SNEDDS Formulation for the Combination of Snakehead Fish (*Channa striata*) and Bitter Melon (*Momordica charantia* L.) Extract

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Abstract. The combination of Snakehead Fish Powder (SFP) and Bitter Melon Extract (BME) was possible to lower blood sugar levels and antidiabetic agents. The combination of SFP and BME through the mechanism of Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) was carried out. The SNEDDS are forms of lipid-based nanoparticle formulation, which is isotropic mixtures of surfactants, cosurfactants, and oil that spontaneously form nanoemulsions upon contact with gastrointestinal fluids. This study aimed to determine the effect of the composition of surfactants, cosurfactants, and oils, and their comparison in the SNEDDS comprising SFP and BME on emulsification time, transmittance, and nanoemulsion droplet size. The formulation used a total of 30 SNEDDS formulas of SFP and BME was prepared with HLB ranging from 11 to 15 and the combination of surfactant (Tween 20 and Span 80), cosurfactant (glycerin), and oil (olive oil) at ratio 8:1:1, 7:2:1, and 7:1:2. The selected SNEDDS formula combined with SFP and BME was characterized through phase separation, emulsification time, transmittance, nanoemulsion stability, nanoemulsion droplet size, and zeta potential employing a particle size analyzer (PSA). The results showed that 30 SNEDDS formulas were selected and combined with SFP and BME using an HLB of 15 with a combination of surfactants (Tween 20 and Span 80), cosurfactant (glycerin), and oil (olive oil) at ratio 8:1:1. It produced a well-dispersed nanoemulsion in aqueous media with droplet sizes of 212.4 ± 58.10 nm, emulsification time of less than 2 min, and transmittance value of 98.2 ± 1.05% .

Keywords: Diabetes Mellitus · Channa striata · Momordica charantia L. · Self-Nanoemulsifying Drug Delivery Systems

1 Introduction

Diabetes Mellitus (DM) is one of the public health challenges in the 21st century. The number of adults diagnosed with diabetes was estimated to triple in the last 20 years. DM

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is a chronic disease due to the insulin resistance in the body. Type 2 Diabetes Mellitus (T2DM) is the most common type of DM among adults. The management of DM involves a healthy diet and the administration of oral drugs [1]. However, the side effects of drugs, especially synthetic drugs, are unavoidable. They frequently affect the patients' daily activities. As a result, natural medicines are commonly used as alternative treatments in Indonesia. The demand for such medicines increases due to their characteristic: lower cost than synthetic drugs, environmentally friendly, and easy to use. Among the natural medicines reported to have antidiabetic properties are snakehead fish (*Channa striata*) and bitter melon (*Momordica Charantia* L.) [2, 3].

Snakehead fish (*Channa striata*) is a species of freshwater fish that is rich in protein, unsaturated fatty acids, albumin, vitamins, and minerals [2]. Albumin is the highest protein content found in snakehead fish that can repair and regenerate the damaged pancreatic beta cells and help insulin secretion normalize blood glucose levels [4, 5]. Bitter melon (*Momordica charantia* L.) is an empirically used plant as a traditional DM treatment medicine. It contains several active compounds such as charantine, flavonoids, and insulin-like protein called polypeptide-p, which has a mechanism to increase insulin secretion [6–8]. The combination of Snakehead Fish Powder (SFP) and Bitter Melon Extract (BME) with a dose of 300 mg/kgBW, respectively, can reduce the blood glucose levels of alloxan-induced rats at a dose of 150 mg/kgBW and reach blood glucose levels of 99.7 $\pm$ 50.4 mg/dL. After treatment, the results showed a decrease in blood glucose levels with the pretest blood glucose level of 583.7 $\pm$ 232.2 mg/dL. The finding indicates the potential effect of the combination of SFP and BME in lowering blood sugar levels and as an antidiabetic agent. However, the availability of snakehead fish and bitter melon in nature is contracting [3]. To overcome this problem, nanoparticle-based drugs are created because the formulation with enhanced oral bioavailability enables a dose reduction and has high drug payloads [9]. Thus, using a low dose of snakehead fish and bitter melon in the formulation can yield the desired effect, overcome the decrease in availability, and potentially reduce the occurrence of side effects.

The oral drug delivery system using natural ingredients frequently encounters problems related to solubility. Approximately 4 to 10% of new drug formulas have relatively low bioavailability. Generally, flavonoid-rich extracts like BME are soluble in water but difficult to penetrate lipid membranes due to their large molecular size, causing low bioavailability and efficacy [10]. Cosurfactants or solubilizers are typically employed to modulate the nanoemulsion droplet size and enhance the drug loading by dissolving the compound with a cosurfactant first [9]. Therefore, it is critical to determine the surfactant with suitable HLB to stabilize and facilitate the cosurfactant in the formulation [11]. In addition, proteins in the oral route of SFP also have low bioavailability due to the enzymatic degradation in the gastrointestinal tract and the poor penetration of the intestinal membrane, resulting in poor protein absorption [12, 13]. One of the efforts to overcome the low bioavailability is reducing the particle size of the drug to a nanometer. Currently, lipid-based nanoparticle formulations are preferred because the active substances contain active compounds with low permeability that can increase the bioavailability of active compounds in the body.

The Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) are forms of lipid-based nanoparticle formulation, namely isotropic mixtures of surfactants, cosurfactants,

and oil that spontaneously create nanoemulsions upon contact with gastrointestinal fluids and has the advantage of improving the solubility and bioavailability of drugs [9]. The combination of SFP and BME as an antidiabetic drug is classified as a novelty because it has not been investigated by previous studies, especially those associated with SNEDDS, which recently have become the most popular formulation method. Therefore, the combination of SFP and BME through the mechanism of SNEDDS needs to be carried out. The best formulation of SNEDDS combination of SFP and BME using the calculation of Hydrophilic-Lipophilic Balance (HLB) is expected to increase the bioavailability level by forming a layer that protects drugs from enzymatic degradation, increases permeability in the gastrointestinal tract, and increases solubility. This study aimed to determine the effect of the composition of surfactants, cosurfactants, and oils and their comparisons in the SNEDDS formula combined with SFP and BME on emulsification time, transmittance, and nanoemulsion droplet sizes.

2 Methods

2.1 Equipment and Materials

Centrifuge, glassware (Pyrex), hot plate, magnetic stirrer (Thermo Scientific Cimarec), micropipette (Socorex), analytical balance (Ohaus), particle size analyzer/PSA (Horiba SZ100), pH meter (Eutech Instruments), spectrophotometer (UV) Mini 1240 SHIMADZU), sonicator (2510 BRANSON), stirrer, and stopwatch was the equipment used in this study. The materials included SFP and BME from CV. Jadiid Herbs Solo, Tween 20 (Merck), Span 80 (Merck), glycerin (Merck), olive oil (Merck), phosphate buffer, Demineralized water, and aqua pro injection (API).

2.2 Preparation of SFP and BME

SFP and BME used in this study were from CV. Jadiid Herbs. BME was made by simplicia bitter melon extracted with maceration method using 70% ethanol as solvent. Then the liquid extract was evaporated with a rotary evaporator until a thick extract was obtained, then powdered. SFP was processed through a steaming process. The snakehead fish was cleaned and steamed for ± 1 h and then dried. After drying process, the snakehead fish was powdered and then sifted until powder [3].

2.3 Surfactant, Cosurfactant, and Oil Phase Selection

Sample 100 mg was dissolved into components of the solvent. The solution mixture was carried out using a magnetic stirrer. After that, the yield was centrifuged for 15 min. The supernatant of 5 mL was taken and dissolved in 10 mL of solvent. The concentration of dissolved compounds was determined using a spectrophotometer with the standard curve [15]. The experiments were performed in triplicate. The concentration of the compound in the solvent was selected as the initial compound content in the determination of drug loading. The solvent with the highest compound content was determined as the cosurfactant used in the SNEDDS formulation with the addition of a sample.

Table 1. SNEDDS Template Formulation

Formula Code	HLB	Ratio (Surfactant: Cosurfactant: Oil)	Tween 20 (mL)	Tween 80 (mL)	Span 80 (mL)	Glycerin (mL)	Olive Oil (mL)
F1	11	8:1:1	4.0	-	4.0	1.0	1.1
F2	11	8:1:1	-	5.0	3.0	1.0	1.1
F3	12	8:1:1	4.5	-	3.0	1.0	1.1
F4	12	8:1:1	-	5.3	2.2	1.0	1.1
F5	13	8:1:1	5.0	-	2.4	1.0	1.1
F6	13	8:1:1	-	6.0	1.5	1.0	1.1
F7	14	8:1:1	6.0	-	1.7	1.0	1.1
F8	14	8:1:1	-	7.0	1.0	1.0	1.1
F9	15	8:1:1	6.3	-	1.2	1.0	1.1
F10	15	8:1:1	-	7.4	0.0	1.0	1.1
F11	11	7:2:1	3.4	-	3.2	1.6	1.1
F12	11	7:2:1	-	4.0	2.6	1.6	1.1
F13	12	7:2:1	4.0	-	2.6	1.6	1.1
F14	12	7:2:1	-	5.0	2.0	1.6	1.1
F15	13	7:2:1	4.5	-	2.1	1.6	1.1
F16	13	7:2:1	-	5.3	1.0	1.6	1.1
F17	14	7:2:1	5.0	-	1.5	1.6	1.1
F18	14	7:2:1	-	6.0	1.0	1.6	1.1
F19	15	7:2:1	5.5	-	1.0	1.6	1.1
F20	15	7:2:1	-	6.5	0.0	1.6	1.1
F21	11	7:1:2	3.4	-	3.2	1.0	2.2
F22	11	7:1:2	-	4.0	2.6	1.0	2.2
F23	12	7:1:2	4.0	-	2.6	1.0	2.2
F24	12	7:1:2	-	5.0	2.0	1.0	2.2
F25	13	7:1:2	4.5	-	2.1	1.0	2.2
F26	13	7:1:2	-	5.3	1.0	1.0	2.2
F27	14	7:1:2	5.0	-	1.5	1.0	2.2
F28	14	7:1:2	-	6.0	1.0	1.0	2.2
F29	15	7:1:2	5.5	-	1.0	1.0	2.2
F30	15	7:1:2	-	6.5	0.0	1.0	2.2

2.4 SNEDDS Template Formulation

The SNEDDS formulation was initiated by formulating the SNEDDS template. The template was produced by calculating the ratio of surfactant components in the form of the HLB value of each desired surfactant in weight percent units. For each HLB value, three surfactant, cosurfactant, and oil ratios were prepared, namely 8:1:1, 7:2:1, and 7:1:2. Thirty formulas were made at room temperature, as shown in Table 1. The selected formula was characterized by emulsification time and transmittance value. The formula eligible was combined with SFP and BME. After 24 h of the absence of phase separation, a further evaluation was performed on the combined SFP and BME formula to identify emulsification time and transmittance value. The evaluation generated the best formula. This formula was characterized by the droplet size and the stability of the nanoemulsion [11].

The SNEDDS formula is prepared from the optimized composition of surfactants, cosurfactants, and oil to create an isotropic mixture. The materials consisted of olive oil, a combination of Tween 20, Tween 80, and Span 80 as a surfactant, and glycerin as a cosurfactant. The SNEDDS formula was produced by mixing Tween and Span (surfactants) according to their respective HLB calculations using a magnetic stirrer at 350 rpm for 10 min. Subsequently, glycerin (cosurfactant) was appended to the surfactant mixture and stirred for 10 min. Following that, olive oil was added dropwise into the mixture and stirred using a magnetic stirrer. The mixture was then sonicated for 15 min employing an ultrasonic homogenizer [11].

2.5 SNEDDS Template Evaluation

The mixtures made from thirty formulas were examined for stability by allowing them to stand at room temperature for 24 h and the phase separation was observed. The visually transparent and stable mixtures without phase separation were taken to identify emulsification time and transmittance. The mixture with the shortest emulsification time (less than 2 min) and the highest transmittance value (more than 90%) was combined with SFP and BME. The stable SNEDDS templates were emulsified in distilled water at a ratio of 1:1000. Stirring was carried out using a magnetic stirrer at 100 rpm at a temperature of 37 $\pm$ 0.5 °C. The auto-emulsification time was mandatory for the template to be completely dispersed in aqueous media. The auto-emulsification time was calculated from template insertion into the media to stop when a homogeneous nanoemulsion system was formed and completely dispersed. Subsequently, the transmittance was read at a wavelength of 650 nm [11, 16].

2.6 SNEDDS Formulation Combination of SFP and BME

Based on the evaluation of the SNEDDS template, several formulas were picked up to be combined with SFP and BME. The combination of 100 mg SFP and 100 mg BME (1:1) was dissolved in glycerin and stirred using a magnetic stirrer at 1,200 rpm. The mixture was centrifuged, and the supernatant was collected. The clear supernatant was added to the surfactant mixture while stirring the mixture using a magnetic stirrer at 350 rpm for 10 min. Consequently, olive oil was added dropwise to the mixture and stirred for 10 min. The mixture was sonicated for 15 min using an ultrasonic homogenizer [11].

2.7 Evaluation of SNEDDS Combination of SFP and BME

Several mixtures from various formulas were combined with SFP and BME. The best combination was characterized by the droplet size and evaluated for the its stability of the nanoemulsion. The selection of the optimum formula was following the evaluation of the SNEDDS combination of SFP and BME, including the evaluation of auto-emulsification time and transmittance value [11].

2.7.1 Auto-emulsification Time and Transmittance

The auto-emulsification capacity of SNEDDS combined with SFP and BME was surveyed. The duration of nanoemulsion was quantified once the SNEDDS was introduced into aqueous media until a homogeneous nanoemulsion system was successfully formed. The SNEDDS combined with SFP and BME was emulsified in the media of distilled water, artificial gastric fluid (AGF) buffer pH 1.2, and artificial intestinal fluid (AIF) phosphate buffer pH 6.8 at ratio 1:1000, at a temperature of 37 $\pm$ 0.5 °C. The media was stirred with a magnetic stirrer at 100 rpm. After that, the SNEDDS with distilled water was read for transmittance value at a wavelength of 650 nm. The experiments were performed in triplicate [11, 16].

2.7.2 Thermodynamic Stability

The SNEDDS formula combined with SFP and BME was subjected to thermodynamic stability tests, including centrifugation and freeze-thaw cycles. Demineralized water was added to the SNEDDS formula combined with SFP and BME at a ratio of 1:20. The mixture was centrifuged at 3,500 rpm for 30 min. The phase separation was monitored. After centrifugation, the stable SNEDDS formula was subjected to freeze-thaw cycles, consisting of freezing at -20 ± 2 °C and thawing at 25 °C (uncontrolled room temperature) for 48 h for two cycles. After 24 h of storage, any phase separation and precipitation activities were observed. Furthermore, the experiments were performed in triplicate [17, 18].

2.7.3 Phase Separation and Nanoemulsion Stability

The SNEDDS formula combined with SFP and BME was stored in two vials. Each vial contained 100 μL of SNEDDS combined with SFP and BME. Then, 10 mL of media demineralized water and AGF (buffer pH 1.2) was included into each vial and stored at room temperature. The mixture was vortexed for 1 min. Separation was observed after 24 h of storage. The experiments were performed in triplicate [17].

2.7.4 Dilution Testing

The SNEDDS formula combined with SFP and BME was diluted 100 times and 1000 times using distilled water, AGF (buffer pH 1.2), and AIF (phosphate buffer pH 6.8). The nanoemulsion was stored for 24 h and observed for precipitation and separation. The experiments were performed in triplicate [17].

2.7.5 Droplet Size, Polydispersity Index, and Zeta Potential

The droplet size, polydispersity index, and zeta potential of SNEDDS formula combined with SFP and BME were estimated using a particle size analyzer (PSA) at a temperature of 25 °C (uncontrolled room temperature). The eligible formula was added with Aqua Pro Injection (API) at a ratio of 1:1000. The experiments were performed in triplicate [19].

2.8 Drug Loading Efficiency

The SNEDDS formula of ± 5 mL was taken and diluted using a solvent of 10 mL. The solution was centrifuged for 15 min. Then, the supernatant was read for absorbance at maximum length wave using a spectrophotometer. The next step was to determine the drug loading efficiency [15].

3 Results and Discussion

3.1 Surfactant, Cosurfactan, and Oil Phase Selection

Olive oil is a group of vegetable oils that can create a sound emulsification system with the help of surfactants. The development of olive oil into stable forms, such as nanoemulsions, has great potential [14]. The choice of surfactant was based on the HLB value required to form an O/W nanoemulsion. The selected surfactant should display a good miscibility with other components in the SNEDDS formula to produce a stable and homogeneous system. The HLB method was used to predict the HLB value of the emulsion and to design a mixture ratio of two or more surfactants in order to produce a system with an HLB > 10. Tween was included because of its structural similarity to Span. To formulate the combination of surfactants with HLB ranging from 11 to 15, the HLB_{mix} of each surfactant was calculated [11, 14]. Glycerin was used as a co-solvent to reduce the interfacial tension to a minimum and even negative value. Therefore, glycerin was chosen as the cosolvent and cosurfactant in this study [11].

3.2 SNEDDS Template Formulation

The SNEDDS template encompassed 30 formulas generated using the HLB method in the mixture of surfactant: cosurfactant: oil in various ratios (8:1:1, 7:2:1, 7:1:2). The stability of the mixtures after 24 h storage at room temperature was monitored. The results are presented in Table 2. The parameter for formula selection was the absence of phase separation following 24 h of storage at room temperature. Based on the parameter, four formulas were selected and evaluated for emulsification time and transmittance. The formula with the shortest emulsification time (less than 2 min) and the highest transmittance value (above 90%) was selected to be combined with SFP and BME.

Table 2. SNEDDS Template Formula (stable after 24 h)

Formula Code	HLB	Surfactants	Surfactant: Cosurfactant: Oil	Description
F5	13	Span 80, Tween 20	8:1:1	not separated
F8	14	Span 80, Tween 80	8:1:1	not separated
F9	15	Span 80, Tween 20	8:1:1	not separated
F27	14	Span 80, Tween 20	7:1:2	not separated

Table 3. SNEDDS Template: Emulsification Time and Transmittance

Formula Code	HLB	Emulsification Time	Transmittance (%)	Description
F5	13	4 min 56 s	75.7	Cloudy
F8	14	1 min 42 s	96.4	Transparent
F9	15	1 min 32 s	98	Transparent
F27	14	3 min 33 s	58.9	Cloudy

3.3 SNEDDS Template Evaluation

The evaluation of the SNEDDS template in form of auto-emulsification time and transmittance values is presented in Table 3. It was conducted on the four selected formulas. The stability of the mixture was noticeable from the absence of phase separation in the mixture. Based on the evaluation of the SNEDDS template, including the evaluation of emulsification time and transmittance value, the template formulas that fulfilled the criteria of the shortest emulsification time (less than 2 min) and the highest transmittance values were F8 and F9. These two formulas were taken to form an optimized SNEDDS comprising SFP and BME [11].

Based on Table 3, the higher the HLB value, the greater the transmittance value is. The higher the transmittance value, the clearer the generated mixture and the smaller the droplet size is. It also indicates that the HLB value influences the hydrophilicity of the SNEDDS and the voltage drop at the oil interface, thereby increasing the solubility and shrinking the droplets. The emulsification time is mediated by surfactants and cosurfactant that can form oil and water interface [11].

3.4 Preliminary Evaluation of the SNEDDS Comprising SFP and BME

An initial evaluation of the SNEDDS formulation with the combination of SFP and BME, including the evaluation of emulsification time and transmittance value, was performed

Table 4. SNEDDS Template of Combination of SFP and BME: Emulsification Time and Transmittance

Formula Code	HLB	Emulsification Time	Transmittance (%)	Description
F8	14	1 min 35 s	93.3 ± 1.12	Cloudy
F9	15	1 min 21 s	98.2 ± 1.05	Transparent

Table 5. Results of Phase Separation and Nanoemulsion Stability Test

Formula Code	Media			Description
	Demineralized Water	AGF (pH 1.2)	AIF (pH 6.8)	
F9	No Sediment	No Sediment	No Sediment	Clear

and presented in Table 4. Based on the results, the SNEDDS comprised SFP and BME with the selected formula code F9 with shortest emulsification time and higher transmittance value was chosen for the thermodynamic stability test, phase separation, dilution, emulsification time in various media, transmittance value, nanoemulsion droplet size, and zeta potential size using PSA.

Emulsification time is the required time period of the nanoemulsion formation process in the body upon mild agitation similar to the movement in the gastrointestinal tract. Surfactants and cosurfactants mediate emulsification time in the SNEDDS formula that forms oil and water interface. Meanwhile, a high transmittance value indicates that the generated solution is transparent with droplet size in the nanometer range. The formula that exhibits a high transmittance value is a formula with a value of 15. The higher the HLB value, the higher the hydrophilicity is, thus allowing the formula to disperse well in water [11].

3.5 Evaluation of SNEDDS for the Combination of SFP and BME

The phase separation and stability test of nanoemulsion are presented in Table 5. The tests were carried out using three different media, including demineralized water, AGF (buffer pH 1.2), and AIF (buffer pH 6.8). The test showed the absence of phase separation or sediment following 24 h of storage at uncontrolled room temperature. It indicated the stability of the nanoemulsion, and the differences in pH showed no effect on the nanoemulsion [11].

Table 6 shows that no sedimentation occurred on the nanoemulsion formula (F9) after 24 h storage on the three media. The result indicates that the F9 will not undergo phase separation when passing through gastrointestinal fluids with different pH and volumes. The SNEDDS formula was diluted in media 100 times and 1000 times dilution. The variation in the volume of three media illustrates the variation in the volume of the body,

Table 6. Results of Dilution Testing

Formula Code	Times Dilution	Media		
		Demineralized Water	AGF (pH 1.2)	AIF (pH 6.8)
F9	100 times	No deposit	No deposit	No deposit
	1000 times	No deposit	No deposit	No deposit

Table 7. Emulsification time in various media

Formula Code	Media		
	Demineralized Water (sec)	AGF pH 1,2 (sec)	AIF pH 6,8 (sec)
F9	102 ± 10.5	24 ± 11.3	77 ± 17.0

for instance, while eating and fasting, in which the formula will experience gradual dilution [11].

Based on Table 7, the emulsification time for nanoemulsion formation in various media was not significantly different. The SNEDDS formula could emulsify within 1 min in all three media. Based on the assessment of the efficiency of emulsification time, the combination of SFP and BME using the F9 is classified in Grade A, namely the emulsification time of 1 min with a transparent appearance. The use of different media was carried out to determine the effect of pH on the emulsification time. The medium of AGF with buffer pH 1.2 was the simulation of gastric fluid, while the medium of AIF with buffer pH 6.8 was the simulation of intestinal fluid [11].

The observation of emulsification time was done to ascertain the required period for the formation of SNEDDS after oral administration. It represents the duration for SNEDDS to form a homogeneous nanoemulsion and good dispersion when introduced into an aqueous medium under light agitation. The required time was calculated once the SNEDDS for the combination of SFP and BME was introduced into an aqueous medium until a homogeneous nanoemulsion system was formed. After emulsification in distilled water, the transmittance value of the formula was established using a spectrophotometer at a wavelength of 650 nm with three repetitions. The results are presented in Table 8. The high transmittance value indicates the transparency level of the formula. The transmittance value above 80% implies that the nanoemulsion droplet size is less than 200 nm. The formula with a high transmittance value is a formula with a value of 15. The higher the HLB value, the higher the hydrophilicity of the formula is [11].

The results of the thermodynamic stability test showed that the F9 remained stable after centrifugation at 3,500 rpm. The unavailability of visible precipitate after centrifugation indicates the protein in the SNEDDS system [15]. The nanoemulsion formula

Table 8. Result of transmittance value

Formula Code	Transmittance (%)	Description
F9	98.2 ± 1.05	Clear

Table 9. Nanoemulsion droplet size, PI, and Zeta Potential

Formula Code	Droplet Size (nm)	Zeta Potential (mV)	PI
F9	212.4 ± 58.10	−40.4	0.44 ± 0.07

was cloudy after storage at −20 ± 2 °C and 25 °C (uncontrolled room temperature). This turbidity indicates the instability of the F9 even though neither phase separation nor precipitation occurs.

At a storage temperature of −20 °C, the hydrophilic group of the surfactant of F9 generally froze, causing the cloudy appearance of the frozen nanoemulsion at −20 °C. Turbidity occurred because the hydrophilic group on the surfactant froze and was unable to cover the oil phase. The appearance of the nanoemulsion at room temperature after freezing was even cloudier due to extreme temperature changes in the freeze-thaw cycles. At a temperature of 25 °C (uncontrolled room temperature), the hydrophilic group of the surfactant returned to its original state and was able to cover the oil phase, however unable to completely cover the oil phase, resulting in a cloudier nanoemulsion. In addition, the storage temperature of −20 °C led to an interaction between the droplets, resulting in the formation of larger and cloudier droplets when returning at a temperature of 25 °C (room temperature). The test showed the aggregation of the nanoemulsion, but the absence of precipitate also indicated the protein had been loaded in the SNEDDS. However, the stability of the nanoemulsion was relatively poor [15, 20].

The analysis results of droplet size, polydispersity index, and zeta potential are listed in Table 9. The SNEDDS sample combined with SFP and BME was emulsified in aqua pro-injection (API) medium first at a ratio of 1:1000 before being analyzed using a Particle Size Analyzer. The use of API in this test aims to avoid bias during measurement due to the presence of foreign particles.

The average droplet size in the SNEDDS formula combined with SFP and BME (F9) was classified appropriate, of 100–500 nm [21]. The formation of droplet size in the nanoscale was inseparable from the surfactant's role, reducing the oil-water interface tension and the part of cosurfactants that formed the oil-water interface layer [22]. Generally, the smaller the droplet size, the larger the absorption area and the faster the drug is released. The polydispersity index value is the standard deviation of the average droplet size, indicating the formulation's uniformity of droplet size. The polydispersity index value of less than 1 signifies a narrow droplet size distribution, showing the uniformity of the droplet size in the nanoemulsion [11].

Zeta potential was utilized to determine the surface charge of the droplets in the nanoemulsion. It becomes one of the critical parameters to estimate the stability of nanodispersion systems. The zeta potential value implied the repulsion between the identically charged particles close to each other in a dispersion system. Particle aggregation was prevented by the zeta potential, in which the more significant the repulsion, the greater the dispersion stability is. Nanoemulsions should possess a zeta potential above +30 mV or less than −30 mV to prevent coalescence [11, 21]. The zeta potential of the F9 indicated that the formula was stable and that the coalescence probability was low.

3.6 Drug Loading Efficiency

Drug loading efficiency was carried out to find out the levels of compounds from samples that entered the SNEDDS system [15]. The resulting drug loading efficiency value did not meet less than 90% of the desired criteria. The results of drug loading efficiency showed ± three-fifths of the number of compounds that entered the SNEDDS system. The effect of solubility and comparison of oil composition used in the formula is a factor that can affect the amount of drug loading produced. The more sample dissolved in the carrier, the larger the sample that can be carried.

4 Conclusion

Based on the study results, as a conclusion, the selected SNEDDS formula with the combination of SFP and BME had an emulsification time of less than 2 min, a transmittance of 98.2 ± 1.05%, and droplet sizes of 212.4 ± 58.10 nm.

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