

Development and Optimization of *Andrographis paniculata* Extract Nanoemulsion by Low-Energy Method Using Simplex Lattice Design

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SUMMARY

Andrographis paniculata extract possesses potent anti-inflammatory activity but suffers from poor water solubility and insufficient bioavailability, restricting its topical application. This research aimed to design and optimize an *Andrographis paniculata* extract nanoemulsion using a low-energy phase-inversion composition method guided by Simplex Lattice Design. Solubility screening identified oleic acid, Tween 80, and propylene glycol as suitable oils, surfactants, and cosurfactants, respectively. Eight formulations were prepared, with droplet size, polydispersity index, and zeta potential as response variables. Simplex Lattice Design optimization predicted an optimal formulation with an oil:Smix ratio of 4:19, a droplet size of 130.707 nm, a polydispersity index of 0.245, and a zeta potential of -22.517 mV, which were in good agreement with the experimental values ($p > 0.05$). The optimized nanoemulsion exhibited low viscosity (48.0 ± 0.67 mPa · s), spherical droplets < 200 nm, and no aggregation, as confirmed by TEM. Thermodynamic stability studies, including centrifugation, heating-cooling, and freeze-thaw cycles, demonstrated robust stability without phase separation or creaming. FT-IR analysis indicated incorporation of andrographolide without significant chemical interactions. The results indicate that the low-energy method successfully produced a stable, homogeneous *Andrographis paniculata* extract nanoemulsion with a favorable droplet size, narrow polydispersity, and physical stability, highlighting its potential for pharmaceutical application, particularly for topical delivery.

Keywords: Herbal formulation, nanomedicine, oleic acid, propylene glycol, topical, tween 80

Andrographis paniculata Ekstraktı Nano Emülsiyonunun Düşük Enerjili Yöntemle Geliştirilmesi ve Basit Latis Tasarımı ile Optimizasyonu

ÖZ

Andrographis paniculata özütü güçlü antiinflamatuvar aktiviteye sahip olmakla birlikte, düşük su çözünürlüğü ve yetersiz biyoyararlanımı nedeniyle topikal uygulaması kısıtlanmaktadır. Bu araştırma, Simplex Lattice Design rehberliğinde düşük enerjili faz inversiyon kompozisyon yöntemi kullanılarak bir *Andrographis paniculata* özütü nanoemülsiyonunun tasarlanması ve optimize edilmesi amacıyla yapılmıştır. Çözünürlük taraması, sırasıyla oleik asit, Tween 80 ve propilen glikolü uygun yağ, yüzey aktif madde ve yardımcı yüzey aktif madde olarak belirlemiştir. Damlacık boyutu, polidispersite indeksi ve zeta potansiyeli yanıt değişkenleri olarak kullanılarak sekiz formülasyon hazırlanmıştır. Simplex Lattice Design optimizasyonu, 4:19 yağ:Smix oranına, 130,707 nm damlacık boyutuna, 0,245 polidispersite indeksine ve -22,517 mV zeta potansiyeline sahip optimal bir formülasyon öngörmüştür; bu değerler deneysel değerlerle iyi bir uyum göstermiştir ($p > 0,05$). Optimize edilmiş nanoemülsiyon, düşük viskozite ($48,0 \pm 0,67$ mPa.s), 200 nm'den küçük küresel damlacıklar ve TEM ile doğrulandığı üzere agregasyon göstermedi. Santrifüjleme, ısıtma-soğutma ve dondurma-çözme döngülerini içeren termodinamik stabilite çalışmaları, faz ayrımı veya krema oluşumu olmaksızın sağlam bir stabilite gösterdi. FT-IR analizi, önemli kimyasal etkileşimler olmaksızın andrografolidin dahil edildiğini gösterdi. Sonuçlar, düşük enerjili yöntemin, uygun damlacık boyutu, dar polidispersite ve fiziksel stabiliteye sahip stabil, homojen bir *Andrographis paniculata* ekstresi nanoemülsiyonu üretmeyi başardığını ve özellikle topikal uygulama için farmasötik uygulama potansiyelini vurguladığını göstermektedir.

Anahtar Kelimeler: Bitkisel formülasyon, nanomedisin, oleik asit, propilen glikol, topikal, tween 80

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INTRODUCTION

Andrographis paniculata (*A. paniculata*) is widely recognized for its anti-inflammatory properties (Mussard et al., 2020). Its major bioactive compound, andrographolide (AND), exhibits anti-arthritic effects (Li et al., 2019) but has an intensely bitter taste, supporting the development of topical formulations to improve patient acceptability and localized delivery (Hossain et al., 2021; Indonesian Herbal Pharmacopoeia, 2017). Topical systems of *A. paniculata* and AND have been investigated for inflammatory skin conditions, wound healing, and skin cancer, with hydrogels showing good skin compatibility (Lapmanee et al., 2025; Mulukuri, Dhara, Gupta, Devi, & Kumar, 2023). *A. paniculata* is widely cultivated in tropical regions, including Indonesia (Ilmi, Pamungkas, Tumewu, Hafid, & Widyawaruyanti, 2021), and extracts standardized to high AND content (>30% w/w) have shown no toxicity in acute studies up to 5000 mg/kg in rodents (Chandrasekaran et al., 2009; Worasutayangkurn et al., 2019). Topical delivery may further reduce systemic exposure and first-pass metabolism (Raman, Murugaiyah, & Parumasivam, 2022). However, oral administration of *A. paniculata* extract (APE) is limited by low solubility (3.29 ± 0.73 mg/mL), poor bioavailability (~2.67%), and fast metabolism, prompting alternative delivery approaches such as self-microemulsifying drug delivery systems (Pandey & Rao, 2018; Pornpitchanarong, Akkaramongkolporn, Nattapulwat, Opanasopit, & Patrojanasophon, 2024; Raman et al., 2022). Nanoemulsions offer improved solubility, stability, and enhanced topical delivery performance (Fang, Zhang, Pang, Thu, & Hussain, 2019). Although various topical systems of AND or *A. paniculata* have been reported, including nanoemulsion-based hydrogels and related carriers, most studies focus on isolated AND or lack systematic optimization for herbal extract-based systems (Indrati, Martien, Rohman, & Nugroho, 2020; Lapmanee et al., 2025; Martihandini, Surini, & Bahtiar, 2021; Mulukuri et al., 2023; Yao et al., 2023). Therefore, this study

aimed to develop and optimize an APE nanoemulsion using Simplex lattice design (SLD), a mixture experimental design that maintains a constant total proportion of formulation components to enable systematic optimization of composition (Bolton & Bon, 2010), with droplet size (DS), polydispersity index (PDI), and zeta potential (ZP) as responses. The optimized formulation was further evaluated for viscosity, morphology through Transmission Electron Microscopy (TEM), molecular interactions via Fourier Transform Infrared (FT-IR), and thermodynamic stability to ensure suitability for topical application.

MATERIALS AND METHODS

Materials

A. paniculata was purchased from Tawangmangu Traditional Health Care Service Unit, Karanganyar, Central Java, Indonesia. Taxonomy identification was conducted in the Pharmaceutical Biology Department of the Faculty of Pharmacy at Universitas Gadjah Mada (identification number 12.30.8/UNI/FFA.2/BF/PT/2023). Tween 20 (BioFroxx), Tween 80 (KAO Corporation), Cremophor RH 40 (BASF), Capryol 90 and Transcutol P (Gattefossé), Miglyol (IOI Oleochemical), oleic acid (AppliChem Panreac), PEG 400 (Shiv Shakti), and propylene glycol (Dow Chemical Pacific).

Preparation of APE

The powdered aerial part of *A. paniculata* (4 kg) was macerated with 96% ethanol at ambient temperature for 24 h. The residue was remacerated twice under the same conditions. The combined filtrates were concentrated at reduced pressure with a vacuum evaporator and subsequently freeze-dried. The resultant freeze-dried extract was preserved in a desiccator for subsequent use (Liang et al., 2023).

Solubility screening of APE

The solubility of APE was determined using previously reported methods with minor modifications. In summary, 10 mg of APE was added to 1 mL of oleic acid, Miglyol, Capryol 90, Tween 80, Tween 20,

Cremophor RH 40, PEG 400, propylene glycol, and Transcutol P in screw cap vials (10 mL; 50 mL for large volumes). Mixtures were vortexed at 3000 rpm for 10 min and left to equilibrate at ambient temperature for 24 h. Solubility was assessed visually for turbidity or precipitation, with additional solvent added, and the procedure repeated until complete dissolution (Chahyani & Zai, 2024). All measurements were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation (SD).

Construction of a pseudo-ternary phase diagram

A 2 mL mixture of oleic acid, Smix (Tween 80 as the surfactant and propylene glycol as the cosurfactant, in 2:1 w/w), and water was prepared, with oleic acid and Smix combined at 0.5:9.5, 0.75:9.25, and 1:9 ratios, based on previous studies showing these ratios produce a transparent and stable nanoemulsion region (Chahyani & Zai, 2024). Mixtures were agitated with a magnetic stirrer at 400 rpm and 37 °C for 20 min. Samples with a transparent, gel-like, or liquid-crystalline appearance were visually identified as nanoemulsions. DLS measured DS and PdI after diluting 100 μ L of each sample in 5 mL of distilled water.

Preparation and optimization of APE nanoemulsion using SLD

APE nanoemulsion was prepared by low-energy emulsification. Oil and Smix were mixed according to the experimental design with Transcutol P as a penetration enhancer, stirred at 400 rpm and 37°C for 20 minutes, followed by dropwise addition of distilled water under continuous stirring until a transparent nanoemulsion formed. Formulations were equilibrated at room temperature before characterization (Pathania, Najda, Chawla, Kaushik, & Khan, 2022). A pseudo-ternary phase diagram was used to define the nanoemulsion region and determine the composition range of oleic acid (oil phase) and Smix (surfactant-cosurfactant) as independent variables. An eight-run SLD, including two center points (Table 2.),

was generated to evaluate the effects of formulation variables. Nanoemulsions (10 mL) were prepared by aqueous titration, and DS, PdI, and ZP were evaluated as response variables. Optimization criteria were set as DS < 200 nm, PdI $\leq$ 0.3, and the highest absolute ZP value to ensure small DS, uniform distribution, and improved stability (Anwer et al., 2023; Maleki et al., 2023; Yang et al., 2019). The optimized formulation was validated in triplicate by comparing experimental and predicted DS, PdI, and ZP using a one-sample t-test at a 95% confidence level.

Characterization of APE nanoemulsion

DS, PdI, and ZP determination

The average of DS, PdI, and ZP was determined using a Zetasizer Nano-ZS (Malvern Instruments, UK) via dynamic light scattering (DLS). Samples were diluted 1:50 with distilled water before testing. Analyses were performed at 25 °C and a 173° scattering angle, and all measurements were conducted in triplicate.

Viscosity Measurement

The viscosity of the optimized APE formulation was assessed using a Brookfield DV-I Prime rotational viscometer (spindle No. 3) at 25 °C without dilution. The spindle was operated at 100 rpm for 15 s, and evaluations were conducted in triplicate. Results were presented in mPa.s (Malik et al., 2022).

TEM analysis

TEM examined the morphology of the optimized APE nanoemulsion and blank nanoemulsion. A sample drop was applied to a carbon-coated copper grid, air-dried, negatively stained with 2% PTA, and subsequently re-dried before observation at 120 kV. Droplet morphology was analyzed at various magnifications (Ramanunny et al., 2022).

FT-IR spectroscopic analysis

Fourier Transform Infrared (FT-IR) spectroscopy was performed to assess potential interactions among nanoemulsion components. Spectra of standard AND, APE, APE nanoemulsion, and blank na-

noemulsion were recorded using the KBr pellet method (2-3 mg; 10,000-15,000 psi) over 500-4000 cm^{-1} at 4 cm^{-1} resolution. Characteristic peaks were analyzed for molecular interactions (Asasutjarit et al., 2021).

Thermodynamic stability

Thermodynamic stability was evaluated using centrifugation, heating-cooling, and freeze-thaw tests. Formulations were centrifuged at 12,000 rpm for 15 minutes over three cycles and visually assessed for phase separation; formulations that remained clear and homogeneous were considered stable (Zhang et al., 2023). For the heating-cooling test, samples were alternately stored at 4°C and 45°C for 24 hours at each temperature over three cycles and observed for physical changes such as phase separation, creaming, or turbidity (Kumar, Bishnoi, Shukla, & Jain, 2021). Freeze-thaw stability was assessed by alternating storage at -20 °C and 25 °C for 24 hours at each temperature over three cycles, followed by evaluation of physical appearance, DS, PdI, and ZP to detect temperature-induced changes (Maleki et al., 2023; Marsup et al., 2020).

Statistical analysis

All experiments were performed in triplicate ($n = 3$), and the results are presented as mean $\pm$ SD. Mixture design analysis was conducted using ANOVA within the SLD model in Design Expert® v13 (Stat-Ease Inc., Minneapolis, MN, USA). Model adequacy was evaluated based on lack of fit, R^2 , adjusted R^2 , predicted R^2 , and adequate precision. Solubility studies were analyzed by an independent t-test, while verification of the optimized formulation was assessed using a one-sample t-test in Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Statistical significance was set at $p < 0.05$.

RESULTS and DISCUSSION

Solubility screening of APE

The solubility of APE in various oils, surfactants, and cosurfactants was evaluated to guide nanoemulsion formulation, as solubility directly affects drug loading and stability, with insufficient solubility potentially causing precipitation (Chahyani & Zai, 2024; Syukri, Fitria, Hanifah, & Idrati, 2020). The results are presented in (Figure 1.). APE showed the highest solubility in oleic acid (0.590 ± 0.035 mg/mL), followed by Miglyol (0.494 ± 0.025 mg/mL) and Capryol 90 (0.268 ± 0.007 mg/mL), with oleic acid significantly higher than Miglyol ($p < 0.05$). Accordingly, oleic acid was selected as the oil phase due to its superior solubilization capacity and established regulatory acceptance, including GRAS status and inclusion in the FDA Inactive Ingredients Database (Rowe, Sheskey, & Quinn, 2009). Among surfactants, solubility was highest in Tween 80 (2.5 ± 0.833 mg/mL), followed by Tween 20 (2.056 ± 0.419 mg/mL) and Cremophor RH 40 (1.639 ± 0.376 mg/mL). Although no significant difference was observed between Tween 80 and Tween 20 ($p > 0.05$), Tween 80 was selected due to its widespread use, regulatory acceptance, and stabilizing ability in O/W nanoemulsions (Pathania et al., 2022; Rowe et al., 2009). For cosurfactant, solubility was highest in propylene glycol (4.444 ± 0.962 mg/mL), followed by Transcutol P and PEG 400. Despite no significant difference between propylene glycol and Transcutol P ($p > 0.05$), propylene glycol was selected due to its pharmacopeial acceptance and role in reducing interfacial tension and enhancing nanoemulsion stability, while Transcutol P was used as a penetration enhancer (Osborne & Musakhanian, 2018; Rowe et al., 2009). Overall, components were selected based on solubility, statistical analysis, and regulatory considerations.

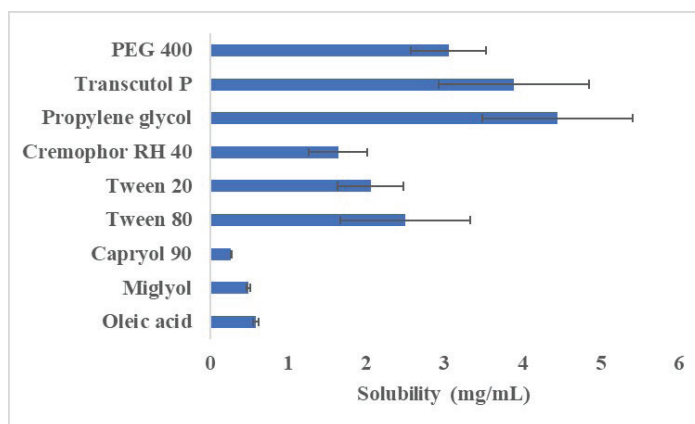


Figure 1. Solubility of APE in various oils, surfactants, and cosurfactants (mean ± SD, n = 3)

Construction of a pseudo-ternary phase diagram

Pseudo-ternary phase diagrams were used to determine the optimal oil-surfactant mixture (Smix, Tween 80:propylene glycol) ratio for the APE nanoemulsion. The phase inversion composition (PIC)

method, a low-energy technique based on compositional changes rather than mechanical energy, was applied. Compared to high-energy methods such as ultrasonication, microfluidization, and high-pressure homogenization, PIC is more energy-efficient, cost-effective, and suitable for scale-up (Singh et al., 2017; Sokolov, 2014).

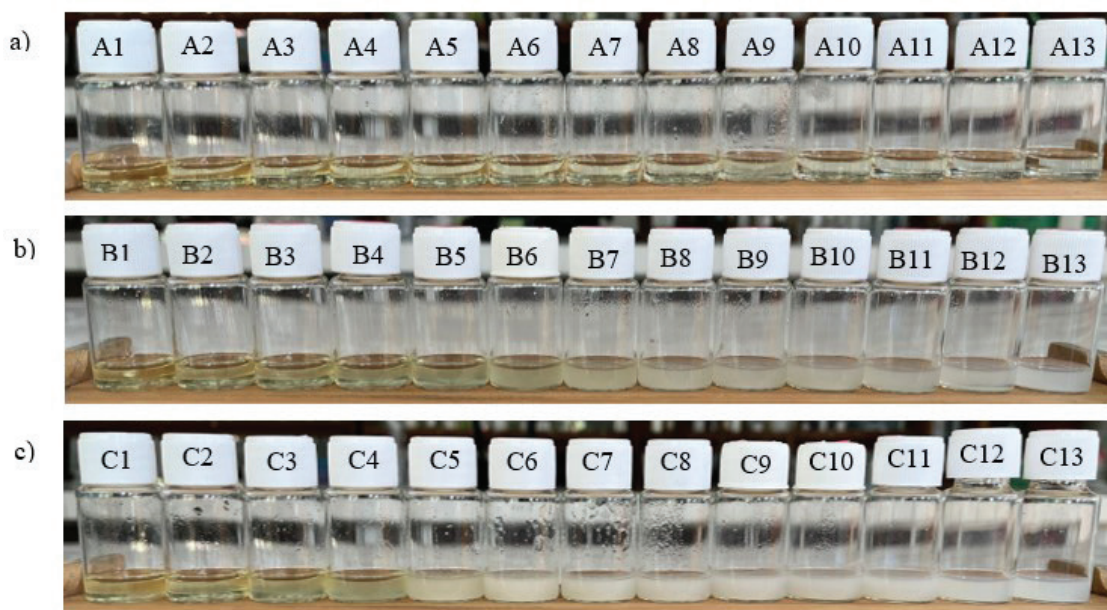


Figure 2. Visual characteristics of nanoemulsion at different oil-Smix ratios: a) 0.5:9.5; b) 0.75:9.25; c) 1:9. The oil content was varied from 20 to 80% (w/w) in 5% increments to construct the pseudo-ternary phase diagram.

Table 1. DS and PdI of LC gel-like samples

Ratio Oil:Smix	Sample Code	Composition ratio		DS (nm)	PdI
		Oil	Smix		
0.5:9.5	A1	4	76	13.09 ± 0.05	0.20 ± 0.002
	A2	3.75	71.25	13.51 ± 0.03	0.19 ± 0.003
	A3	3.5	66.5	13.11 ± 0.06	0.21 ± 0.001
	A4	3.5	61.75	13.57 ± 0.06	0.21 ± 0.003
	A5	3	57	10.98 ± 0.04	0.26 ± 0.009
	A6	2.75	52.25	13.18 ± 0.06	0.23 ± 0.001
	A7	2.5	47.5	13.49 ± 0.10	0.20 ± 0.007
	A8	2.25	42.75	14.52 ± 0.06	0.28 ± 0.005
	A10	1.75	33.25	17.16 ± 0.53	0.35 ± 0.008
	A11	1.5	28.5	12.81 ± 0.14	0.22 ± 0.010
	A12	1.25	23.75	13.85 ± 0.10	0.23 ± 0.013
	A13	1	19	12.56 ± 0.18	0.24 ± 0.005
0.75:9.25	B1	6	74	114.27 ± 0.06	0.31 ± 0.007
	B2	5.625	69.375	110.33 ± 1.45	0.53 ± 0.038
1:9	C1	8	72	220.53 ± 0.67	0.49 ± 0.007
	C2	7.5	67.5	142.03 ± 0.57	0.46 ± 0.005

Data are presented as mean ± SD (n = 3)

Visual observation showed that formulations A1-A8, B1-B2, and C1-C2 were clear or LC gel-like, while A9, B3-B6, and C3-C4 appeared slightly turbid. Samples A10-A13 were clear with low-viscosity LC characteristics, whereas B7-B10 and C5-C8 were highly cloudy and viscous, and B11-B13 and C9-C13 formed turbid dispersion, indicating composition-dependent phase behavior (Figure 2.). Transparent LC

gel-like samples were further evaluated for DS and PdI to identify nanoscale characteristics. Based on these results, the composition limits were defined using formulations with DS < 200 nm and low PdI (Table 1.). The optimal nanoemulsion region was established at 1-6% oil and 19-76% for Smix, which served as the boundaries for subsequent optimization using SLD.

Table 2. DS, PdI, and ZP of APE nanoemulsion for eight experimental runs

Run	Composition ratio					DS (nm)	PdI	ZP (mV)
	Oil (%)	Smix (%)	APE (mg)	Enhancer* (%)	Water (v/v)			
1	4	19	100	30	46	129.833 ± 0.208	0.238 ± 0.004	-22.3 ± 1.908
2	4	19	100	30	46	134.867 ± 0.208	0.233 ± 0.001	-23.4 ± 0.500
3	2.5	20.5	100	30	46	24.313 ± 0.087	0.204 ± 0.006	-15.267 ± 3.430
4	1	22	100	30	46	13.277 ± 0.070	0.15 ± 0.008	-11.903 ± 2.492
5	1.75	21.25	100	30	46	17.127 ± 0.067	0.173 ± 0.005	-12.333 ± 0.451
6	3.25	19.75	100	30	46	63.393 ± 0.532	0.253 ± 0.004	-22.267 ± 1.193
7	2.5	20.5	100	30	46	39.923 ± 0.182	0.201 ± 0.009	-8.637 ± 0.137
8	1	22	100	30	46	12.873 ± 0.100	0.176 ± 0.026	-6.277 ± 0.647

Data are presented as mean ± SD (n = 3); Enhancer = Transcutol P

Formulation and optimization of APE nanoemulsion using SLD

The APE nanoemulsion was prepared using the SLD (Table 2.), and the absence of phase separation confirmed adequate solubility of APE in the system. All formulations produced transparent nanoemulsions, with DS below 150 nm. Increasing oil concentration led to larger DS, while higher surfactant concentration reduced it. These DS values are consistent with previous herbal nanoemulsions, including 138-234 nm (Pathania et al., 2022), 13-45 nm (Syukri et al., 2020), and 12.96-37.32 nm (Chahyani & Zai, 2024), although variations may arise from differences in formulation components and preparation methods. DS is a critical parameter influencing solubility, bioavailability, and stability (Syukri et al., 2020), with values

below 200 nm generally preferred for topical delivery due to improved surface area and kinetic stability, although larger droplets may still support transdermal permeation (Danaei et al., 2018). The DS response followed a quadratic model with a significant p-value and a non-significant lack of fit, indicating a good model fit (Table 3.). High R² and adjusted R² values confirmed agreement between predicted and experimental data, while adequate precision indicated a reliable signal (Jusril et al., 2022). The model showed positive effects of oil and Smix and a negative interaction term, indicating that increasing either component individually increased DS, whereas their interaction reduced it. This trend was consistent with experimental results (Table 2.), where higher oil content produced larger droplets, while lower oil combined with higher Smix resulted in smaller droplets.

Table 3. Regression equations and statistical parameters derived from SLD for nanoemulsion characteristics.

Parameter	Response		
	DS	PdI	ZP
Model	Quadratic	Linear	Linear
p-value	0.0001 (significant)	0.0014 (significant)	0.0049 (significant)
Lack of fit (p-value)	0.4814 (not significant)	0.1879 (not significant)	0.5175 (not significant)
Final equation	Y=130.71A+14.4B-165.53AB (1)	Y = 0.24 A + 0.16 B (2)	Y = -22.52 A - 8.08 B (3)
R ²	0.9879	0.8389	0.7583
Adjusted R ²	0.9831	0.8121	0.7180
Predicted R ²	0.9739	0.7113	0.6144
Adequate Precision	29.1738	10.5403	8.1804

A = oil, B = Smix, AB = interaction A and B

The PdI reflects DS distribution and uniformity, with values ≤ 0.3 indicating acceptable homogeneity (Danaei et al., 2018). In this study, PdI values indicated narrow size distribution and good homogeneity (Table 2.) (Jusril et al., 2022), and were lower than those reported in similar systems, such as *Phyllanthus niruri* nanoemulsion (0.2-0.4) and beta-carotene SNEDDS (up to 0.541), suggesting improved size control and physical stability (Chahyani & Zai, 2024; Pathania et al., 2022). The PdI response followed a linear model with a significant p-value and non-significant lack of fit, indicating adequate model performance (Table 3.).

The R² and adjusted R² values confirmed good agreement between predicted and experimental data, while adequate precision indicated sufficient signal (Ananingsih, Pratiwi, Soedarini, & Putra, 2024). The model showed a positive contribution of oil and Smix, with oil exerting a stronger effect on size distribution. This was consistent with experimental observation (Table 2.), where higher oil content resulted in broader distributions, while lower oil levels produced more uniform droplets.

ZP reflects droplet surface charge and contributes to nanoemulsion stability. All formulations exhibited

negative ZP values, indicating moderate electrostatic stabilization, supported by steric stabilization from Tween 80 (Balakumar, Raghavan, Selvan, Prasad, & Abdu, 2013; Dash, Mohammed, Humaira, & Ramesh, 2015; Yang et al., 2019). These values are comparable to *Phyllanthus niruri* nanoemulsion (-15.0 to -18.4 mV) and slightly lower than propolis-based systems (< -38 mV), suggesting sufficient repulsion to minimize aggregation. Values below ± 30 mV indicate moderate electrostatic stabilization, with combined electrostatic-steric effects supporting stability (Pathania et al., 2022; Syukri et al., 2020). The ZP response followed a linear model with a significant p-value and a non-significant lack of fit, indicating good model fit (Table 3.). The R^2 and adjusted R^2 values showed acceptable agreement, while adequate precision confirmed model reliability. The model indicated that both oil and Smix increased the negative surface charge, with oil as the dominant factor. This was consistent with experimental results (Table 2.), where higher oil content produced more negative ZP, while lower oil content

resulted in less negative values, indicating oil as the primary contributor to electrostatic stabilization.

Optimization and verification of formulation

Determination of optimum APE nanoemulsion formula

Optimization of the nanoemulsion formulation using the SLD approach was performed, and the optimum formulation was selected based on a desirability value close to 1. The optimization criteria were defined to minimize DS and PdI while maximizing the absolute ZP, with parameter ranges established from experimental data. The SLD model generated an optimal formulation with a desirability value of 0.948, indicating a high level of agreement between the predicted responses and the defined targets (Chahyani & Zai, 2024). The optimal composition consisted of oil and Smix at a ratio of 4:19, with predicted DS, PdI, and Zp values of 130.707 nm, 0.245, and -22.517. The corresponding instrument output report confirming these predictions is presented in (Figure 3.).

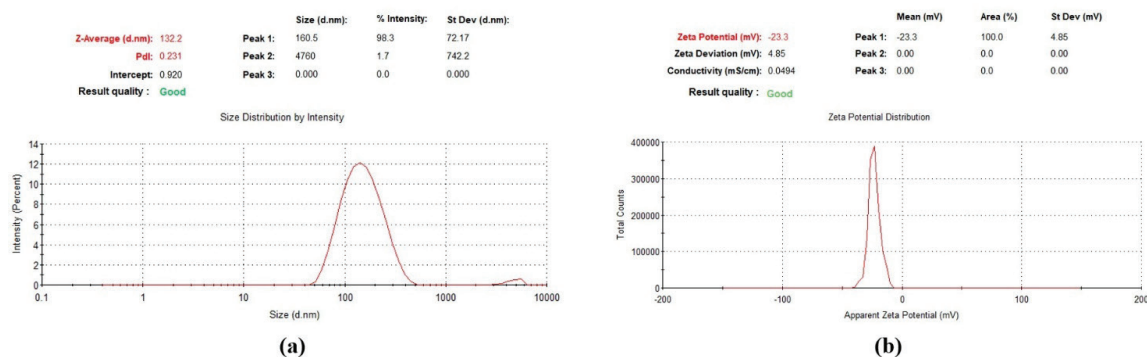


Figure 3. DS and PdI distribution (a), ZP distribution (b) of optimum APE nanoemulsion

Verification of optimum APE nanoemulsion

The verification of the optimal formula focused on comparing experimental outcomes with predicted SLD results, thereby confirming the accuracy and reproducibility of the formulation. The one-sample t-test at the 95% confidence level revealed no statisti-

cally significant difference between the experimental findings and the SLD-predicted outcomes (Table 4.). These findings indicate that the SLD model accurately predicted the optimal APE nanoemulsion, with experimental DS, PdI, and ZP closely matching the predicted values.

Table 4. Comparison between predicted and experimental parameter values (n = 3)

Parameter	Predicted value	Experimental value	Sig. 2-sided p	Significance
DS (nm)	130.707	132.678 ± 1.802	0.199	Not significant
PdI	0.245	0.241 ± 0.006	0.306	Not significant
ZP (mV)	-22.517	-23.2 ± 0.569	0.173	Not significant

Viscosity analysis of optimized APE nanoemulsion

The viscosity of the optimized APE nanoemulsion was 48.00 ± 0.67 mPa·s (mean $\pm$ SD, n = 3), a relatively low value that may be influenced by the proportion of oil, Smix, and water in the formulation. Higher water content generally lowers viscosity, while lower Smix levels increase it by raising interfacial tension, affect-

ing both stability and drug release (Malik et al., 2022). Compared with previous studies, *Phyllanthus niruri* extract nanoemulsion exhibited viscosities of 38 ± 7 mPa·s, and AND nanoemulsion showed 28 mPa·s, indicating that low to moderate viscosity values are common among nanoemulsion systems (Pathania et al., 2022).

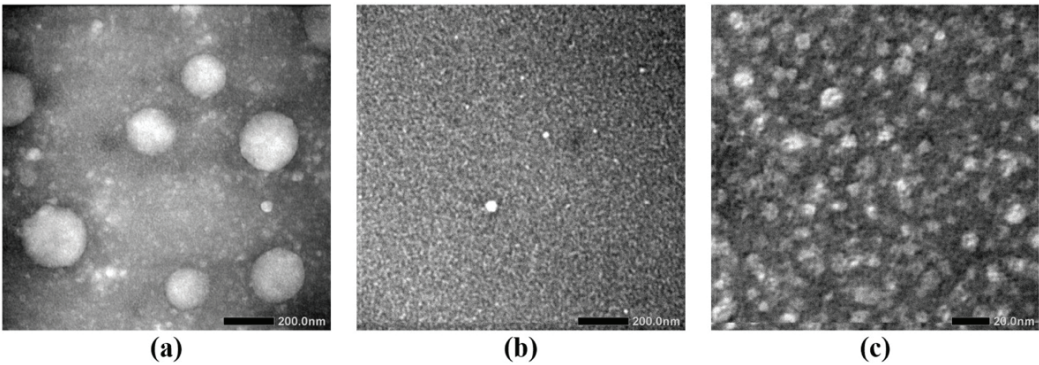


Figure 4. TEM images of optimized APE nanoemulsion at magnification 20,000x (a); blank nanoemulsion at 20,000x (b); blank nanoemulsion at 150,000x magnification (c)

TEM analysis

The morphology of the optimized APE nanoemulsion observed by TEM (Figure 4a.) showed spherical droplets with smooth surfaces and a relatively uniform distribution, with an apparent size of approximately 150–200 nm, in good agreement with the DLS result (132.68 nm). In contrast, the blank nanoemulsion appeared more homogeneous at 20,000x (Figure 4b.), while finer nanoscale structures became more visible at higher magnification of 150,000x (Figure 4c.). The larger DS in the APE nanoemulsion can be attributed to the incorporation of APE into the oil phase, which may alter interfacial properties, disrupt surfactant packing, and increase the effective volume of the dispersed phase. Similar increases in droplet size upon drug incorporation have been reported in nanoemul-

sion systems (Najlah et al., 2016), while the extent of this effect depends on formulation composition and interfacial characteristics (Deniz et al., 2025; Mishra, Kaushik, Sharma, & Alam, 2023).

FT-IR analysis

FT-IR is a rapid and reliable analytical technique for identifying functional group characteristics and assessing potential drug-excipient interaction (Verma, Zaman, Verma, Verma, & Sarwa, 2020). FT-IR spectroscopy was conducted to verify the incorporation of AND from APE into the nanoemulsion and to detect possible interactions with formulation components. The spectra of the AND standard, APE, blank nanoemulsion, and APE nanoemulsion are presented in (Figure 5.).

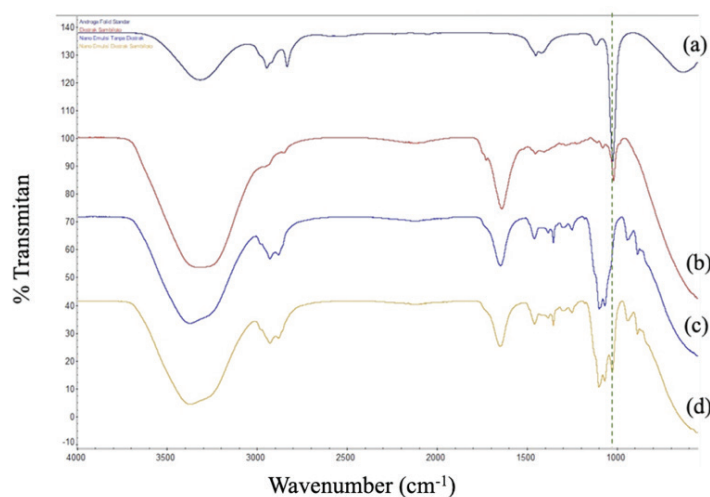


Figure 5. Overlay of FT-IR spectra of AND standard (a), APE (b), blank nanoemulsion (c), and APE nanoemulsion (d)

The AND standard displayed a characteristic band at 1021.82 cm^{-1} , corresponding to $\text{C}=\text{O}$ stretching of the lactone group. Similar peaks appeared at 1016.31 cm^{-1} in the extract and 1024.74 cm^{-1} in the APE nanoemulsion, but were absent in the blank nanoemulsion. The slight peak shifts indicate the absence of significant chemical interactions or new bond formation, suggesting that AND was incorporated in the nanoemulsion mainly through physical interaction, such as encapsulation or adsorption at the interface.

Thermodynamic stability evaluation

To assess the shelf-life of nanoemulsion formulations, thermodynamic stability studies are essential. These tests evaluate the durability of APE nanoemul-

sion under extreme conditions and intense kinetic stress, ensuring stability during storage (Senapati, Sahoo, & Sahu, 2016). The results are summarized in (Figure 6.). The optimal formulation successfully passed the centrifugation, heating-cooling, and freeze-thaw tests, exhibiting no phase separation or creaming and maintaining a clear appearance. Centrifugation confirmed the nanoemulsion's physical integrity and transparency, while heating-cooling and freeze-thaw cycles demonstrated its stability under temperature variations (Syukri et al., 2020). These findings confirm that the optimized APE nanoemulsion possesses robust thermodynamic and kinetic stability, distinguishing it from conventional emulsions.

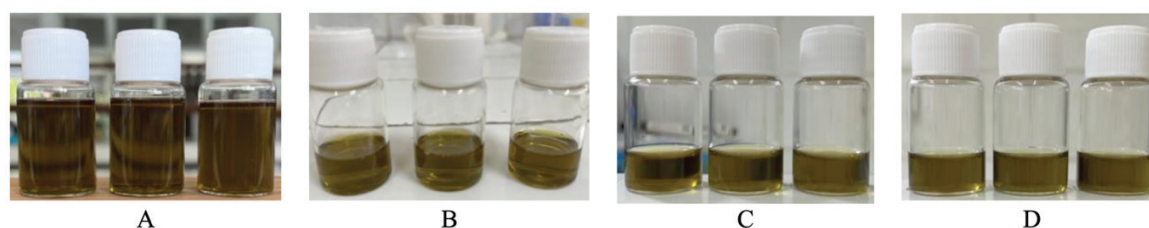


Figure 6. Thermodynamic stability of the optimum APE nanoemulsion: (A) before test; (B) after centrifugation; (C) after heating-cooling cycle; (D) after freeze-thaw cycle

Study limitations

This study has several limitations. First, APE solubility was assessed primarily by visual inspection,

which may not detect minor undissolved particles or subtle phase separation. Second, viscosity was measured only for the optimized formulation, limiting

the evaluation of flow behavior across all runs. Future studies could apply more sensitive quantitative methods for solubility determination and include viscosity measurements for all formulations to strengthen formulation design.

CONCLUSION

The low-energy method combined with SLD successfully optimized an APE nanoemulsion with a DS of 132.678 ± 1.802 nm, PdI of 0.241 ± 0.006 , and ZP of -23.2 ± 0.569 mV. The optimized formulation showed low viscosity (48.0 ± 0.67 mPa-s), spherical morphology without aggregation, and good stability under centrifugation, heating-cooling, and freeze-thaw conditions. FT-IR analysis confirmed the successful incorporation of APE without chemical interaction with formulation components. The nanoscale DS and narrow PdI suggest improved solubility of APE and potential enhancement of topical bioavailability. Overall, the developed nanoemulsion represents a promising carrier system for the topical delivery of APE.

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AUTHOR CONTRIBUTION STATEMENT

Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization (MAA). Methodology, Review & editing, Conceptualization (AKN). Review & editing, Conceptualization (TH). Review & editing, Conceptualization (KZ).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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