakehead fish nanoemulsion has nanoparticle size which is physically stable according to characterization and evaluation results.

TABLE 4: ONE-WAY ANOVA FOR pH OF SNAKEHEAD FISH POWDER NANOEMULSION

ANOVA						
		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
pH of snakehead fish powder nanoemulsion on stability test of freeze thaw method	Between Groups	.000	1	.000	.000	1.000
	Within Groups	.194	12	.016		
	Total	.194	13			
The treatment tests	Between Groups	.000	1	.000	.000	1.000
	Within Groups	56.000	12	4.667		
	Total	56.000	13			

TABLE 5: ONE-WAY ANOVA FOR VISCOSITY OF SNAKEHEAD HEAD POWDER NANOEMULSION

ANOVA						
		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Viscosity of snakehead fish powder nanoemulsion on stability test of freeze thaw method.	Between Groups	.000	1	.000	.000	1.000
	Within Groups	9.669	12	.806		
	Total	9.669	13			
The treatment tests	Between Groups	.000	1	.000	.000	1.000
	Within Groups	56.000	12	4.667		
	Total	56.000	13			

CONFLICT OF INTEREST: The researchers declare no conflict of interest.

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