

Formulation and Characterization Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) Chloroform Extract of Agarwood Leaves (*Gyrinops versteegii* (Gilg.) Domke)

Siska Noviana Dewi¹, Ronny Martien¹, Laras Novitasari¹, & Tri Rini Nuringtyas*²

¹Department of Pharmaceutics, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia

²Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia

ABSTRACT: Agarwood (*Gyrinops versteegii* (Gilg.) Domke), an indigenous Indonesian species, contains potentially beneficial compounds, such as terpenoids, in its leaf chloroform extract, however, these compounds exhibit low water solubility. Self-nanoemulsifying drug delivery system (SNEDDS) technology offers a means to enhance the solubility and bioavailability of these active compounds within cellular environments. The research methodology involved leaf extraction through soxhletation and the optimization of the formulation using various oils (migliol® 812N, olive oil, and virgin coconut oil (VCO)), surfactants (Tween 80 and Tween 20), and cosurfactants (polyethylene glycol (PEG-400)). The SNEDDS preparations were characterized by assessing the globule size, polydispersity index, zeta potential, percent transmittance, and globule morphology. The optimization of SNEDDS revealed that a formulation comprising miglyol® 812N oil and Tween 80 (32%:68%) resulted in a globule size of 63.25 ± 5.93 nm, a polydispersity index of 0.272 ± 0.016 , a transmittance percentage of $98.33 \pm 0.23\%$, and spherical globule morphology. This study concluded that the optimal formula for SNEDDS chloroform extract of agarwood leaves had good characteristics.

Keywords: *Gyrinops versteegii* (Gilg.) Domke; SNEDDS agarwood; miglyol® 812N; Tween 80.

Introduction

Agarwood (*Gyrinops versteegii* (Gilg.) Domke) is a native Indonesian plant that is widely used as a resin-producing plant in medicine, cosmetics, and perfumes. The chloroform extract of agarwood leaves has high antioxidant activity against HeLa cell lines with IC_{50} values of 1.66 ± 2.11 $\mu\text{g}/\text{mL}$ [1]. A previous study has revealed that *G. versteegii* has cytotoxic activity against colon cancer (WiDr), breast cancer (T47D), and cervical cancer (HeLa). The GC-MS results showed that the extract contained terpenoids and fatty acids as the main compounds [2]. However, because this compound is included in the nonpolar fraction, it has low solubility in aqueous media which can inhibit its absorption in cells and its therapeutic effects [3]. Nonpolar compounds dissolve in nonpolar solvents. Drugs or compounds with low water solubility are difficult to dissolve in Gastrointestinal Tract (GIT) fluids, which can inhibit their permeability and bioavailability [4].

Nanotechnology can solve these problems because it can coat active substances that are hydrophobic to nano size, so that their bioavailability in cells can increase [5].

One is the manufacture of self-nanoemulsifying drug delivery systems (SNEDDS), which are mixtures of oils, surfactants, and cosurfactants that can spontaneously form nanoemulsions in water [6]. SNEDDS can improve the solubility of nonpolar active substances and hydrophobic drugs, and their delivery into cells [7]. Previous research showed that SNEDDS of ethyl acetate extract of *Pandanus tectorius* fruit showed DPPH scavenging activity seven times higher than crude extract and had cytotoxicity with an IC_{50} values of 13.2 $\mu\text{g}/\text{mL}$ against HeLa cell line [8]. Curcumin SNEDDS at a dose of 6.25 μM showed an increase in cytotoxicity of $29.45 \pm 4.59\%$ against CaCo-2 cell line compared to free curcumin which has not shown an increase in cytotoxicity at that dose [6]. Another study of tamoxifen SNEDDS also showed better therapeutic and internalization effects in the MCF-7 cell line and increased oral bioavailability by 4.16-folds more with tamoxifen suspension [9].

This study aimed to optimise the formulation and characterization of SNEDDS of the chloroform extract

Article history

Received: 02 Nov 2024

Accepted: 12 Jan 2025

Published: 10 Apr 2025

Access this article



*Corresponding Author: Tri Rini Nuringtyas
Faculty of Biology, Universitas Gadjah Mada,
Yogyakarta, Indonesia, 55281 | Email: tririni@ugm.ac.id

of agarwood leaves according to pharmaceutical characteristics, including globule size, polydispersity index, zeta potential, percent transmittance, and globule morphology.

Methods

Materials

The materials used were Agarwood leaves (*G. versteegii*) were obtained from Losari Village, Comal District, Pemalang. Oils (miglyol® 812N, olive oil, and virgin coconut oil (VCO)) (PT. Brataco, Indonesia), surfactants (Tween 80 and Tween 20) (PT. Brataco, Indonesia), cosurfactants (polyethylene glycol-400 (PEG-400) and propylene glycol) (PT. Brataco, Indonesia), chloroform (PT. Smart Lab, Indonesia).

Extraction of Agarwood Leaves (*G. versteegii* (Gilg.) Domke)

Fresh young leaves were chosen as leaf samples. The samples were washed with running water, dried for 5 d, and dried in a 40 °C oven until they reached a constant weight. Dried leaves were powdered using a blender. Ten grams of leaf powder were extracted with 150 mL of chloroform using a Soxhlet apparatus at a current of 300 Watts at a temperature of 75-80 °C. The extract was dried to obtain a paste extract [1].

Mixing Test of Agarwood Leaves Extract with Oils, Surfactants and Cosurfactants

A total of 100 mg of agarwood leaf chloroform extract was placed in a vial, each containing a carrier total of 10 mL of oils (olive oil, miglyol® 812N, VCO), surfactants (Tween 80, Tween 20), and cosurfactants (PEG-400, propylene glycol), and then stirred using a vortex for 5 min. The mixture was magnetically stirred for 24 h and left at room temperature for 24 h. The solubility was visually observed [10].

Optimization of Oil and Surfactant

A total of 10 mg of agarwood leaf chloroform extract was added to 5 mL of each of the selected oils and surfactants in ratios of 2:8, 3:6, 4:5, 5:5, and 1:8. The mixture was homogenised by vortexing for 5 min and sonicated for 30 min at 45 °C. The resulting mixture was then stirred at 350 rpm for 24 h. One hundred microlitres of the formula were dripped into 5 mL of distilled water for visual observation. The clear samples were then measured using a Malvern Zetasizer instrument with parameters of globule size and polydispersity index.

SNEDDS Optimization and Formulation Using Simplex Lattice Design

The selected oils, surfactants, and cosurfactants were optimised using Design Expert software version 13.0.5.0, employing the Simplex Lattice Design method. The specified lower and upper limits for oil (10-50%), surfactant (30-70%), and cosurfactant (20-60%) were combined with 10 mg of agarwood leaf extract in 5 mL. This mixture was homogenised by vortexing for 5 min, followed by sonication for 30 min at 45 °C. Subsequently, the mixture was stirred at 350 rpm for 24 h at room temperature. Visual observations were conducted to assess the formula. Mixtures that did not undergo phase separation were further analysed using a Malvern Zetasizer Instrument to measure globule size (nm), polydispersity index, zeta potential (mV), and percent transmittance (%). The optimal formula results were reevaluated for each response variable to compare the measured values with the actual values to determine the percentage bias (%).

Characterization of SNEDDS Agarwood Leaves Extract

The SNEDDS preconcentrate of the agarwood leaf chloroform extract was diluted to 100 µL in 50 mL of distilled water to measure the globule size (nm) and determine polydispersity (PDI) using a Zetasizer instrument (Malvern Instruments, Worcestershire, UK) and percent transmittance (%) with a UV-Vis spectrophotometre (GENESYS™ 150 UV-Vis Spectrophotometre, USA) at a wavelength of 650 nm [11].

SNEDDS Morphology Analysis

The SNEDDS of the chloroform extract of the agarwood leaves was analysed using transmission electron microscopy (JEOL JEM-1400 TEM, USA). SNEDDS was diluted 1:100 in distilled water. Samples (20 µL) were collected, dripped onto a copper microgrid, and stained with 1% phosphotungstic acid for 30 s. Morphological analysis was performed by capturing images at a magnification of 10.000–80.000 [12].

Result and Discussion

The agarwood leaves (*G. versteegii* (Gilg.) Domke) sampled were fresh green leaves that were not diseased (Figure 1). Agarwood leaf samples were not dried under direct sunlight to avoid degradation of secondary metabolite compounds by UV radiation [13]. The dried leaf samples were then placed in an oven at 45-50 °C to remove water and avoid the possibility of extracts from



Figure 1. A. Agarwood (*Gyrinops versteegii* (gilg.) Domke) in Losari Village, Comal District, Pemalang, B. Fresh agarwood leaves.

contaminated fungi. Agarwood leaf samples were extracted using the Soxhletation method and a chloroform solvent. The results of chloroform extraction from agarwood leaves (*G. versteegii*) using the Soxhletation method are shown in [Table 1](#).

Based on [Table 1](#). The chloroform extract of agarwood leaves was obtained as a solid dark green paste with an average weight of chloroform extract of agarwood leaves of 0.61 ± 0.02 g and the yield percentage of 6,1%. These results are similar to those of previous studies that obtained an average weight of 0.55 g of agarwood leaf extract and a yield percentage of 5,5% [\[1\]](#).

The mixing test results of the chloroform extract of agarwood leaves with oils, surfactants, and cosurfactants are important for obtaining a stable formula. The mixing test was visually observed by selecting the result with the least amount of precipitate. The visual observations are presented in [Table 2](#).

Based on the observations, olive oil and miglyol® 812N were selected as oils. Tween 80 and PEG-400 were selected as the surfactant and co-surfactant, respectively. Based on these results, the oil was mixed with the surfactant by visually observing the clarity, and a clear sample was measured using a Malvern Zetasizer instrument. The

Table 1. Extraction of agarwood leaves (*Gyrinops versteegii* (gilg.) domke) with 150 ml chloroform.

Solvent	Weight of simplicia (g)	Extract weight (g)	Average Extract Weight (g)	% rendemen	Organoleptic test
Chloroform	10	0.63	0.61 ± 0.02	6.3	Paste, dark green, thick
		0.61		6.1	
		0.59		5.9	

Table 2. Results of observation of mixability of chloroform extract of agarwood leaves (*G. versteegii* (gilg.) domke) with oils, surfactants, and cosurfactants.

Extract	Oil			Surfactant		Cosurfaktan	
	VCO	Olive oil	Miglyol® 812N	Tween 80	Tween 20	PEG-400	Propylene glycol
Agarwood Leaves Extract	+	-	-	-	+	-	+

v = precipitate, = no precipitate

Table 3. Results of mixing of olive oil and miglyol® 812n with tween 80.

Sample	Ratio (oil:surfactant)	Visual observation	Clarity	Globule size (nm)	PDI
A1	1:8	-	clear	23.33	0.459
A2	2:8	-	turbid		
A3	3:6	-	turbid		
A4	4:5	-	turbid		
A5	5:5	-	turbid		
F1	1:8	-	clear	30.99	0.364
F2	2:8	-	clear	14.71	0.194
F3	3:6	-	clear	55.09	0.521
F4	4:5	-	turbid		
F5	5:5	-	turbid		

results of olive oil and miglyol® 812N emulsification with Tween 80 are shown in [Table 3](#).

As shown in [Table 3](#), all samples of olive oil or miglyol® 812N with Tween 80 formed preparations that did not separate. However, miglyol® 812N with Tween 80 was able to form a clear emulsion when dispersed into the water more than olive oil, namely at the oil:surfactant ratio 1:8; 2:8; 3:6. These results indicate that Mygliol® 812N oil and Tween 80 are more capable of forming nanosized preparations than olive oil. The globule size was <100 nm and the PDI value was <0.7. The nanoemulsion requirement was 20-200 nm. PDI values <0.3 are

monodispersed because they represent a narrow size distribution and uniformity in globule size. PDI values of 0.3-0.7 are polydispersed but are still considered good because they have a uniform globule size but different shapes and a wide distribution [\[14\]](#).

Based on the above results, miglyol® 812N oil was chosen as the carrier oil and Tween 80 was chosen as the SNEDDS surfactant for the agarwood leaf chloforom extract. Tween 80 is a surfactant with low toxicity and is not easily affected by changes in the pH and ionic charge. The HLB value of Tween 80 is 15, lower than that of Tween 20, and is more hydrophobic, which can facilitate

Table 4. Results of mixing miglyol® 812n, tween 80, and PEG 400 in SNEDDS formula optimization of agarwood leaves extract (*G. versteegii* (Gilg.) Domke).

Run	Miglyol® 812N	Tween 80	PEG 400	Size (nm)	PDI	%T	Visual observation
1	50.0	30.0	20.0	-	-	-	+
2	36.7	36.7	26.7	-	-	-	+
3	10.0	30.0	60.0	-	-	-	+
4	30.0	30.0	40.0	35.84	0.281	99.4	-
5	10.0	70.0	20.0	18.48	0.377	99.6	-
6	16.7	36.7	46.7	-	-	-	+
7	10.0	50.0	40.0	-	-	-	+
8	10.0	30.0	60.0	-	-	-	+
9	16.7	56.7	26.7	-	-	-	+
10	10.0	70.0	20.0	27.28	0.292	99.6	-
11	30.0	50.0	20.0	-	-	-	+
12	23.3	43.3	33.3	-	-	-	+
13	50.0	30.0	20.0	-	-	-	+

PDI: Polydispersity index, %T: Percent Transmittance, (-) no phase separation, (+) phase separation

Table 5. Results of SNEDDS response test of agarwood leaves extract with design expert version 13.0.5.0.

Run	Component v/v (%)			Response		
	Oil	Surfactant	Globule size (nm)	PDI	Zeta potential (mV)	transmittance (%)
1	25	75	22.39	0.379	-14.60	99.50
2	45	55	158.80	0.151	-19.10	89.10
3	25	75	25.35	0.410	-14.20	99.40
4	40	60	120.10	0.174	-9.63	95.40
5	35	65	78.21	0.213	-10.80	98.60
6	30	70	57.50	0.348	-14.70	99.20
7	35	65	87.41	0.218	-12.80	97.90
8	45	55	134.80	0.174	-19.20	86.70

PDI : polydispersity index

the formation of oil-in-water (o/w)-type nanoemulsions [15]. Miglyol® 812N is also an oil with better solubilization potential and stability than long-chain triglycerides such as oleic acid, olive oil, coconut oil, and virgin coconut oil, miglyol 812 has better solubilization potential and stability [6].

Furthermore, the orientation of the oil and surfactant with PEG-400 cosurfactant was determined using Design Expert version 13.0.5.0 software using the simplex lattice design (SLD) method. Eleven formulas were obtained by optimizing the lower and upper limits of Mygliol® 812N (10-50%), Tween 80 (30-70%), and PEG-400 (20-60%). The formulas were then visually observed. Table 4 shows the orientation results for the PEG-400 cosurfactant.

Based on Table 4, the mixing results between agarwood leaves chloroform extract, miglyol 812N oil, tween 80, and PEG-400 show that the provision of PEG-400 in various ratios causes the preparation to experience

phase separation except in run 4,5,10. However, the oil ratio in these run formulas is very small compared to the ratio of surfactants to cosurfactants. An excessively high surfactant ratio can have a cytotoxic effect on cells by disrupting the fluidity of the cell membrane [7]. The SNEDDS formulation of lamotrigine was selected as the minimum surfactant because of its potential toxicity, while maintaining optimal emulsification properties [16]. The SNEDDS formula should be clear and transparent, and form a homogeneous mixture when placed in the water phase at room temperature. Therefore, the next step of the SNEDDS manufacturing process was not performed with the addition of a cosurfactant.

Optimization of the SNEDDS agarwood leaf chloroform extract without cosurfactants was performed using the upper and lower limits of miglyol® 812N (25-45%) and Tween 80 (55-75%) [17,18]. The responses tested were globule size (nm), PDI, and percentage transmittance

Table 6. Numerical optimization results.

Name	Goal	Lower limit	Upper limit
A:Oil	<i>is in range</i>	25	45
B:Surfactant	<i>is in range</i>	55	75
Globule Size	<i>is target = 68.83</i>	22.39	158.8
Zeta Potential	<i>is in range</i>	-30	30
PDI	<i>minimize</i>	0.151	0.41
Transmittance	<i>maximize</i>	90	99.5

PDI : polydispersity index

Table 7. Results of SNEDDS optimal formula for agarwood leaves extract.

Oil (%)	Surfactant (%)	Globule size (nm)	PDI	Zeta Potential (mV)	Transmittance (%)	Desirability
32.28	67.72	68.83	0.267	-13.63	99.51	0.863

PDI : polydispersity index

(%). The SNEDDS formula optimization of chloroform extract of agarwood leaves was performed by mixing 10 mg of chloroform extract of agarwood leaves with 3 ml of SNEDDS, as shown in [Table 5](#).

[Table 5](#) shows the response test results for the SNEDDS globule size 22.39-158.80 nm, PDI 0.151-0.410, zeta potential -9.63 – -19.20 mV, and percent transmittance 86.70%- 99.50%. The target globule size in SNEDDS of the chloroform extract of the agarwood leaf extract was <200 nm, because a smaller size can increase the inter-surface area of SNEDDS to increase drug absorption and bioavailability in cells [\[19\]](#). When the percentage of surfactant increased, the average globule size decreased, but if the percentage of oil increased, a larger globule size was produced. Surfactants provide a mechanical barrier to protect globules formed from aggregates. The more surfactants in the formula, the tighter the surfactant film thus, its stability will be higher, and the interfacial tension between oil and water will be reduced [\[20\]](#). Even without the use of cosurfactants, the SNEDDS formula of agarwood leaf chloroform could form nanosized particles with good uniformity. The surfactant Tween 80 reduced the surface and interfacial tension with miglyol® 812N. The decrease in interfacial tension also causes a decrease in Gibbs free energy, which causes a decrease in globule size [\[21\]](#).

The second response was PDI, which was used to

observe the uniformity of the globule size distribution in the nanoemulsion. The more the PDI value approaches 0, the more homogeneous is the SNEDDS preparation [\[20\]](#). The PDI results are presented in [Table 5](#). was still below 0.5, indicating that the preparation was uniform or monodispersed [\[23\]](#).

The third variable, zeta potential, indicated the potential stability of the emulsion system. A good zeta value is generally more positive than +30 mV or more negative than -30 mV, therefore, particles repel each other and create dispersion stability [\[23\]](#). The optimization results of 8 run formulas obtained zeta potential values from -9.63 mV to -19.20 mV. When dispersed in water, the non-ionic surfactant Tween 80 produces a negative interfacial charge owing to the differential adsorption between hydroxyl ions (OH⁻) and hydronium ions (H₃O⁺) [\[24\]](#). The lower zeta potential was attributed to the larger nonionic surfactants sterically stabilizing the system by forming a surface layer. The negative zeta potential values of all formulas indicate a stable system [\[20\]](#).

The fourth response was the percent transmittance value, which is the clarity value of an emulsion. Transmittance values close to 100% indicate that the globule is nano-sized (nanodispersion), which also indicates that the formula has a large surface area for drug release and a high capacity to undergo increased absorption in the biological matrix, thus increasing

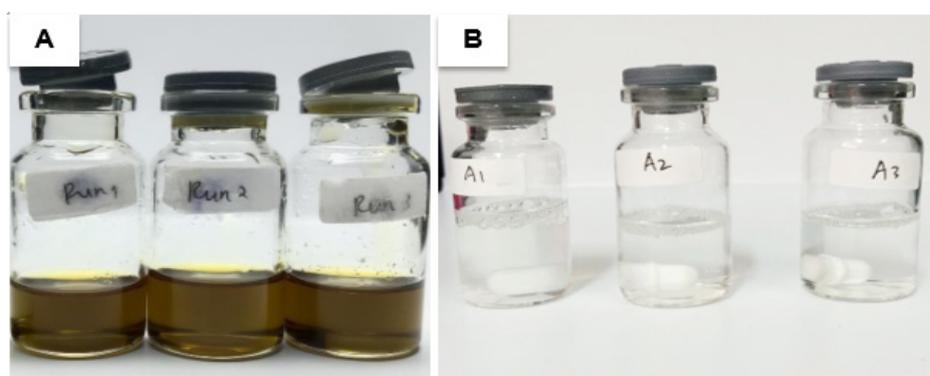


Figure 2. Results of making the optimal formula for SNEDDS of the chloroform extract of agarwood leaves (*G. versteegii* (Gilg.) Domke). A. SNEDDS pre-emulsion. B. SNEDDS nanoemulsion.

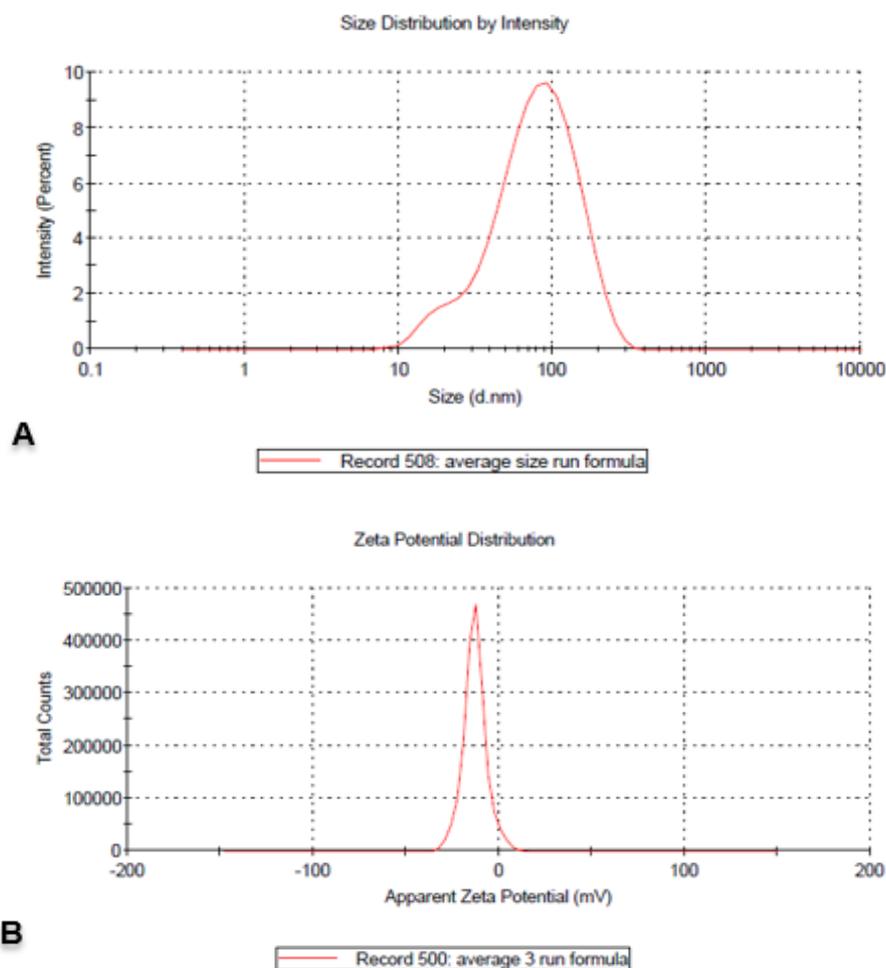


Figure 3. Results of measuring the optimal formula of SNEDDS chloroform extract of agarwood leaves (*G. versteegii* (Gilg.) Domke). A. Average globule size. B. Average zeta potential.

bioavailability [20,25]. The results of Table 5. shows that the percent transmittance value was good. The ratio between the oil and surfactant influences the difference in clarity. The lower the oil ratio and the higher the surfactant ratio, the higher the percentage transmittance obtained. Furthermore, numerical optimization was performed to analyze the composition in accordance with the expected target, as shown in Table 6.

The oil component (25-45%) and surfactant (55-75%) were optimized with the target in range. This is because the oil and surfactant components can obtain the required SNEDDS to form nanoemulsions in that range. The targeted globule size response was ± 68.83 , with a lower limit of 22.39 and an upper limit of 158.8. Based on previous research, curcumin SNEDDS with a globule size of 68.83 nm has a cytotoxic effect on HT29 colon cancer cells with an IC_{50} of 21.1 $\mu\text{g}/\text{mL}$ [26]. The targeted PDI response was minimised with a lower upper limit of 0.151 and upper limit of 0.41. The target zeta potential is in the

range of +30 mV to -30 mV. The target transmittance percentage was maximized, indicating that the optimal formula was expected to have the highest transmittance. The formula was obtained based on this optimization, as shown in Table 7.

As shown in Table 7, the optimal SNEDDS formula for agarwood leaf extract consisted of mygliol® 812N (32.28%) and Tween 80 (67.72%). The results of the optimal formula for the SNEDDS are shown in Figure 2. SNEDDS is a pre-emulsion which is then emulsified into water to form a nanoemulsion spontaneously in a short time (< 1 min). The expected target response for globule size was 68.83 nm, PDI 0.267, zeta potential -13.63 mV, and percent transmittance 99.51%, with a desirability value of 0.863. If the desirability value is close to 1, the selected response variable reaches an optimum value [27]. The results of the optimal formula prediction obtained were then replicated three times for verification, and the percentage bias (%) was calculated, as shown in Table 8.

Table 8. Response, prediction target, test results and SNEDDS bias percentage of agarwood leaves extract.

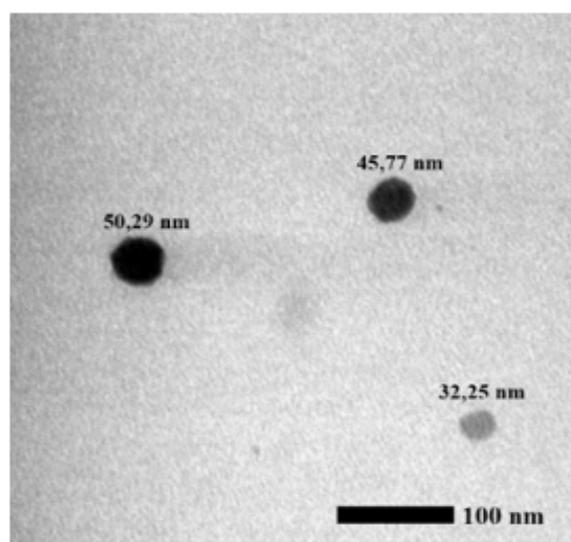
Response	Target	Experimental Results	95% CI low	95% CI high	Bias
Globule size (nm)	68.83	63.25 ± 5.93 nm	61.59	76.07	8.10%
PDI	0.267	0.272 ± 0.02	0.231	0.303	1.87%
Zeta Potential (mV)	-13.63	-12.4 ± 1.98	-15.3	-11.9	8.82%
Transmittance (%)	99.51	98.3 ± 0.23%	98.13	100.88	1.21%

PDI : polydispersity index

The verification results of the optimal formula of three replications obtained an average globule size of 63.25 ± 5.93 nm, PDI of 0.272 ± 0.016 , zeta potential of -12.4 ± 1.98 mV, and percent transmittance of $98.3 \pm 0.23\%$. The verification results showed an average globule size of <100 nm, PDI <0.4 , and percent transmittance $>95\%$. These values were still within the 95% Confidence Interval (CI) range, and the percent bias in the response between each verification sample and the target prediction was also below 10%. A deviation value below 10% is still categorized as good [28].

The results of measuring the globule size, PDI, and zeta potential of the SNEDDS verification sample of agarwood leaf chloroform extract using the Malvern Zetasizer instrument showed good nanoemulsion characteristics as shown in Figure 3. Globule sizes of less than 100 nm indicate that the particles have a larger interfacial surface area for drug absorption. The surfactant concentration influences the globule size in SNEDDS

because the more surfactant, the more the hydrophilic part of the surfactant will interact with the water phase, and the lipophilic part interacts with miglyol® 812N oil to provide stronger stabilization so that coalescence between oil droplets does not occur and the globule size decreases [12]. The globule size distribution was less than 4, indicating that the particle distribution was homogeneous. A PDI value of less than 5 indicated that the preparation was stable [29]. The zeta potential value obtained was negatively charged with an average of -12.4 ± 1.98 mV. The zeta potential is the potential difference between the electronegative part of the solution and the surface layer of ions strongly bound to the solid surface. Dispersions with zeta potential values $> +30$ mV or < -30 mV are considered stable systems, however, dispersions with lower or near-zero zeta potentials can remain stable owing to the formation of a surface layer by nonionic surfactants. Negative zeta potential values indicated a stable system. SNEDDS brigatinib with oleic acid, Tween

**Figure 4.** The results of TEM analysis of the SNEDDS optimal formula 100 nm scale (mag. 40000).

20, and diethylene glycol monoethyl ether has a zeta potential of -3.73 and -6.46 mV [16]. SNEDDS tamoxifen with olive oil, tween 80, and PEG-400 had a zeta potential of -17.0 mV and an IC_{50} cytotoxic effect of $5.98 \pm 0.9 \mu\text{g/ml}$ against MCF-7 breast cell line [29].

Figure 4 shows that the morphology of SNEDDS is spherical with globule size sizes ranging from 32.25 nm to 55.78 nm. These results indicate that SNEDDS of the chloroform extract of agarwood leaves can form nanoparticles <100 nm in size. These results were also confirmed by the globule size distribution in the Malvern Zetasizer instruments that the particle diameter size distribution of SNEDDS of agarwood leaf chloroform extract as much as 10.4% was dominated by the size of 78.82 nm. The range of values from 32.25 nm to 55.78 nm from TEM analysis results is also measured in this distribution, the size of 43.82 (5.9%), 50.75 nm (7.5%), 58.77 nm (9.0%), target size 68.06 nm (10.0%). Therefore, it can be concluded that the particle diameter of SNEDDS of agarwood leaf chloroform extract with miglyol® 812N oil and Tween 80 can form nanoparticles <100 nm with a negatively charged zeta potential, so that no aggregation is formed.

Conclusion

The optimal formula of SNEDDS agarwood leaves (*Gyrinops versteegii* (Gilg.) Domke) chloroform extract had good characteristics formula contained miglyol® 812N oil and tween 80 (32%:68%) with globule size 63.25 ± 5.93 nm, polydispersity index 0.272 ± 0.016 , zeta potential 12.4 ± 1.98 mV, percent transmittance $98.33 \pm 0.23\%$, and spherical globule morphology.

Conflict of Interest

The authors declare no conflicts of interest regarding this investigation.

Acknowledgement

This research was supported and financed by Lembaga Pengelola Dana Pendidikan in 2024. The authors would like to thank the Faculty of Biology and Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, for providing laboratory facilities.

References

- [1]. Nuringtyas TR, Isromarina R, Septia Y, Hidayati L, Wijayanti N, Moeljopawiro S, The Antioxidant and Cytotoxic Activities of The Chloroform Extract of Agarwood (*Gyrinops versteegii* (Gilg.) Domke) Leaves on Hela Cell Lines, AIP Conference Proceedings. 2018;2002(1):1-9. <https://doi.org/10.1063/1.5050163>
- [2]. Wardana TAP, Nuringtyas TR, Wijayanti N, Hidayati L. Phytochemical Analysis of Agarwood (*Gyrinops versteegii* (Gilg.) Domke) Leaves Extracts as Anticancer Using GC-MS, AIP Conference Proceedings. 2019;2194(1):1-9. <https://doi.org/10.1063/1.5139868>
- [3]. Rizqiana A, Sudarmin, S. Analysis of Antioxidant Activity on the Ethanol Extract of Indonesian Tropical Forest Plants. Indonesian Journal of Chemical Science. 2023;12(1): 47-57. DOI 10.15294/ijcs.v12i1.65147
- [4]. Kumari L, Choudhari Y, Patel P, Gupta GD, Singh D, Rosenholm JM, Bansal KK, Kurmi BD. Advancement in solubilization approaches: A step towards bioavailability enhancement of poorly soluble drugs. Life. 2023; 13(5): 1099. <https://doi.org/10.3390/life13051099>
- [5]. Noore S, Rastogi NK, O'Donnell C, Tiwari B. Novel Bioactive Extraction and Nano-Encapsulation. Encyclopedia. 2021;1(3):632-664. <https://doi.org/10.3390/encyclopedia1030052>
- [6]. Annisa R, Mutiah, R, Yuwono, M, Hendradi E. Nanotechnology Approach-Self Nanoemulsifying Drug Delivery System (SNEDDS). International Journal of Applied Pharmaceutics. 2023;15(4): 12-19. <https://doi.org/10.22159/ijap.2023v15i4.47644>
- [7]. Kanwal T, Saifullah S, ur Rehman J, Kawish M, Razzak A, Maharjan R, Imran M, Ali I, Roome T, Simjee SU, Shah MR. Design of Absorption Enhancer Containing Self-Nanoemulsifying Drug Delivery System (SNEDDS) for Curcumin Improved Anti-Cancer Activity and Oral Bioavailability. Journal of Molecular Liquids. 2021;324:114774. <https://doi.org/10.1016/j.molliq.2020.114774>
- [8]. Kholieqoh AH, Kassim MNI, Muhammad TST, Anam K, Sung YY, Amir H, Praja HN, Andriani Y. SNEDDS to improve the bioactivities of Pandanus tectorius leaves: Optimization, antioxidant, and anticancer activities via apoptosis induction in human cervical cancer cell line. Journal of Applied Pharmaceutical Science. 2024;14(10):175-189. <http://doi.org/10.7324/JAPS.2024.168694>
- [9]. Shrivastava N, Parikh A, Dewangan RP, Biswas L, Verma AK, Mittal S, Ali J, Garg S, Baboota S, Solid Self-Nano Emulsifying Nanoplatfrom Loaded with Tamoxifen and Resveratrol for Treatment of Breast Cancer. Pharmaceutics. 2022;14(7):1-34 <https://doi.org/10.3390/pharmaceutics14071486>
- [10]. Mustika A, Fatimah N, Sari GM. Formulation and characterizations of self-nanoemulsifying drug delivery system of extract Petiveria alliacea (Singawalang) leaves. International Journal of Applied Pharmaceutics. 2019;11(5):61-65. <https://doi.org/10.22159/ijap.2019.v11s5.T0050>
- [11]. Reddy MS, Sravanthi B. Formulation and In Vitro Characterization of Solid-Self Nanoemulsifying Drug Delivery System of Atorvastatin Calcium. Asian Journal of Pharmaceutics (AJP). 2017;11(04):991-999. <https://doi.org/10.22377/ajp.v11i04.1771>
- [12]. Senapati PC, Sahoo SK, Sahu AN. Mixed surfactant based (SNEDDS) self-nanoemulsifying drug delivery system presenting efavirenz for enhancement of oral bioavailability. Biomedicine & Pharmacotherapy. 2016;8:42-51. <http://dx.doi.org/10.1016/j.biopha.2016.02.039>
- [13]. Vania I, Tita N, Nur R. Uji Aktivitas Ekstrak Etanol Daun Pandan Wangi (*Pandanus amaryllifolius* Roxb.) Sebagai Hair Tonic pada Kelinci Jantan Galur Lokal. Pharmacoscrypt. 2019;1(2):57-67. DOI: <https://doi.org/10.36423/pharmacoscrypt.v2i1.148>
- [14]. Syukri Y, Nugroho BH, Istanti I. Penggunaan D-optimal Mixture Design untuk Optimasi Dan Formulasi Self-Nano Emulsifying Drug Delivery System (SNEEDS) Asam Mefenamat. JSFK (Jurnal Sains Farmasi & Klinis). 2020;7(3):180-187. DOI:10.25077/jsfk.7.3.180-187.2020

- [15]. Ansari MJ, Alnakhli M, Al-Otaibi T, Al Meanazel O, Anwer MK, Ahmed MM, Alshahrani SM, Alshetaibi A, Aldawsari MF, Alalawi AS, Alazani AZ, Zahrani MA, Ahmad N. Formulation and Evaluation of Self-Nanoemulsifying Drug Delivery System of Brigatinib: Improvement of Solubility, In Vitro Release, Ex-Vivo Permeation and Anticancer Activity. *Journal of Drug Delivery Science and Technology*. 2021;61:102204. <https://doi.org/10.1016/j.jiddst.2020.102204>
- [16]. Abdelmonem R, Azer MS, El-Nabarawi M, Makky A, Zaghoul A, Nada A. Development, Characterization, and in-vivo Pharmacokinetic Study of Lamotrigine Solid Self-Nanoemulsifying Drug Delivery System. *Drug Design, Development and Therapy*. 2020;14(4):4343–4362. <https://doi.org/10.2147/dddt.s263898>
- [17]. Chai F, Sun L, Ding Y, Liu X, Zhang Y, Webster TJ, Zheng C. A Solid Self-Nanoemulsifying System of The BCS Class IIB Drug Dabigatran Etexilate to Improve Oral Bioavailability. *Nanomedicine*. 2016;11(14):1801–1816. <https://doi.org/10.2217/nnm-2016-0138>
- [18]. Sindi AM, Hosny KM, Alharbi WS. Lyophilized composite loaded with meloxicam-peppermint oil nanoemulsion for periodontal pain. *Polymers*. 2021;13(14):1-17. <https://doi.org/10.3390/polym13142317>
- [19]. Yin HF, Yin CM, Ouyang T, Sun SD, Chen WG, Yang XL, He X, Zhang CF. Self-Nanoemulsifying Drug Delivery System of Genkwanin: A Novel Approach for Anti-Colitis-Associated Colorectal Cancer. *Drug Design, Development and Therapy*. 2021;15:557-576. doi: 10.2147/DDDT.S292417
- [20]. Nasr A, Gardouh A, Ghorab M. Novel Solid Self-Nanoemulsifying Drug Delivery System (S-SNEDDS) for Oral Delivery of Olmesartan Medoxomil: Design, Formulation, Pharmacokinetic and Bioavailability Evaluation. *Pharmaceutics*, 2016;8(3):20. doi: 10.3390/pharmaceutics8030020
- [21]. Zingale E, Bonaccorso A, D'Amico AG, Lombardo R, D'Agata V, Rautio J, Pignatello R. Formulating Resveratrol and Melatonin Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) for Ocular Administration Using Design of Experiments. *Pharmaceutics*. 2024;16(1):1-27. <https://doi.org/10.3390/pharmaceutics16010125>
- [22]. Sapiun Z, Imran AK, Dewi STR, Pade DFM, Ibrahim W, Tungadi R, Abdulkadir WS, Banne Y, Sartini S, Permana AD, Rifa'i Y, Ysrafil Y, Slamet NS. Formulation and Characterization of Self Nano-Emulsifying Drug Delivery System (SNEDDS) Fraction of N-Hexane: Ethyl Acetate from Sesewana Leaf (*Clerodendrum fragrans* Wild.). *International Journal of Applied Pharmaceutics*. 2023;15(2):72-77. DOI: <https://dx.doi.org/10.22159/ijap.2023v15i2.46365>
- [23]. Fitriani H, Fitria A, Miladiyah I, Syukri Y. Pengembangan Self-Nano Emulsifying System (SNES) Ekstrak Temulawak (*Curcuma xanthorrhiza*): Formulasi, Karakterisasi, dan Stabilitas. *Jurnal Sains Farmasi & Klinis*. 2021;8(3):332-339. <https://doi.org/10.25077/jsfk.8.3.332-339.2021>
- [24]. Choi KO, Aditya NP, Ko S. Effect of Aqueous pH and Electrolyte Concentration on Structure, Stability and Flow Behavior of Non-Ionic Surfactant Based Solid Lipid Nanoparticles. *Food chemistry*. 2014;147:239-244. <http://dx.doi.org/10.1016/j.foodchem.2013.09.095>
- [25]. Suhery WN, Sumirtapura YC, Pamudji JS, Mudhakir D. Development and Characterization of Self-Nanoemulsifying Drug Delivery System (SNEDDS) Formulation for Enhancing Dissolution of Fenofibric Acid. *Journal of Research in Pharmacy*. 2020;24(5):738-47. <https://doi.org/10.35333/jrp.2020.227>
- [26]. Karthika C, Sureshkumar R, Suhal A. Formulation Development and In Vitro Evaluation of Curcumin-Loaded Solid Selfnanoemulsifying Drug Delivery System for Colon Carcinoma. *Asian Journal of Pharmaceutical and Clinical Research* 2019;12(7):243-247. <https://doi.org/10.22159/ajpcr.2019.v12i7.33231>
- [27]. Yadav P, Rastogi V, Verma A. Application of Box–Behnken design and desirability function in the development and optimization of self-nanoemulsifying drug delivery system for enhanced dissolution of ezetimibe. *Future Journal of Pharmaceutical Sciences*. 2020;6:1-20. <https://doi.org/10.1186/s43094-020-00023-3>
- [28]. Fitria A, Hanifah S, Chabib L, Uno AM, Munawwarah H, Atsil N, Pohara HA, Weuanggi DA, Syukri Y. Design and Characterization of Propolis Extract Loaded Self-Nano Emulsifying Drug Delivery System as Immunostimulant. *Saudi Pharmaceutical Journal*. 2021;29(6):625-634. <https://doi.org/10.1016/j.jsps.2021.04.024>
- [29]. Batool A, Arshad R, Razaq S, Nousheen K, Kiani, MH, Shahnaz G. Formulation and Evaluation of Hyaluronic Acid-Based Mucoadhesive Self Nanoemulsifying Drug Delivery System (SNEDDS) of Tamoxifen for Targeting Breast Cancer. *International Journal of Biological Macromolecules*. 2020;152:503-515. <https://doi.org/10.1016/j.ijbiomac.2020.02.275>



Copyright © 2025 The author(s). You are free to share (copy and redistribute the material in any medium or format) and adapt (remix, transform, and build upon the material for any purpose, even commercially) under the following terms: Attribution — You must give appropriate credit, provide a link to the license, and indicate if changes were made. You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use; ShareAlike — If you remix, transform, or build upon the material, you must distribute your contributions under the same license as the original (<https://creativecommons.org/licenses/by-sa/4.0/>)