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Original Article

FORMULATION AND CHARACTERIZATION OF SELF NANO-EMULSIFYING DRUG DELIVERY SYSTEM (SNEDDS) FRACTION OF N-HEXANE: ETHYL ACETATE FROM SESEWANUA LEAF (*CLERODENDRUM FRAGRANS* WILD.)

ZULFIAYU SAPIUN[1,2*], ARLAN K. IMRAN[1,2], SISILIA TERESIA ROSMALA DEWI[3], DHEA FADILA MASITA PADE[1], WIDYAWATI IBRAHIM[1], ROBERT TUNGADI[4], WIDY SUSANTI ABDULKADIR[4], YOS BANNE[5], SARTINI SARTINI[6], ANDI DIAN PERMANA[6], YUSNITA RIFAI[6], YSRAFIL YSRAFIL[7], NANGSIH SULASTRI SLAMET[1,2]

[1]Department of Pharmacy, Health Polytechnic of Gorontalo, Indonesia, [2]Center of Excellent for Cardiovascular and Endocrine, Health Polytechnic of Gorontalo, Indonesia, [3]Department of Pharmacy, Health Polytechnic of Makassar, Indonesia, [4]Department of Pharmacy, State University of Gorontalo, Indonesia, [5]Department of Pharmacy, Health Polytechnic of Manado, Indonesia, [6]Department of Pharmacy, Hasanuddin University, Indonesia, [7]Faculty of Medicine, Universitas Palangka Raya, Palangka Raya, Indonesia
*Email: zulfiayu@poltekkesgorontalo.ac.id

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ABSTRACT

Objective: Sesewanua leaves contain alkaloid compounds as antioxidants, and its leaves can be used to formulate SNEDDS dosage forms, which can effectively deliver the medicine.

This study intended to determine the variation of surfactant concentration (Tween 80) and cosurfactant (PEG 400) towards pH, viscosity, nano-emulsion duration and characterization using PSA method (particle size and polydispersity index).

Methods: This study employed a quasi-experimental method and the independent variables in this study were variations in the concentration of surfactant (Tween 80) and cosurfactant (PEG 400), which consist of 3 formulas, such as SFS 1 (6:3), SFS 2 (7:2), and SFS 3 (8:1). The dependent variables in this study including pH, viscosity, nano-emulsion time, particle size and polydispersity index which utilized One Way Anova Post Hoc LSD ($p>0.05$) and Tamhane ($p<0.05$) tests as the data analysis.

Results: The pH test SFS1-SFS3 has a pH value of 7.92, 8.30 and 8.35, followed by Viscosity test SFS1-SFS3, which has a viscosity value of 1.00 cP, 1.38 cP and 2.91 cP. Further, the SFS1-SFS3 nano emulsified time test had nano emulsified time in gastric and intestinal fluids 35.18s and 43.96s, 43.54s and 47.13s and 44.00s and 50.29s. Characterization of SFS1-SFS3 particle size in gastric and intestinal fluids 23.9 nm and 23.0 nm, 18.5 nm and 22.7 nm and 19.1 nm and 22.9 nm, while characterization of SFS1-SFS3 polydispersity index in gastric and intestinal fluids were 0.433 and 0.348, 0.451 and 0.440 and 0.568 and 0.462.

Conclusion: The increase of variations in surfactant concentration and decreased cosurfactant significantly affected pH, viscosity, nano-emulsion time, and particle size of SFS preparations. However, the polydispersity index was not considerably affected.

Keywords: Surfactant, Polydispersity index, Particle size, Inflammation, Rheumatoid arthritis

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INTRODUCTION

Inflammation is derived from inflammatory reactions were injured and occur occurs because of the invasion of swelling and pain [1]. One rheumatoid arthritis and inflammatory diseases, Rheumatoid arthritis (RA) is a cause of chronic inflammatory joint steering. A corticosteroid orally in the low dose is part of the treatment of RA but it should be noted. caused side effects the use of (oains) drug antiinflamasi nonsteroidal has adverse side effects erosion the stomach on the use of long-term. Oains do not affect the course of a disease or prevent mischief. Joints Oains are only capable of relieving the pain of RA. Because it needs to be an alternative treatment that could be a choice in the treatment of RA [2]. One of the plants that have the potential for the treatment of RA is sesewanua.

Empirically plant Sesewanua (*Clerodendrum fragrans* Wild.) has been used by the community for the treatment of various diseases. Part of a plant sesewanua. leaves often used is extract ethanol leaves sesewanua has tested emulgel antiinflamasi activity in the form of preparation that can hinder inflammation in mice by 34 % with the concentration of ethanol leaves extract sesewanua 500 ppm. Sesewanua leaves have also been tested in the form of fractions obtained from the ethanolic extract of Sesewanua leaves, found the most active fraction containing alkaloids with an Rf value of 0.9 which has potential as an antioxidant, namely in fraction 1 (9:1, 8:2, 7: 3, 6:4) n-Hexane: ethyl acetate has high antioxidant activity with an IC50 value of 2.5 ppm with a fraction concentration of 500 ppm, this fraction can be categorized as a very strong antioxidant because the smaller the IC_{50} value, the higher the antioxidant activity [3–7]. The mechanism of alkaloids as antioxidants is by donating H atoms to free radicals. This mechanism indicates that the alkaloids work as primary antioxidants [8]. It is important to achieve the therapeutic effect of utilizing the high-effectiveness antioxidant content of the n-Hexane: ethyl acetate fraction of Sesewanua leaves, namely to formulate it into the Self Nano-emulsifying Drug Delivery System (SNEDDS).

SNEDDS is a nanoparticle that can improve the solubility of a compound to increase drug penetration to reach the target. The improvement of the alkaloid absorption process in the SNEDDS formula is expected to increase the bioavailability of the drug so that it is more effective as an anti-inflammatory RA and can be commercially profitable. SNEDDS preparations also have high stability compared to nanoemulsion preparations. SNEDDS can be further formulated into soft capsules with good SNEDDS characterization requirements [9–13]. One of the factors for the formation of a good SNEDDS is the correct composition of surfactants and cosurfactants.

The characterization of SNEDDS preparations can be influenced by the constituent components consisting of oil, surfactant and cosurfactant.

Surfactants are substances that in their molecular structure, have lipophilic and hydrophilic parts that can reduce surface tension, while cosurfactants are emulsifiers that help surfactants maintain stability in the film layer between oil and water [14, 15].

Based on the results of research by [16], showed that the concentration of Tween 80 as a surfactant and PEG 400 as a cosurfactant (6:3) had a particle size of 12.4 nm, zeta potential 30.8 mV, nano emulsified time 49.55 seconds and percent transmittance 97.7%. Another study from [12], succeeded in showing the concentration of Tween 80 and PEG 400 (7:2) having a particle size of 46.1 nm, zeta potential 0.46 mV, emulsification time of 47.44 seconds, and percent transmittance 99.3%. The latest research by [17], succeeded in showing that the concentration of Tween 80 and PEG 400 (8:1) had a particle size of 15.06 nm, a zeta potential of-14.3 mV, a nano-emulsion time of 24.93 seconds and a percent transmittance of 98.6%.

Based on the description above, the researchers wanted to formulate the n-Hexane: ethyl acetate fraction of Sesewanua (*Clerodendrum fragrans* Wild.) leaves in the SNEDDS preparation for the treatment of Rheumatoid Arthritis using various concentrations of surfactants and cosurfactants (6:3), (7:2), (8:1) to see the physical preparation of SNEDDS produced.

MATERIALS AND METHODS

Material

The tools used are watch glass, Pyrex glass tool®, dropper, maceration jar, acid cupboard ESCO Frontier®, stirring rod, column, vial, horn spoon, spatula, Thermo Scientific® magnetic stirrer, Mettler Toledo Multi Parameter® pH meter, Krisbow® digital ultrasonic and Sartorius®analytical balance.

Plant material

The ingredients used are Sesewanua leaf (*Clerodendrum fragrans* Wild.) that was picked up form Talaga Biru village, 96% ethanol (Pharmapreneur-store), ethyl acetate (ProShied), VCO (Beorganikshop), tween 80 (Pharmapreneur-store), PEG 400 (Pharmapreneur-store), sand, cotton, filter paper, citric acid (Pharmapreneur-store), phosphoric acid (Pharmapreneur store), n-Hexane (ProShied), Silica gel_{60}, sodium citrate (Pharmapreneur-store), sodium phosphate (Pharmapreneur-store), sodium chloride (Pharmapreneur-store).

Ethical approval

Before conducting the research, the research protocol was approved by the Ethics Committee of the Health Polytechnic of the Ministry of Health of Gorontalo with No. LB. 01.01/KEPK/152/2022.

SNEDDS preparation

This study refers to the best formula from three studies, namely the research of [17] with the title of formulation, characterization and evaluation of the Self-Nano Emulsifying Drug Delivery System (SNEDDS) ethanol extract of pineapple peel as an antibacterial Streptococcus mutans, [12] entitled Optimization of Tween 80 and Polyethylene Glycol 400 in Self-Nanoemulsifying Drug Delivery System (SNEDDS) Basil (Ocimum basilicum) Leaf Essential Oil Nanoemulsifiying Drug Delivery System (SNEDDS) Basil Essential Oil (Ocimum basilicum) and research by [16] with the title Development of Kersen Leaf Extract Nanoparticles (*Muntingia calabura* L.) with Self Nano Emulsifying Drug Delivery System (SNEDDS) Technique for Antibacterial Applications. Researchers will compare the best formulas from the three studies and use the active substance of the n-Hexane: ethyl acetate fraction of Sesewanua leaves (*Clerodendrum fragrans* Wild.) with variations in surfactant and cosurfactant concentrations, namely SFS1 (6:3), SFS2 (7:2) and SFS3 (8:1) to produce preparations that are by the quality of the physical stability of the preparation. The design formula in this study can be seen in table 1.

SNEDDS preparation components

Active substance

n-hexane: ethyl acetate fraction was obtained from the ethanol extract of sesewanua leaves, which was used as the active substance with a concentration of 500 ppm, which had a high antioxidant activity with an IC50 value of 2.5 ppm.

Table 1: Design formula

Ingredient	Concentration (g)		
	SFS1	SFS2	SFS3
Fraction n-Hexane: ethyl acetate sesewanua leaf extract	0.005	0.005	0.005
VCO	1	1	1
Tween 80	6	7	8
PEG 400	3	2	1

SFS1: *Sesewanua* SNEDDS with 6g Surfactant and 3g Co-surfactant, SFS2: *Sesewanua* SNEDDS with 7g Surfactant and 2g Co-surfactant, SFS3: *Sesewanua* SNEDDS with 8g Surfactant and 1g Co-surfactant

Oil phase

VCO is widely chosen as a phase oil in nanoemulsion formulations because VCO contains medium-chain fatty acids so that it is easier to emulsify and can produce nanometer-sized preparations.

Surfactant

Tween 80 is used as a surfactant in SNEDDS preparations, where tween 80 is an emulsifier of a nonionic surfactant type which has the advantage of being nontoxic and non-irritating [18].

Cosurfactant

PEG 400 was used as a co-surfactant by showing good stability in the SNEDDS formula, which was characterized by the non-separability of the mixture of oil, surfactant and co-surfactant.

Preparation of SNEDDS

The preparation of this preparation using the high-pressure homogenization (HPH) method of making high-pressure nanoemulsion homogenizers using a hot plate with a magnetic stirrer into the emulsifier mixture is carried out in stages at a temperature of 50 °C [19].

Mixed the oil phase (VCO) and the active substance (n-Hexane fraction: ethyl acetate sesewanua leaf extract) with the help of a magnetic stirrer at 400 rpm for 5 min, then added surfactant (Tween 80) and stirred with a magnetic stirrer at 400 rpm for 5 min, added co-surfactant (PEG 400) and then stirred with a magnetic stirrer at 400 rpm [20].

Physical evaluation of preparations

pH test

According to [12] the pH meets the requirements of the SNEDDS preparation, namely pH 1.2-7.4. pH measurements were carried out using a pH meter with electrodes calibrated with standard buffers of pH 4 and pH 7 [21, 22].

Viscosity test

This test is carried out using a viscometer and the viscosity measurement starts when the rotor needle moves and is stable. The

trick is to fill the tube with SNEDDS and then set it in a capillary tube to the line limit with the help of pressure or suction. Open the capillary tube to allow SNEDDS to flow freely. The time required for SNEDDS to flow from the upper limit to the lower limit of the capillary tube was recorded in seconds [23].

Nano emulsified time

Emulsification was carried out in citrate buffer pH 3.5 (stomach) and phosphate buffer pH 6.6 (intestine). This test was carried out to obtain an overview of the ease with which SNEDDS can form emulsions while in the body (Syukri *et al.*, 2018). A good emulsification time for SNEDDS is less than 1 minute [9].

Particle size

A good nanoemulsion particle size is less than 100 nm; the smaller the particle size of an active substance in the SNEDDS preparation will further increase its stability and distribution in the dissolution medium [24, 25].

Polydispersity index

Lower the polydispersity index value, the higher the uniformity of globule size in the preparation. The polydispersity index describes the uniformity distribution of globules in the nanoemulsion. A good polydispersity index has a value below 0.5, while a value above 0.5 indicates that the globule distribution is non-uniform [26].

Data analysis

The data for each test preparation with pH, viscosity, nano emulsified time as well as particle size and polydispersity index using a Particle Size Analyzer. Arranged in a table by clearly describing the results of the n-Hexane: ethyl acetate fraction of Sesewanuwa leaves, analyzed by One Way ANOVA (Analysis of Variance) method using SPSS 16.0.

RESULTS AND DISCUSSION

Rheumatoid arthritis (RA) is the most common cause of chronic joint inflammation. One of the plants that have the potential for the treatment of RA is sesewanua. Empirically, the sesewanua plant (Clerodendron fragrans Wild.) has been used by the community for the treatment of various diseases. Sesewanua plant parts that are often used are leaves. Sesewanua leaves have also been tested in the form of fractions obtained from the ethanolic extract of Sesewanua leaves, found the most active fraction containing alkaloids with an Rf value of 0.9 which has potential as an antioxidant, namely in fraction 1 (9:1, 8:2, 7:3, 6:4) n-Hexane: ethyl acetate has high antioxidant activity with an IC_{50} value of 2.5 ppm with a fractional concentration of 500 ppm, this fraction can be categorized as a very strong antioxidant. Strong because the smaller the IC_{50} value, the higher the antioxidant activity [6]. The use of antioxidants in this fraction can be developed into the Self Nano-Emulsifying Drug Delivery System (SNEDDS). SNEDDS is a preparation consisting of active substances, surfactants, co-surfactants and oils.

pH test

Table 2: pH test results SFS

Composition	Formula	pH value
VCO: Tween 80: PEG 400	SFS 1 (1:6:3)	7.92±0.04
	SFS 2 (1:7:2)	8.30±0.05*
	SFS 3 (1:8:1)	8.35±0.06*

Description: -*Significantly Different value Against SFS1 (level 0.05), Data represents mean±SD (n=3)

Tween 80 has a pH of 6.8–8.0, while PEG 400 has a pH of 4.0–7.5 [18]. Based on [27] the less variation in the concentration of PEG 400, the pH of the SNEDDS preparation will be more alkaline this is because PEG 400 is acidic. Meanwhile, according to [12, 28], SNEDDS preparations with a large concentration of tween 80 will increase the pH value of the preparation because tween 80 is alkaline. This is in line with the pH results in table 2, it can be seen that the pH test results for SFS preparations have an average pH value of SFS1 7.92, SFS2 8.35 and SFS3 8.30 according to the results of [29], the SNEDDS formulation can maintain stability. nanoemulsion in acidic pH, alkaline pH and the influence of electrolytes in the gastrointestinal tract.

Based on the One Way Anova test using SPSS 16.0, the homogeneity value of significance was 0.702>0.05 and continued with the LSD test; the significance value was<0.05. This means that there is a significant difference in the increase in pH, it can be interpreted that SFS1 to SFS2 with variations in the concentration of surfactant increase and decrease in cosurfactant affect the increase in pH. Meanwhile, for SFS2 to SFS3, the value is not significant>0.05. This means that there is no significant difference in the increase in pH, it can be interpreted that variations in the concentration of surfactant increase and decrease in cosurfactant do not affect increasing pH.

Viscosity test

Table 3: Viscosity test results SFS

Composition	Formula	Viscosity (cP)
VCO: Tween 80: PEG 400	SFS 1 (1:6:3)	1.000±0.12
	SFS 2 (1:7:2)	1.384±0.25
	SFS 3 (1:8:1)	2.915±0.52*

Description: -*Significantly different value against SFS1 (level 0.05), Data represents mean±SD (n=3)

A good standard of viscosity for SNEDDS preparations is less than 1,00 cP [14]. The mixing temperature of the material is a factor that can affect the viscosity value. According to [30], the process of mixing materials using low temperatures can increase the viscosity value. The choice of the type of surfactant is also one of the factors that can affect the viscosity value. Tween 80, which is used as a surfactant in the formulation of SNEDDS preparations with a concentration that is increasing, can increase the viscosity value of the preparation; this is because the greater the concentration of tween 80 will decrease the size of the globule diameter so that it will increase the surface area and resistance of the nanoemulsion to flow and increase the viscosity value [21]. Meanwhile, based on the results of the study [27], the use of a single surfactant (tween 80) made the viscosity increase, whereas when cosurfactant was added, there was an interaction between tween 80 and PEG 400 to make the viscosity of the preparation stable. This is the same as the mixing temperature, surfactants and cosurfactants used with the results obtained in table 3, namely SFS1 1.000 cP, SFS2 1.384 cP and SFS3

2.915 cP all formulas have increased viscosity but are still within the standard.

Based on the One Way Anova test, the homogeneity value was not significant, namely 0.002<0.05 and continued with the Tamhane test; the significance value was<0.05. This means that there is a significant difference in the increase in viscosity, it can be interpreted that SFS1 to SFS2 and SFS2 to SFS3 with variations in the concentration of surfactant increase and decrease in cosurfactant affect the increase in viscosity.

Nano-emulsified time test

Table 4: Emulsified time test results SFS

Composition	Formula	Emulsified time (Second)	
		Gastric fluid	Intestinal fluid
VCO: Tween 80: PEG 400	SFS 1 (1:6:3)	35.18±5.35	43.96±0.65
	SFS 2 (1:7:2)	43.54±1.80	47.13±1.26*
	SFS 3 (1:8:1)	44.00±2.27	50.29±0.81*

Description: -*Significantly different value against SFS1 (level 0.05), Data represents mean±SD (n=3)

To obtain an overview of the ease with which SNEDDS forms an emulsion while in the gastrointestinal tract where there is a stomach and intestines in the gastrointestinal tract, a nano-emulsified time test was carried out on gastric fluid and artificial intestinal fluid [24]. A good standard of emulsion time for SNEDDS preparations is less than 1 minute [12]. The nano-emulsion time that is more than standard causes a decrease in the absorption rate of the drug [31]. Meanwhile, according to [32], the faster the nano-emulsion time, the higher the absorption of the drug. The addition of tween 80 and PEG 400 also affected the nano-emulsion time. A good emulsified time is produced by a formula with a decreased concentration of PEG 400, which has a faster emulsifying time [33]. Meanwhile, based on [34] research, increasing variations in the concentration of tween 80 can also increase the nano-emulsified time produced. PEG 400 will slip and form a space between the tween 80 so that the structure is more swollen but has high fluidity and can form nanoemulsions faster [35]. This is in line with the results obtained in table 4; namely all formulas have good emulsified times in gastric and intestinal fluids, respectively, SFS1 35.18 seconds and 43.96 seconds, SFS2 43.54 seconds and 47.13 seconds and SFS3 44.00 seconds and 50.29 seconds.

Based on the One Way Anova test, the homogeneity value was not significant in gastric fluid, namely 0.001<0.05 and continued with the Tamhane test; the significance value was 0.05. This means that there is no significant difference in the increase in nano-emulsified time in gastric fluid, it can be interpreted that variations in the concentration of increasing surfactant and decreasing cosurfactant do not affect increasing emulsified time in gastric juice.

For the One Way Anova test on intestinal fluid, the homogeneity value was not significant, namely 0.009<0.05 and continued with the Tamhane test; the significance value was<0.05. This means that there is a significant difference in increasing the nano emulsified time in intestinal fluid, it can be interpreted that SFS1 against SFS2 and SFS2 against SFS3 with variations in surfactant concentration increasing and decreasing cosurfactant affect the increase in emulsified time in intestinal fluid.

Particle size test

Table 5: Particle size test results SFS

Composition	Formula	Particle size (nm)	
		Gastric fluid	Intestinal fluid
VCO: Tween 80: PEG 400	SFS 1 (1:6:3)	23.9±1.61	23.0±0.23
	SFS 2 (1:7:2)	18.5±0.56	22.7±0.49*
	SFS 3 (1:8:1)	19.1±1.01	

Description: -*Significantly different value against SFS1 (level 0.05), data represents mean±SD (n=3)

Polydipsia index test

Table 6: Polydipsiatity index test results

Composition	Formula	*Polydispersity Index* (PI)	
		Intestinal fluid	Gastric fluid
VCO: Tween 80: PEG 400	SFS 1 (1:6:3)	0.348±0.09	0.433±0.01
	SFS 2 (1:7:2)	0.440±0.02	0.451±0.02*
	SFS 3 (1:8:1)	0.462±0.03	0.568±0.04*

Description: -*Significantly different value against SFS1 (level 0.05), Data represents mean±SD (n=3)

Based on table 5, the results of the particle size test show that formula 2 produces smaller particle sizes than formulas 1 and 3. This is influenced by the concentration of surfactant and cosurfactant 7:2. According to [36], the use of a 7:2 concentration of cosurfactant combination surfactant can reduce interfacial tension because surfactants will enclose oil droplets when emulsified in water so that they will form a nanometer size. The particle size of SNEDDS is in the range of less than 100 nm with a polydispersity index (PI) value of nano-emulsion droplets less than 1 [24, 37, 38]. A polydispersity index value close to 0 indicates homogeneous particle size dispersion, while a polydispersity index of more than 0.5 indicates high heterogeneity. Samples with a polydispersity index value >0.7 have a very wide size distribution. The smaller the polydispersity index number, the more uniform the particle size because if the difference in size between particles is greater, the results will affect the characterization of stable nanoemulsion particles with uniform particle size and clear, homogeneous and yellow SNEDDS preparations [39]. To produce SNEDDS preparations

that have uniform particle size and small PI numbers, this can be influenced by variations in surfactant (tween 80) and cosurfactant (PEG 400) concentrations.

Tween 80 with higher concentrations resulted in uniform particle size and small PI numbers [12]. Meanwhile, based on the research of [40], the use of PEG 400 as a cosurfactant with lower concentration variations, the smaller the PI number and uniform particle size. This is because the increasing concentration of tween 80 and decreasing concentration of PEG 400 can reduce the interfacial tension between the oil-in-water layers, which can produce nanoemulsions that have a very wide particle size distribution [38, 41]. This is compared with the particle size of SFS preparations in intestinal fluid and gastric fluid with the average results of SFS1 23.0 nm and 23.9 nm, SFS2 22.7 nm and 18.5 nm and SFS3 22.9 nm and 19.1 nm all formulas have a particle size of<100 nm. Meanwhile, for the polydispersity index in gastric and intestinal fluids, the average results were SFS1 0.433 and 0.348, SFS2 0.451 and 0.440 and SFS3 0.568 and 0.462. All formulas had a PI value close to 0, which indicates homogeneous particle size.

Based on the One Way Anova test, it was found that the homogeneity value was not significant in gastric fluid, namely 0.008<0.05 and continued with the Tamhane test, the significance value was<0.05. This means that there is a significant difference in the decrease in particle size in gastric fluid, it can be interpreted that SFS1 against SFS2 with variations in the concentration of surfactant increase and decrease in cosurfactant affect the decrease in particle size in gastric fluid. Meanwhile, for SFS2 to SFS3, the value is not significant>0.05. This states that there is no significant difference in the increase in particle size in gastric fluid, so it can be It was concluded that the variation of surfactant concentration increasing and decreasing cosurfactant did not affect the increase in particle size in gastric fluid.

For the One Way Anova test, the homogeneity value was not significant in the intestinal fluid, namely 0.022<0.05 and continued with the Tamhane test; the value was not significant>0.05. This indicates that there is no significant difference in the decrease and increase in particle size in intestinal fluid, it can be stated that SFS1 against SFS2 and SFS2 against SFS3 with variations in surfactant concentration increasing and decreasing cosurfactant do not affect the decrease and increase in particle size in intestinal fluid.

For the polydispersity index of gastric fluid using the One Way Anova test, the homogeneity value was not significant, namely 0.003<0.05, then the Tamhane test was continued. obtained an insignificant value>0.05. This means that there is no significant difference in increasing the polydispersity index in gastric fluid, it can be interpreted that SFS1 against SFS2 and SFS2 against SFS3 with variations in the concentration of increasing surfactant and decreasing cosurfactant do not affect decreasing and increasing the polydispersity index in gastric juice. Meanwhile, in the intestinal fluid, the non-homogeneity value was 0.003<0.05 and continued with the Tamhane test, the value was not significant>0.05. This means that there is no significant difference in increasing the polydispersity index, it can be interpreted that SFS1 to SFS2 with variations in the concentration of surfactant increase and decrease in cosurfactant affect the increase in the polydispersity index of intestinal fluid. Meanwhile, for SFS2 to SFS3, the significance value is<0.05. This means that there is a significant difference in increasing the polydispersity index in intestinal fluids, it can be interpreted that variations in the concentration of surfactant increase and decrease in cosurfactant affect increasing the polydispersity index in intestinal fluid.

CONCLUSION

Based on the results of variations in surfactant concentration, increasing and decreasing cosurfactant have a significant effect on pH, viscosity, nano emulsified time and particle size of SFS preparations but have no significant effect on polydispersity index of SFS preparations. The best formula results were SFS1 in testing pH, viscosity, emulsified time and polydispersity index and SFS2 is best at particle size testing.

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AUTHORS CONTRIBUTIONS

ZS and AI were involved in planning and supervised of the work, ZS and AD drafted the manuscript and designed the figures, ST and WS aimed in data analysis, DF and WI collect the sampel and performed the measurement, RT and YB formulated the drug, SS performed the calculation, AD performed the characterization, YR and YY aided in interpreting the results and worked on the manuscript, NS performed the statistical analysis. All authors discussed the results and commented on the manuscript.

CONFLICT OF INTERESTS

All authors declare that they have no conflicts of interest.

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