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Red Palm Oil Submicron Emulsions Formulated by D-Phase Emulsification: A D-Optimal Mixture Design Study Using Sucrose Palmitate and Glycerol

(Emulsi Submikron Minyak Sawit Merah Diformulasikan melalui Pengemulsian Fasa-D: Kajian Reka Bentuk Campuran D-Optimal menggunakan Sukrosa Palmitat dan Gliserol)

Elsa Fitria Apriani[1], Mahdi Jufri[1,*], Tommy Julianto Bustami Effendi[2,3], Windy Keumala Budianti[4], Nurzalina Abdul Karim Khan[5], Budiawan[6] & Adilah Marwa[1]

[1]*Laboratory of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Indonesia, Depok, 16424, Indonesia*
[2]*Faculty of Pharmacy, Universiti Teknologi MARA Selangor, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia*
[3]*Department of Pharmacy Mitra Bunda Health Institute, Batam, 29454, Indonesia*
[4]*Department of Dermatology and Venereology, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo National Central General Hospital, Jakarta, 10430, Indonesia*
[5]*Discipline of Pharmaceutical Technology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia*
[6]*Department of Chemistry, Faculty of Sciences, Universitas Indonesia, Depok, 16424, Indonesia*

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ABSTRACT

Red palm oil (RPO) contains carotenoids, which are highly beneficial to human health owing to their antioxidant properties. RPO has low stability during storage; hence, it must be formulated in a submicron emulsion. This study aims to enhance the formulation of RPO submicron emulsion using a D-phase emulsification method with D-optimal mixture design methodology. The effect of the concentration of RPO (45%-60%), sucrose palmitate (10%-25%), and glycerol (25%-40%) on globule size, polydispersity index (PDI), and zeta potential was investigated. The optimum formula was then subjected to a short-term accelerated stability test at 40 °C for 7 days, comparing the carotenoid retention in the submicron emulsion versus bulk RPO and characterized using Transmission Electron Microscopy (TEM) and Fourier Transform Infrared Spectroscopy (FTIR). The data showed that an increase in component concentrations below a preset limit resulted in a significant change in response cubically, thus yielding high coefficients of determination ($R^2>0.96$) for all three responses ($p<0.05$). The optimum formula was achieved with components in the proportions of 55.70% RPO, 12.05% sucrose palmitate, and 32.25% glycerol, with globule size, PDI, and zeta potential of 184.20±2.75 nm, 0.079±0.011, and -40.40±0.53 mV, respectively. Notably, the short-term stability study showed that the submicron emulsion system significantly improved carotenoid stability compared to bulk RPO after 7 days at 40 °C. The TEM study confirmed that the emulsion globules form spheres and are uniformly distributed, whereas the FTIR study showed no chemical interactions. In summary, the D-phase emulsification method effectively produced a stable RPO submicron emulsion.

Keywords: D-optimal mixture design; D-phase emulsification; optimization; red palm oil; submicron emulsion

ABSTRAK

Minyak sawit merah (MSM) mengandungi karotenoid yang sangat bermanfaat untuk kesihatan manusia kerana sifat antioksidannya. MSM mempunyai kestabilan yang rendah semasa penyimpanan; oleh itu, ia mesti diformulasikan dalam emulsi submikron. Kajian ini bertujuan untuk meningkatkan formulasi emulsi submikron MSM menggunakan kaedah pengemulsian fasa-D dengan metodologi reka bentuk campuran D-optimum. Kesan kepekatan RPO (45%-60%), sukrosa palmitat (10%-25%) dan gliserol (25%-40%) ke atas saiz globul, PDI dan potensi zeta telah dikaji. Formula optimum kemudiannya tertakluk kepada ujian kestabilan dipercepatkan jangka pendek pada suhu 40 °C selama 7 hari, membandingkan pengekalan karotenoid dalam emulsi submikron berbanding MSM pukal dan dicirikan menggunakan Mikroskopi Elektron Pengimbasan (TEM) dan spektroskopi Fourier transformasi inframerah (FTIR). Data menunjukkan bahawa peningkatan kepekatan komponen di bawah had pratetap menyebabkan perubahan ketara dalam tindak balas secara kubik, dengan itu menghasilkan pekali penentuan yang tinggi ($R^2>0.96$) untuk ketiga-tiga tindak balas ($p<0.05$). Formulasi terbaik dicapai dengan komponen dalam perkadaran 55.70% RPO, 12.05% sukrosa palmitat dan 32.25% gliserol dengan saiz globul, PDI dan potensi zeta masing-masing adalah 184.20±2.75 nm, 0.079±0.011 dan - 40.40±0.53 mV. Terutamanya, kajian kestabilan jangka pendek menunjukkan bahawa sistem emulsi submikron meningkatkan kestabilan karotenoid dengan ketara berbanding MSM pukal selepas 7 hari pada suhu 40 °C. Kajian TEM telah mengesahkan bahawa globul emulsi membentuk sfera dan diedarkan secara seragam, manakala kajian FTIR menunjukkan tiada interaksi kimia. Ringkasnya, kaedah pengemulsian fasa-D berkesan menghasilkan emulsi submikron MSM yang stabil.

Kata kunci: Emulsi submikron; minyak sawit merah; pengemulsian fasa D; pengoptimuman; reka bentuk campuran D-optimum

INTRODUCTION

Red palm oil (RPO) is obtained from the mesocarp of the palm oil fruit, *Elaeis guineensis*, which is mainly grown in tropical regions such as Malaysia and Indonesia. RPO is a significant source of bioactive elements, especially carotenoids (α- and β-carotene) with a concentration in the range of 500-700 mg/kg (Chong et al. 2018; Tan et al. 2021). The carotenoid structure protects the cell from oxidative damage by delocalizing electrons and transferring a hydrogen atom from a radical to an unpaired electron (Sharoni et al. 2012). In addition to its antioxidant function, β-carotene is a provitamin A and is important for the health of the eyes, skin, and the immune system (Ellison 2015).

Carotenoids enhance the significance of RPO as a key component in multiple formulations involving pharmaceutical, nutraceutical, and cosmetic applications. However, incorporating RPO into formulation systems presents significant challenges, especially due to its limited water solubility and diminished stability under various storage conditions. Previous research indicates that carotenoid concentrations significantly diminish when subjected to elevated temperatures (60 °C) or prolonged storage (12 weeks) (Ping, Idris & Maurad 2020; Tan et al. 2017). A delivery system is essential for preserving bioactive compounds from external environmental influences and improving their bioavailability.

Submicron emulsions are colloidal systems of dispersions of either oil-in-water (O/W) or water-in-oil (W/O) with active agents, lipophilic materials, surfactants, co-surfactants, and possibly cosolvents with globules between 10 and 500 nm (Mundada, Patel & Sawant 2016). This technology has been proven to improve emulsion stability, control the release of active agents, increase the passage of bioactive compounds through biological membranes, and prolong emulsion shelf life (Abdul Wahab et al. 2022; Lavanya, Namasivayam & John 2024; Ozogul et al. 2022). Therefore, submicron emulsions are an appropriate delivery system for sensitive bioactive compounds of RPO, including carotenoids.

An appropriate formulation technique is vital for developing a stable, homogeneous emulsion system that yields emulsions with a uniform, consistent globule size. The D-phase emulsification method provides a suitable formulation method that demands neither excessive thermal energy nor considerable mechanical energy, thus preserving the integrity of temperature-sensitive compounds. With this method, a D phase is created from a mixture of oil, surfactant, and co-surfactant, and then diluted to form an emulsion with reduced, uniformly dispersed globule size (Zhang et al. 2019). The choice of surfactant and co-surfactant is critical to the success of this method.

This study used sucrose palmitate as a surfactant and glycerol as a cosurfactant and cosolvent. Sucrose palmitate is a natural non-ionic surfactant composed of a hydrophilic part (sucrose) and a lipophilic part (palmitic acid). Compared to cyclodextrin and polysorbate surfactants, sucrose palmitate is a better interfacial tension decreasing, entrapment efficiency higher, globule size, and polydispersity index smaller, zeta potential higher, increasing surfactant at greater than 10% concentration (Anarjan & Tan 2013; Ariyaprakai & Tananuwong 2015). On the other hand, glycerol is a co-surfactant and co-solvent that, when used at more than 40% concentration, promotes the formation of phase D, increases the solubility of the active ingredients in the continuous phase, and also functions as a humectant that stabilizes the globule size during storage (Rao & McClements 2013; Saberi, Fang & McClements 2013).

A D-optimal mixture design was applied to determine the optimum formulation of an RPO submicron emulsion by evaluating the effect of different concentrations of RPO, sucrose palmitate, and glycerol on the globule size, polydispersity index (PDI), and zeta potential. The optimum formulation was then evaluated in a short-term accelerated stability test at 40 °C for 7 days. This involved comparing carotenoid retention in the submicron emulsion and in bulk RPO. The optimum formulation was also characterized by Transmission Electron Microscopy (TEM), and the chemical interactions were evaluated by Fourier-Transform Infrared Spectroscopy (FTIR).

MATERIALS AND METHODS

MATERIALS

Red palm oil was obtained from Harvist (Malaysia). Sucrose palmitate was purchased from Compass Food (Singapore), while glycerol and demineralized water were supplied by Bratachem (Indonesia).

EXPERIMENTAL DESIGN

The submicron emulsions were formulated using the D-phase emulsification method, which was conducted in two sequential stages (Yukuyama et al. 2018). First, RPO was mixed slowly with sucrose palmitate and glycerol on a magnetic stirrer at 500 rpm to produce a pre-concentrated RPO emulsion. After that, demineralized water was used to dilute the pre-concentrated RPO emulsion at room temperature while stirring gently with a magnetic stirrer to create the final submicron emulsion.

A D-optimal mixture design approach was employed to optimize the formulation of the pre-concentrated emulsion. This statistical framework facilitated the assessment of the synergistic effects of three independent variables: RPO concentration (X_1: 45-60%), sucrose palmitate concentration (X_2: 10-25%), and glycerol concentration (X_3: 30-45%). These variables were systematically examined for their impact on three critical response parameters: globule size (Y_1), PDI (Y_2), and zeta potential (Y_3).

A total of 16 experimental runs, including five replicates at the center point to ensure model validity and reproducibility, were generated and randomized using the Design-Expert® software (Stat-Ease Inc., Minneapolis, USA). Table 1 shows the composition of each formulation in the experimental matrix.

DETERMINATION OF GLOBULE SIZE, POLYDISPERSITY INDEX, AND ZETA POTENTIAL

Using a Particle Size Analyzer (PSA), a 1 mL sample of the submicron emulsion was inserted into an optical polystyrene cuvette, and the globule size, PDI and zeta potential were measured (Apriani, Mardiyanto & Hendrawan 2022; Mardiyanto, Apriani & Helyken 2023).

DETERMINATION OF OPTIMUM FORMULA

The optimum formula is determined from the analytical results on globule size, PDI, and zeta potential, in line with the objectives outlined in Table 1. The Design Expert program will use a desirability value approaching 1 to determine the most appropriate formula for these parameters (Apriani et al. 2023; Miksusanti, Apriani & Bihurini 2023).

CONFIRMATION OF MODEL

The optimum formula, obtained through model simulation using expert design analysis, was replicated experimentally in triplicate. The globule size, PDI, and zeta potential were measured, and the experimental values were compared with the predicted values to calculate the percentage error (% error), which served as an indicator for validating the model's predictive accuracy.

SHORT-TERM STABILITY STUDY OF OPTIMUM FORMULA

The stability of optimized RPO submicron emulsions was evaluated both physically, by observing globule size, PDI, and zeta potential, and chemically, by measuring beta carotene levels at day 0, 3, 5, and 7. To ensure a fair comparison in chemical stability, the bulk RPO was diluted with an inert carrier to match the exact beta carotene concentration present in the optimized submicron emulsion formula. Both the optimized submicron emulsion and the standardized bulk RPO were placed in light-resistant, airtight glass vials to prevent photo-oxidation. The samples were then stored at an accelerated temperature of 40 ± 2 °C for 7 days. The beta-carotene content was measured by High-Performance Liquid Chromatography at 450 nm, as reported by Barba et al. (2006). The degradation rate was expressed as the percentage of beta carotene retention, calculated using the following formula:

$$\textit{Retention of beta carotene}\ (\%) = \frac{ct}{c0} \times 100\% \qquad (1)$$

where C0 is the Initial beta carotene concentration at day 0 and Ct is the beta carotene concentration at day t.

MORPHOLOGY OF OPTIMUM FORMULA

The optimum formula's globule morphology was examined with a transmission electron microscope (TEM) at 80 kV. The TEM staining procedure consists of the following steps: The submicron emulsion formulation was diluted with demineralized water at a ratio of 1:50, and a drop of

TABLE 1. Coded levels of independent and dependent variables used in D-optimal mixture design

Independent variables	Code	Low limit (%)	High limit (%)
Red palm oil concentration	A	45	60
Sucrose palmitate concentration	B	10	25
Glycerol concentration	C	30	45
Dependent variables	**Code**	**Goals**	
Globule size	Y1	In range: 150 - 200 nm	
Polydispersity index	Y2	Minimize: Below 0.1	
Zeta potential	Y3	Less than -30 mV or greater than + 30 mV	

the sample was put into a copper grid. The sample was soaked in a uranyl acetate solution for 10 s. The object was drained with water for two 1-s intervals, and the exposed surfaces were wiped with filter paper (Visetvichaporn et al. 2020).

FTIR ANALYSIS

FTIR analysis studied potential chemical interactions between RPO, sucrose palmitate, and glycerol in the optimal formulation for the RPO submicron emulsion. The measurements were taken using an FTIR spectrophotometer in the region of 4000-500 cm^{-1}. The emulsion was prepared by placing a thin layer on the ATR (Attenuated Total Reflectance) crystal and gently pressing it to ensure proper contact (Almeida et al. 2019). In this study, the submicron emulsion spectra were analyzed alongside the spectra of each individual component (pure RPO, sucrose palmitate, glycerol) to identify the occurrence of potential shifts, or changes in the submicron emulsion spectra, to capture the presence of absorption bands to demonstrate a possible molecular interaction or new bond formations in the formulation.

DATA ANALYSIS

A statistical analysis was conducted to assess the effects caused by the concentration variation of RPO (X_1: 45-60%), sucrose palmitate (X_2: 10-25%), and glycerol (X_3: 30-45%) on three response variables globule size (Y_1), polydispersity index (Y_2) and zeta potential (Y_3). Data design and analysis were conducted using Design-Expert® software version 13 (Stat-Ease Inc., Minneapolis, USA) using the D-optimal mixture design methodology. The mathematical models used in the analysis include several forms of polynomial regression models, specifically:

Linear Model: $Y=\beta_1X_1+\beta_2X_2+\beta_3X_3$

Quadratic Model: $Y=\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3$

Cubic Model:

$Y=\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3+\beta_{123}X_1X_2X_3$

As regards model fitting, the appropriate model is selected based on the following criteria: F statistic (ANOVA), R^2, Adjusted R^2, Predicted R^2, and the p-value from the model significance test. The lack of a Fit test establishes the model's validity; the model is considered acceptable if the p-value is greater than 0.05.

RESULTS AND DISCUSSION

RPO SUBMICRON EMULSION

In this study, RPO submicron emulsions were prepared by dispersing a preconcentrated emulsion into distilled water. The results for preconcentrated and final submicron emulsions for all 16 formulations are shown in Figure 1. RPO, sucrose palmitate, and glycerol yielded preconcentrated emulsions that were thick and homogeneous, with a yellow or orange hue, as shown in Figure 1(A). The process starts with the formation of the D-phase, a surfactant-rich phase produced by the reaction of sucrose palmitate with glycerol. This phase facilitates the spontaneous formation of an isotropic, thermodynamically stable system upon mixing with RPO, resulting in an oil-in-D-phase (O/D-phase), also called a pre-concentrated emulsion (Zhang et al. 2019). Upon water dilution, sucrose palmitate moves quickly towards the o/w interface, decreasing interfacial tension and allowing oil to be stabilized. This leads to the spontaneous formation of a submicron emulsion with nanoscale globule size and a transparent yellow to slightly turbid appearance, as shown in Figure 1(B).

GLOBULE SIZE, POLYDISPERSITY INDEX, AND ZETA POTENTIAL OF RPO SUBMICRON EMULSION

The RPO submicron emulsion formula was tested for globule size, PDI, and zeta potential, which are shown in Table 2. The results of the globule size of the 16 formulas ranged from 145.67 ± 1.19 to 265.77 ± 6.79 nm, PDI ranged from 0.035 ± 0.023 to 0.194 ± 0.024, and zeta potential ranged from -24.63 ± 0.15 to -38.63 ± 0.40 mV. Variations in the results of globule size, PDI, and zeta potential from the 16 formulas were due to differences in the concentration of RPO (X_1), sucrose palmitate (X_2), and glycerol (X_3) used. The characteristic results obtained in Table 2 were continued with the D-optimal mixture design analysis.

ANOVA AND FIT STATISTIC MODEL

The primary step in the formula optimization analysis involves reviewing the model fit using ANOVA and other relevant fit measures, including the model p-value, lack-of-fit p-value, R^2, adjusted R^2, adequate precision, SD, and CV. In statistical terms, the model is acceptable if the model p-value is less than 0.05, the lack of fit p-value is greater than 0.05, R^2 is greater than 0.70, and the difference between R2 and adjusted R^2 is less than 0.2, indicating overfitting and adequate precision is greater than 4; this indicates an adequate signal-to-noise ratio. In addition, a well-defined model will have minimal SD and a CV of less than 10%, indicating precision and high reproducibility.

Based on the results in Table 3, the Cubic Model was chosen as the best-fit model for all responses (globule size, PDI, and zeta potential). Compared to other models, the Cubic model best described the data variation, with significant sequential p-values ($p < 0.0001$) and a non-significant lack of fit ($p > 0.05$). In contrast to this model, several others (linear, quadratic and special cubic) demonstrated a significant lack of fit ($p<0.05$). Thus, in order to obtain optimum results for the RPO submicron emulsion, the Cubic model was used.

The cubic model was statistically significant, with all three dependent variables having p-values < 0.05. This shows a positive regression correlation with strong relationships with the independent variables. Additionally, the lack-of-fit p-values between 0.7012 and 0.9811 indicate that the model fits the experimental data well, with no significant unexplained variation. The data show that the determination coefficient (R2) values indicate the model explains 96.76% - 99.89% of the data. R2 values range from 0.9676 to 0.9989. The adjusted R2 values were within 0.2 of the R2, suggesting that the model is robust and reliable with multiple factors and interactions. Adequate precision values exceeded 4 across all dependent variables, confirming the model shows an adequate signal with no noise. The model's predictive capability is solidified with the consistency and reproducibility of the experimental results with low SD and CV values across all responses.

PREDICTED VS. ACTUAL

The predicted vs. actual graph compares the model's predicted and experimental values, assessing the model's ability to accurately predict the data. The predicted vs. actual graph results are shown in Figure 2. The model has a reasonable fit (the values of y and x are nearly equal) because most of the three dependent variables have data points clustered around the diagonal line (y = x). The three dependent variables have data points close to the diagonal line (y = x). In contrast to the PDI data, the data points in the globule size and zeta potential data appear to be extremely near to the diagonal line. The data do not have significant outliers, as the R2 and adjusted R2 values are nearly 1.

CONTOUR PLOT AND 3D SURFACES

Contour plots and 3D surfaces illustrate the correlation between the independent variables (X_1: RPO, X_2: sucrose palmitate, and X_3: glycerol) and the dependent variables: globule size, PDI, and zeta potential. The 3D-surface graph is three-dimensional, whereas the contour plot is a two-dimensional representation of the 3D surface. Figure 3 displays the contour plots and 3D surface graphs for the three dependent variables. Glycerol (X_3) is point C, marked in the lower right corner (45%); sucrose palmitate (X2) is point B, marked in the lower left corner (25%); and RPO (X_1) is point A and occupies the top vertex of the triangle (60%). The red areas exhibit a stronger response, while the blue areas in Figure 3 exhibit a weaker response. The zeta potential value can be reduced, and the size of the globule and PDI can be increased by using RPO at a high concentration of up to 60%, as indicated by the graph. The zeta potential value can be increased, and the globule size can be reduced using sucrose palmitate at a high concentration (25%). In the interim, the PDI and zeta potential values can be reduced by using a high concentration of glycerol.

INFLUENCE OF INDEPENDENT VARIABLES ON ZETA POTENTIAL, PDI, AND GLOBULE SIZE

The relationship between RPO's concentration (X_1), sucrose palmitate (X_2), and glycerol (X_3) and the response of globule size (Y_1), PDI (Y_2), and zeta potential (Y_3) is defined by a derived response equation, which is based upon the regression coefficient and p-value of each considered factor. Each response's p-value and regression coefficient are shown in Table 4. Only models with p-values considered significant ($p < 0.05$) were used in the final models. The response equations produced in this research are as follows:

$$\text{Globule Size } (Y_1) = 265.73X_1 + 167.66X_2 + 190.65X_3 - 217.6X_1X_2 - 29.38X_1X_3 - 24.36X_2X_3 - 472.46X_1X_2X_3 - 522.15X_1X_2(X_1-X_2) + 184.15X_1X_3(X_1-X_3) - 358.14X_2X_3(X_2-X_3)$$

$$\text{PDI } (Y_2) = 0.195X_1 + 0.051X_2 + 0.084X_3 - 0.262X_1X_2 - 0.477\,X_1X_2\,(X_1-X_2) + 1.238X_2X_3\,(X_2-X_3)$$

$$\text{Zeta Potential } (Y_3) = -38.63X_1 - 26.27X_2 - 38.22X_3 + 15.91X_1X_2 + 2.95X_1X_3 - 15.97X2X3 - X_1X_2\,(X_1-X_2) - 64.05\,X_1X_3\,(X_1-X_3) - 53.07X_2X_3\,(X_2-X_3)$$

The regression coefficient can be positive (+) or negative (-). The notation explains how factors influence the response. An increase in the value of a factor (+) indicates that the coefficient will positively affect the response value, whereas a decrease (-) indicates the opposite. Moreover, the magnitude of the regression value also enables analysis to reveal the absolute impact of the regression factor on the response. Thus, if the factor in concern has a greater absolute value, the response will be more sensitive to larger changes.

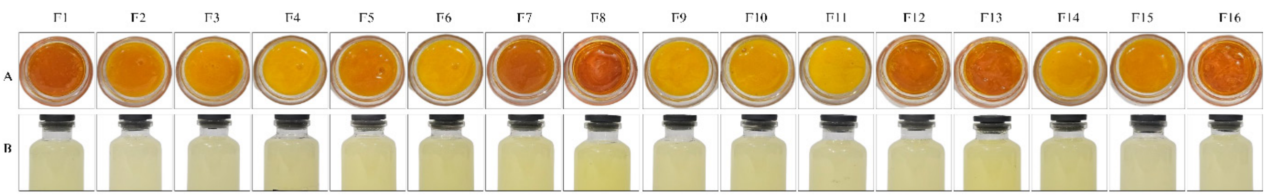

FIGURE 1. Pre-concentrated emulsion (A) and submicron emulsion (B) of 16 formulas

TABLE 2. Characteristics of red palm oil submicron emulsion

Run	Independent variables			Dependent variables		
	X_1	X_2	X_3	Y_1	Y_2	Y_3
1	52.5	10	37.5	220.40 ± 4.21	0.176 ± 0.019	-37.67 ± 1.31
2	47.5	20	32.5	160.50 ± 0.10	0.139 ± 0.004	-31.60 ± 0.35
3	52.5	10	37.5	219.73 ± 1.56	0.164 ± 0.018	-38.10 ± 0.26
4	45	17.5	37.5	170.23 ± 3.42	0.065 ± 0.009	-36.20 ± 0.53
5	52.5	10	37.5	222.40 ± 2.05	0.143 ± 0.008	-37.30 ± 0.00
6	45	10	45	189.90 ± 3.34	0.071 ± 0.028	-38.27 ± 0.12
7	50	15	35	160.37 ± 1.40	0.079 ± 0.018	-34.20 ± 0.10
8	60	10	30	265.77 ± 6.79	0.194 ± 0.024	-38.63 ± 0.40
9	45	17.5	37.5	173.73 ± 2.12	0.046 ± 0.005	-36.17 ± 0.97
10	45	10	45	191.40 ± 1.21	0.096 ± 0.020	-38.17 ± 0.85
11	47.5	12.5	40	188.23 ± 0.15	0.049 ± 0.033	-30.87 ± 1.10
12	50	20	30	190.33 ± 1.30	0.078 ± 0.018	-24.63 ± 0.15
13	55	15	30	145.67 ± 1.19	0.055 ± 0.014	-33.20 ± 0.10
14	45	17.5	37.5	175.23 ± 1.88	0.035 ± 0.023	-36.33 ± 0.47
15	45	25	30	167.70 ± 0.56	0.050 ± 0.031	-26.27 ± 0.25
16	52.5	17.5	30	162.90 ± 1.60	0.053 ± 0.027	-28.47 ± 0.25

X_1 = Red palm oil concentration (%), X_2 = Sucrose palmitate concentration (%), X_3 = Glycerol concentration (%), Y_1 = Globule size (nm), Y_2 = Polydispersity Index, Y_3 = Zeta Potential (mV). Data are given as mean±SD, n=3

TABLE 3. ANOVA and fit statistic model

	Response	Model	Model (p-value)	Lack of fit (p-value)	SD	% CV	R_2	Adjusted R_2	Adequate precision
Y_1	Globule size	Cubic	<0.0001	0.7012	1.77	0.94	0.9987	0.9967	85.6824
Y_2	PDI	Cubic	0.0008	0.7829	0.0148	15.88	0.9676	0.9190	12.4350
Y_3	Zeta potential	Cubic	<0.0001	0.9811	0.2385	0.6989	0.9989	0.9972	74.2625

SD = Standard Deviation, CV = Coefficient of Variations

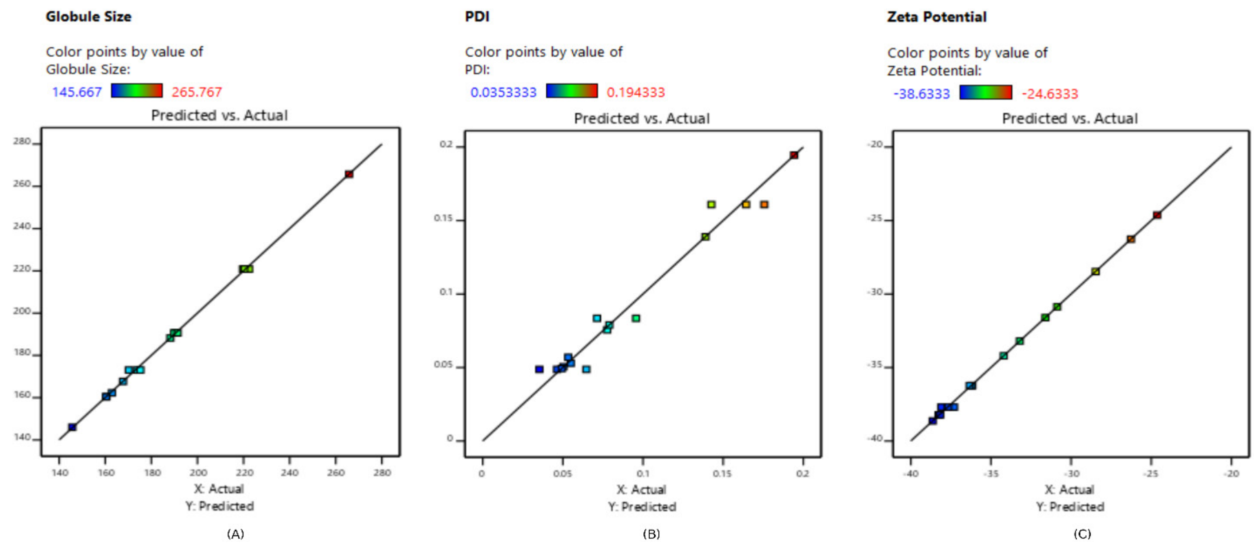

FIGURE 2. Predicted vs actual graph of globule size (A), PDI (B), and zeta potential (C)

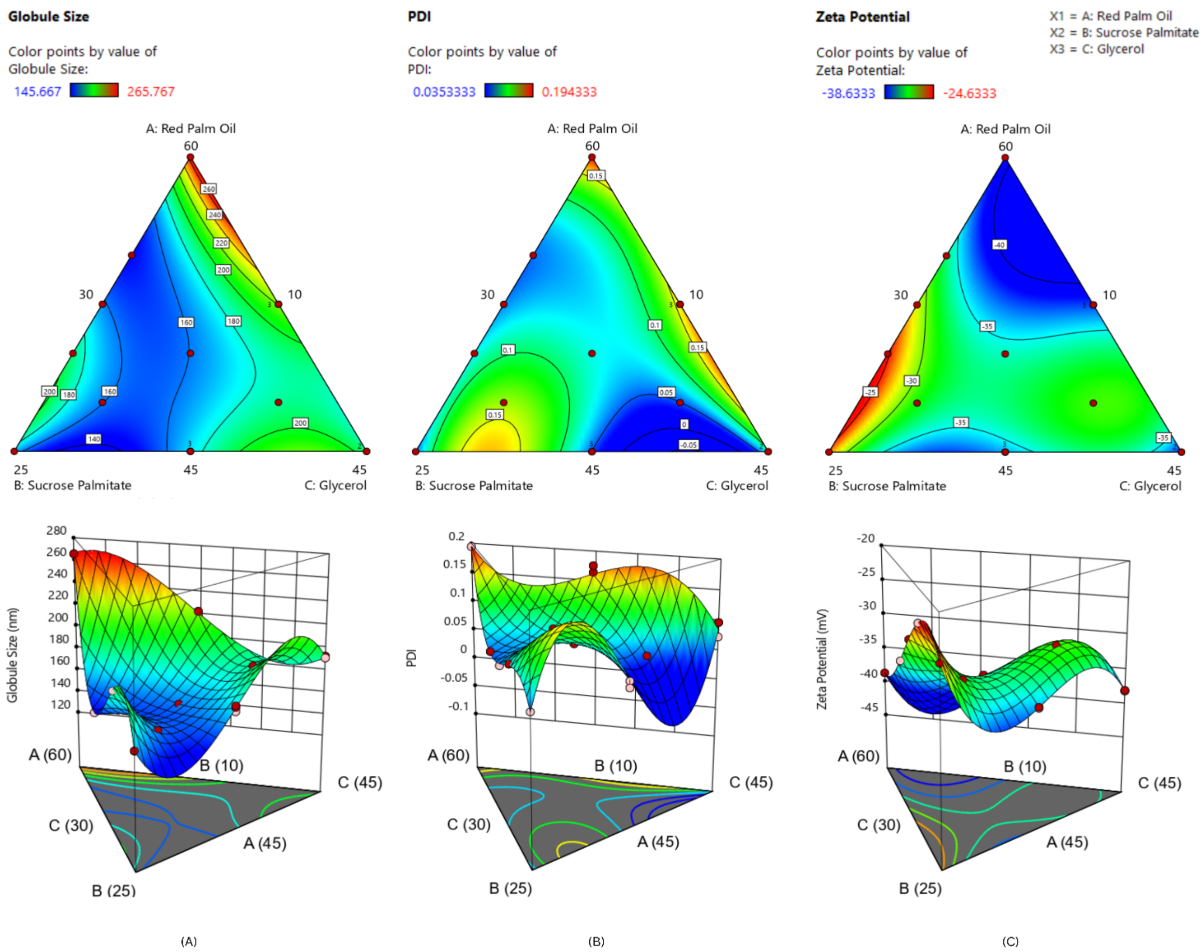

FIGURE 3. Contour plot and 3D-surface of globule size (A), PDI (B), and zeta potential (C)

The regression results indicate that the main factors and their interactions significantly affect all three responses. The globule size (Y_1) was most influenced by the higher-order interaction between red palm oil and sucrose palmitate, denoted by $X_1(X_1-X_2)$, with the highest absolute coefficient (522.15). Similarly, PDI (Y_2) was primarily influenced by the interaction $X_2(X_2-X_3)$, with a coefficient of 1.238. Moreover, the zeta potential (Y3) was heavily affected by the $X_1(X_1-X_3)$ high-level interaction, as it contained the −64.05 coefficient.

Data interpretation indicates that increased levels of red palm oil (X_1), sucrose palmitate (X_2), and glycerol (X_3) are likely the cause of increased globule size and PDI, resulting from increased viscosity that hinders emulsification. RPO hinders emulsification by increasing viscosity and reducing shear effectiveness, leading to larger, more uneven globule sizes (Wang et al. 2017). This is best seen in run 8 (60% RPO, 10% SP, and 30% Glycerol), which has the largest globule size (265.77 nm) and PDI (0.194) of all experimental runs. This is a good example of system destabilization when the surfactant-oil ratio is too low to overcome the viscosity of the internal phase. While sucrose palmitate is good at stabilizing globules at low concentrations, it can destabilize and promote coalescence (Henry et al. 2009). Increasing the concentration of sucrose palmitate may increase the absolute value of the zeta potential due to adsorption of sucrose palmitate at the oil-water interface, which is a cause of increased surface charge (Zhang et al. 2022). Glycerol, like RPO and sucrose palmitate, increases system viscosity and alters zeta potential and electrostatic interactions (Mirhosseini & Tan 2009).

Interactions between factors are important in moderating emulsion properties. For example, the interaction between sucrose palmitate and glycerol (X_2X_3) contributes to zeta potential enhancement and globule stabilization by creating a denser interfacial film. By forming junction sites during fat crystallization, sucrose palmitate can improve the structural properties of RPO and yield a more stable, consistent emulsion (Shu et al. 2023). Glycerol can stabilize small globules by forming hydrophobic contacts and hydrogen bonds with oil molecules and emulsifiers. These interactions maintain the emulsion's integrity and prevent an increase in globule size (Lu et al. 2023).

OPTIMUM FORMULA OF RPO SUBMICRON EMULSION

The optimum formula of RPO submicron emulsion was determined using Design-Expert® version 13, based on the desirability value approaching 1. According to the optimization goals outlined in Table 1, the program identified the optimum formulation as comprising 55.70% red palm oil (X_1), 12.05% sucrose palmitate (X_2), and 32.25% glycerol (X_3), with a desirability value of 1.000. The resulting pre-concentrated emulsion exhibited a thick, homogeneous texture and a yellowish-orange color, while the final submicron emulsion appeared as a clear yellow dispersion, as shown in Figure 4.

Importantly, this optimal formula reflects a RPO-to-surfactant ratio of approximately 4.6:1, a proportion shown to balance emulsification efficiency and system stability. Even though the oil content was relatively high, the sucrose palmitate surfactant was sufficient to stabilize the interface of the emulsion globule, whilst being fortified with a glycerol co-surfactant to maintain dispersed-phase consistency and hinder particle aggregation. This ratio allowed fine, well-stabilized globules to form without excessive surfactant that might otherwise destabilize the system.

The prediction model was validated using experimental measurements of globule size, polydispersity index (PDI), and zeta potential for the optimal formula. The results are presented in Table 5 and Figure 5. The globule size of the optimized submicron emulsion formula was 184.20 ± 2.75 nm, PDI was 0.079±0.011, and zeta potential was -40.40 ± 0.53 mV. All measured parameters fell within the model's predicted range. The submicron emulsion system is well-defined and stable, with globule sizes below 200 nm and a PDI of less than 0.1, indicating high uniformity and low instability.

In addition to a small globule size, a decrease in globule size in the submicron emulsion system results in increased surface area, a higher dissolution rate, and improved bioavailability of the hydrophobic active ingredient. These factors are vital for the development in pharmaceutical, cosmetic, and nutraceutical products (Wilson et al. 2021). In addition, a zeta potential greater than -40 mV indicates strong electrostatic stability and can prevent globule agglomeration, thereby extending shelf life (Ozogul et al. 2022). Furthermore, the intensity, volume, and zeta potential distribution curves shown in Figure 5 exhibit a single peak, indicating their dominant nature. This suggests a homogeneous population of globules that does not exhibit coalescence or aggregation, indicating that the emulsion system formed is very stable.

In addition, multiple studies indicate that systems within the range of 20 to 200 nm improve retention of active ingredients in the skin, thus improving the efficacy of the treatment and cosmetics as it retains the skin's ability to help (Abd et al. 2018; Chen et al. 2025; Vater et al. 2020). Submicron-sized emulsions (<200 nm) have unique properties that facilitate their utilization as delivery systems in the topical and systemic routes. For example, in topical applications, emulsions with globule sizes of 20 nm or less can be trapped in the stratum corneum (the outermost layer of the skin) and diffuse into deeper skin layers and hair follicles; thus, they have great potential. In addition, for systemically administered emulsions with globule sizes of less than 200 nm, they can cross the gastrointestinal (GI) mucus barrier and reach the intestinal epithelium, thereby improving the bioavailability of the active pharmaceutical ingredients (APIs) (Haddadzadegan, Dorkoosh & Bernkop-Schnurch 2022).

STABILITY OF OPTIMUM FORMULA

The stability study conducted under accelerated conditions (40 °C±2 °C for 7 days) showed that storage time had a significant overall effect on all evaluated parameters, including globule size, PDI, zeta potential, beta-carotene levels, and beta-carotene retention, as confirmed by Repeated Measures ANOVA ($p<0.05$). However, from a pharmaceutical performance perspective, the optimized formula demonstrated excellent physical robustness throughout the study period. At the end of day 7, the submicron emulsion maintained ideal characteristics, with a mean globule size remaining well below 200 nm (193.13±0.38 nm), a PDI under 0.2 (0.129±0.022), and a zeta potential more negative than -30 mV (-35.77±0.35) (Table 6). These results indicate that, despite statistically detectable changes over time, the D-phase emulsification system effectively prevented drastic globule coalescence and maintained the electrostatic stability required for high-quality topical formulations.

Regarding chemical preservation, the optimized RPO submicron emulsion exhibited a superior protective capacity compared to bulk RPO at an equivalent concentration (55.70%). While a slight initial reduction of 2.75% in beta-carotene levels was observed immediately after the emulsification process, likely due to the high-shear energy or thermal exposure during production, the subsequent retention during storage was significantly higher in the submicron emulsion system (95.96±0.33%) than in the bulk oil (87.56±1.91%) at day 7. This confirms that the submicron globules, stabilized by the sucrose palmitate and glycerol interfacial film, act as a functional barrier that shields beta-carotene from accelerated thermal degradation. The submicron emulsion's ability to preserve 55.70% RPO effectively demonstrates its superiority as a delivery vehicle for unstable lipophilic bioactives such as red palm oil.

MORPHOLOGY OF OPTIMUM FORMULA

The morphological analysis of the optimized RPO submicron emulsion using Transmission Electron Microscopy (TEM) showed that the resulting globules are uniformly spherical and within the size range of <200 nm (Figure 6), thus confirming the formation of a submicron

TABLE 4. Regression analysis

	X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3	$X_1X_2X_3$	$X_1X_2(X_1-X_2)$	$X_1X_3(X_1-X3)$	$X_2X_3(X_2-X_3)$
Globule size	265.73	167.66	190.65	-217.60	-29.38	-24.36	-472.46	-522.15	184.15	-358.14
p-values	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0026	0.0064	0.0001	< 0.0001	0.0200	0.0001
PDI	0.195	0.051	0.084	-0.262	0.087	-0.073	-0.081	-0.477	-0.518	1.238
p-values	0.0002	0.0002	0.0002	0.0041	0.1303	0.1938	0.8622	0.0187	0.3325	0.0105
Zeta potential	-38.63	-26.27	-38.22	15.91	2.95	-15.97	-4.02	-30	-64.05	-53.07
p-values	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0105	< 0.0001	0.5960	< 0.0001	0.0002	< 0.0001

TABLE 5. Comparison predicted value and observed value of optimum formula

	Response	Replicate	SD	Predicted value	Observed value
Y_1	Globule size (nm)	3	2.75	$181.78 < Y_1 < 200.74$	184.20
Y_2	PDI	3	0.011	$0.002 < Y_2 < 0.161$	0.079
Y_3	Zeta potential (mV)	3	0.53	$-42.221 < Y_3 < -39.662$	-40.40

A B

FIGURE 4. Pre-concentrated emulsion (A) and submicron emulsion (B) of optimum formula

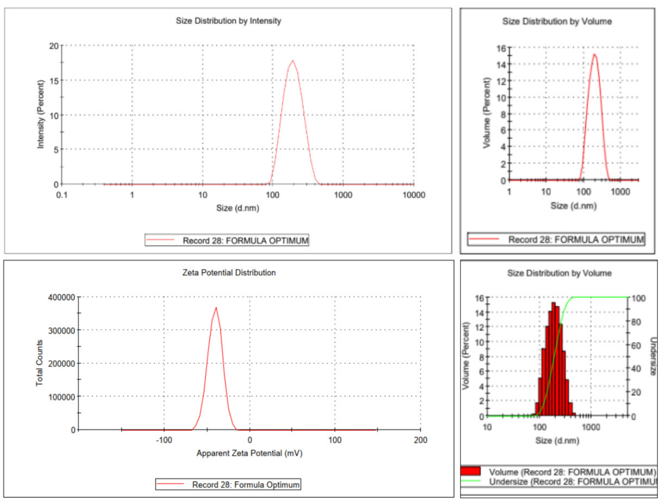

FIGURE 5. Size distribution by intensity, volume and zeta potential distribution graphs

emulsion system. The results and the globule size analysis are comparable, reinforcing the D-optimal mixture design model. The results of the TEM analysis support the hypothesis that a structured surfactant phase and mild mechanical dispersion used in the D-phase emulsification method yield submicron emulsions with a narrow size distribution.

FTIR ANALYSIS

The Fourier-Transform Infrared (FTIR) spectrum analysis results were based on the optimum formula of RPO submicron emulsion and each component such as RPO, sucrose palmitate and glycerol. The analysis results of the RPO submicron emulsion formula, indicated by the black line in Figure 7, showed that there is no major shift or the emergence of significant new peaks compared to the spectrum of each component, namely RPO (green line), sucrose palmitate (blue line), and glycerol (red line). The characteristic peaks are still clearly identified, such as the hydroxyl group (O–H) absorption band at around 3400 cm^{-1} from glycerol and sucrose, the aliphatic C–H band at 2920–2850 cm^{-1} from the oil and ester hydrocarbon chains, and the carbonyl ester (C=O) band at around 1740 cm^{-1} originating from red palm oil triglycerides and sucrose palmitate. In addition, the absorption in the 1100–1000 cm^{-1} region indicates the presence of the C–O–C group from sucrose and glycerol compounds. The absence of significant changes in the chemical structure in the final formula spectrum indicates that the interaction between components in the submicron emulsion system is physical rather than chemical. This supports the conclusion that emulsion formation occurs through physical mechanisms, such as dispersion, without degradation or formation of new compounds. Thus, FTIR confirms that the D-phase emulsification method produces a physically stable system and maintains the chemical integrity of the active components.

TABLE 6. Stability of optimum formula

Parameters		Day				p-value
		0	3	5	7	
Globule size (nm)	Optimum Formula	184.20±2.75	191.23±0.47	192.50±0.69	193.13±0.38	0.001
	Bulk RPO	NA	NA	NA	NA	-
PDI	Optimum Formula	0.079±0.011	0.101±0.020	0.128±0.006	0.129±0.022	0.018
	Bulk RPO	NA	NA	NA	NA	-
Zeta potential (mV)	Optimum Formula	-40.40±0.53	-37.10±0.62	-36.63±0.35	-35.77±0.35	0.000
	Bulk RPO	NA	NA	NA	NA	-
Beta carotene levels (µg/mL)	Optimum Formula	358.58±0.90^{a}	355.55±1.65^{a}	350.34±4.23^{a}	344.09±0.84^{a}	0.000
	Bulk RPO	368.73±7.54^{a}	339.94±0.94^{b}	332.54±2.85^{b}	322.77±1.40^{b}	0.000
Retention of beta carotene (%)	Optimum Formula	100.00±0.00^{a}	99.16±0.41^{a}	97.70±1.07^{a}	95.96±0.33^{a}	0.000
	Bulk RPO	100.00±0.00^{a}	92.22±1.72^{b}	90.20±1.51^{b}	87.56±1.91^{b}	0.000

p-value column indicates the statistical significance of changes over time (Day 0 to Day 7) within the same group that analyzed using Repeated Measures ANOVA. Different superscript letters (a, b) in the same parameters indicate a significant difference between the Optimum Formula and Bulk RPO at that specific time point, determined by Independent Sample T-test ($p < 0.05$). NA indicates not applicable. Data are given as mean±SD, n=3

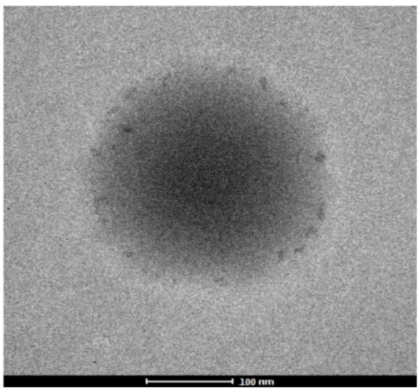

FIGURE 6. TEM of optimum formula with magnification of 19,500×

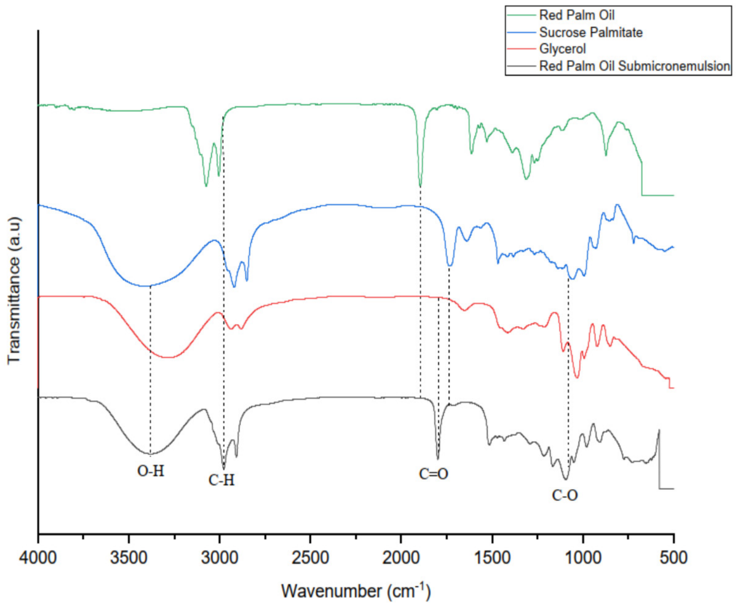

FIGURE 7. FTIR spectra of red palm oil, sucrose palmitate, glycerol, and RPO submicron emulsion

CONCLUSIONS

This research has successfully optimized a Red Palm Oil (RPO) submicron emulsion using the D-phase emulsification technique in combination with the D-optimal mixture design methodology. With the high predictive capacity of the chosen cubic model, the optimal composition of 55.70% RPO, 12.05% Sucrose palmitate, and 32.25% Glycerol was identified, which provided a stable system with a globule size of 184.20 ± 2.75 nm, PDI of 0.079 ± 0.011, and a high zeta potential of -40.40 ± 0.53 mV. Further, the accelerated stability study at 40 °C (for 7 days) showed that the submicron emulsion improved carotenoid retention to 95.96±0.33% compared with bulk RPO (87.56±1.91%). Moreover, the developed delivery system provided a better physical and chemical barrier (against thermal degradation) as evidenced by the consistent globule size and PDI during the storage duration. While the TEM analysis verified that the submicron emulsions had a uniform, spherical globule distribution of less than 200 nm, the formation of submicron emulsions was confirmed by FTIR analysis, which showed no significant changes or new absorption bands. Therefore, the RPO submicron emulsion is stable and likely to be a suitable intermediate for the formulation of pharmaceuticals, nutraceuticals and cosmeceuticals.

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*Corresponding author; email: mahdijufri@farmasi.ui.ac.id