Science and Technology Indonesia
e-ISSN:2580-4391 *p*-ISSN:2580-4405
Vol. 9, No. 3, July 2024

Research Paper

Optimization of Nanoemulsion Formula Containing Erythromycin with VCO and Varying Concentrations of Tween-80 and PEG-400

Mardiyanto[1*], Risfidian Mohadi[2], Najma Annuria Fithri[1], Gilang Kurniawan[1]

[1]*Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Indralaya, 30862, Indonesia*
[2]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Indralaya, 30862, Indonesia*
***Corresponding author**: mardiyanto@mipa.unsri.ac.id*

Abstract
Erythromycin, a macrolide antibiotic is classified into Biopharmaceutical Classification System (BCS) class II which has low solubility in water. The low solubility corresponds to the bioavailability in the blood. One strategy to increase the solubility of Erythromycin is the formulation of erythromycin in nanoemulsion. This research aims to form nanoemulsion using the PIT (Phase Transition Temperature) method for obtaining the optimum formula of erythromycin nanoemulsion using virgin coconut oil (VCO) can be found with varying concentrations of Tween 80 surfactant and PEG 400 cosurfactant. The selection of the optimum formula was assisted by Design Expert software with the Factorial design method 2^2. The basis for determining the optimum formula is based on the results of organoleptic characterization tests, adsorption efficiency (%EE), percent transmittance, viscosity test, pH test, and stability test. The optimum formula was nanoemulsion which had a concentration of Tween 80 25% and PEG 400 25% as a desirability value of <1. The results of the optimum formula showed that the particle size was 170.6±12.8594 nm, polydispersity index (PDI) 0.403±0.04406, and zeta potential -8.8667±0.25697 mV and had an appropriate stability without phase separation during stability test.

Keywords
Optimization, Nanoemulsion, Erythromycin, VCO, Surfactant, Cosurfactant

Received: 27 March 2024, Accepted: 4 June 2024
https://doi.org/10.26554/sti.2024.9.3.697-709

1. INTRODUCTION

Macrolide group antibiotics including erythromycin are insoluble in water, therefore currently most pharmaceutical dosage forms found in trade are in the form of tablets and capsules. Research over the past five years has attempted to increase the solubility of erythromycin by forming polymeric and solid lipid nanoparticles as well as solid dispersions. Research conducted by Tron et al. (2017) succeeded in forming nanoemulsion loading erythromycin using phospholipids and lecithin surfactant. None has yet evaluated the oil phase of virgin coconut oil (VCO) and combined use of surfactants and cosurfactants to increase the stability of nanoemulsions loading erythromycin. The chemical structure of erythromycin, surfactant and co surfactant was displayed in Figure 1.

Erythromycin is an antibiotic that is classified as a macrolide, which is usually used to inhibit Staphylococcus aureus bacteria as one of the causes of acne (Zaenglein, 2016). Erythromycin is classified as a BCS (Biopharmaceutical Classification System) class II drug which has poor solubility in water due to its hydrophobic nature (Wang et al., 2006). This poor solubility disrupts the penetration and release of topical drugs due to not being able to penetrate the stratum corneum, while the S.aureus bacteria are in the sebaceous gland area of the skin. Sebaceous glands are located in the dermis layer so they need better drug penetration ability to reach target cells such as bacterial cells in the dermis layer (Mollerup et al., 2016).

Currently, erythromycin in conventional topical dosage forms is widely used in Indonesia to treat acne. However, the main drawback of conventional topical preparations is that it is difficult to reach specific targets because they cannot penetrate the stratum corneum the skin's main barrier. This makes it difficult for the drug to reach the target site and the effective drug concentration decreases over a certain period (Haque and Talukder, 2018). Therefore, the formulation was modified into a nanoemulsion preparation to increase absorption effectiveness and achieve the desired target.

Nanoemulsion is an effective nanocarrier delivery system for increasing the penetration of poorly soluble drugs such as erythromycin by reducing the droplet size, then increasing absorption through the skin, and making it possible to reduce

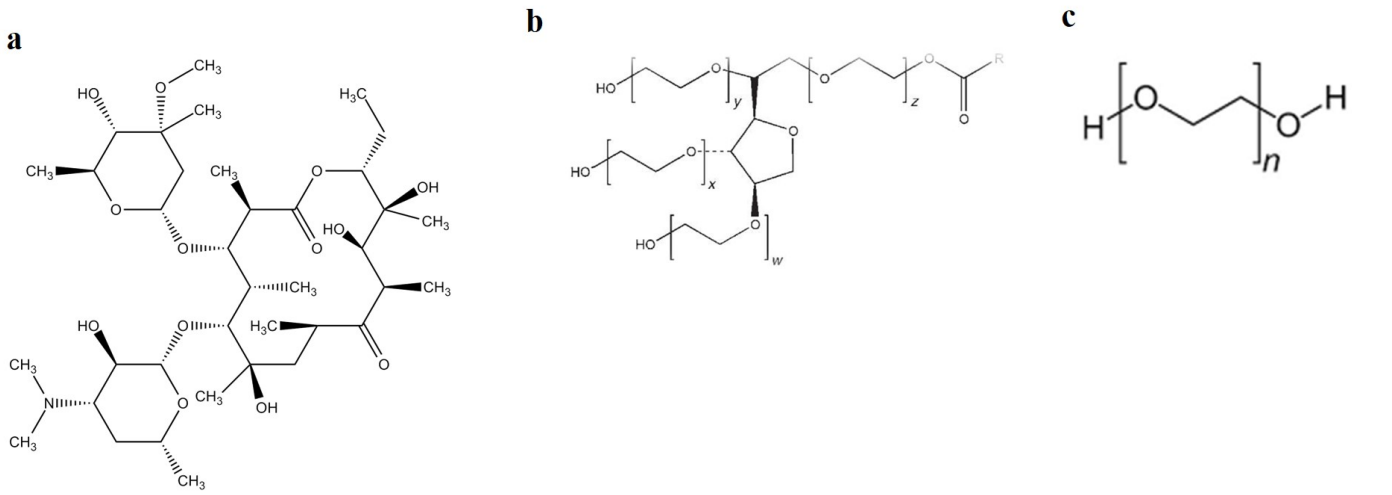

Figure 1. Molecular Structure of Erythromycin (a), Tween-80 (b), and PEG-400 (c)

the total dose of the drug thereby minimizing side effects (Keleb et al., 2015). According to research by Dhillon et al. (2019), encapsulating Erythromycin using SLN and then forming it into a Carbopol-based gel has an antimicrobial effect against S.aureus much more efficiently after 24 and 30 hours compared to conventional erythromycin gel preparations.

Various studies have stated that the nanoemulsion system is a potential delivery system for drugs that cannot penetrate the stratum corneum in sufficient quantities by increasing drug penetration into the skin to provide a therapeutic effect (Nastiti et al., 2017). According to Mortazavi et al. (2013), nanoemulsion with a droplet size of <500 nm allows more effective penetration of drugs into the skin. The smaller droplet size facilitates drug penetration to penetrate the stratum corneum and the release of the drug through the gaps between cells, then the drug enters the sebaceous glands in the dermis layer and reaches the targeted bacterial cells.

The main components of nanoemulsion consist of oil, surfactant, and cosurfactant phases. The oil phase functions to dissolve hydrophobic molecules and increase their absorption through the lipid layer of the body, as well as prolonging contact between the preparation and the skin. Surfactants function as stabilizers that can reduce interfacial tension and prevent droplet aggregation so that all liquid phases in the nanoemulsion can be dispersed evenly. Cosurfactants are needed to improve surfactant performance to produce smaller droplet sizes and more stable nanoemulsions (Singh et al., 2017). Increasing the concentration of surfactants and cosurfactants in nanoemulsions can form aggregates after reaching Critical Micelle Concentration (CMC), which can increase droplet size and the toxic effects of non-ionic surfactants. Therefore, it is necessary to optimize the concentration of surfactants and cosurfactants to avoid excessive aggregate formation and reduce toxic effects (Perinelli, 2020).

Based on the description above, it is necessary to carry out research in the form of Optimization and Characterization of Erythromycin Nanoemulsion with Varying Concentrations of Tween 80 and PEG 400 as Surfactants and Cosurfactants using Factorial design. The resulting erythromycin nanoemulsion was then subjected to various characterization and stability tests, then to obtain the optimum formula, optimization was required with the help of design-expert software using the Factorial design method 2^2. The optimum formula obtained was then characterized, including droplet size, polydispersity index, and zeta potential value.

2. EXPERIMENTAL SECTION

2.1 Materials

Eritromisin was obtained from Hec Pharm Co LTD, Virgin coconut oil (Nutiver®), Tween 80 and PEG-400 (Sigma Aldrich®), and Etanol 96% (Amersam®).

2.2 Methods

2.2.1 Formula Design

The formula design was conducted by using Design-Expert® series 12 software. Optimization of the nanoemulsion formula was developed using the Regular Two-Level Factorial design. There are upper limit values and lower limit values for determining the surfactant and co-surfactant formula used so 4 run formulas were obtained. The concentration range of Tween 80 as a surfactant is 15-25%, and PEG 400 as a co-surfactant is 15-25% (Rismarika et al., 2020). Erythromycin is dissolved in ethanol to reach a concentration of 1% (Mardiyanto et al., 2022). 5% virgin coconut oil was added as the oil phase to the nanoemulsion preparation. The nanoemulsion formulation design can be seen in Table 1.

Table 1. The Formula of Nanoemulsion Containing Erythromycin

Components	Concentration (%)			
	F1	F2	F3	F4
Erythromycin	1	1	1	1
Tween 80	15	25	15	25
PEG-400	15	15	25	25
Virgin Coconut Oil	5	5	5	5
Aquadest	ad 100 mL	ad 100 mL	ad 100 mL	ad 100 mL

2.2.2 Preparation of Oil and Water Phase

The preparation of the oil phase in forming Erythromycin nanoemulsion follows the research of Mardiyanto et al. (2022) with slight modifications in the procedure. Virgin coconut oil is heated on a magnetic stirrer at a temperature of 60°C, for 15 minutes on a magnetic stirrer (IKA®). After that, an erythromycin solution was made with a concentration of 1% using 96% ethanol solvent, then 1 mL of 1% erythromycin solution was taken and added to 5 mL of virgin coconut oil as the oil phase. The mixture of erythromycin and VCO was then stirred using a hotplate magnetic stirrer at a speed of 3000 rpm at a temperature of 60°C.

The water phase in the erythromycin nanoemulsion formula consists of Tween 80, PEG-400, and distilled water. Tween 80 and PEG-400 are measured according to the formula using a measuring cup. Mix Tween 80 and PEG 400 then add distilled water until the 100 mL. Mixing the water phase was carried out using a hotplate magnetic stirrer at a speed of 3000 rpm for 15 minutes at a temperature of 60°C (Khan et al., 2012).

2.2.3 Preparation of Nanoemulsion

O/W type nanoemulsion was prepared by spontaneous emulsification method. The mixture of the oil phase, namely erythromycin and virgin coconut oil, was stirred using a magnetic stirrer at 3000 rpm for 15 minutes at a temperature of 60°C. The water phase mixture, namely Tween 80, PEG 400, and distilled water was stirred using a magnetic stirrer at 3000 rpm for 15 minutes at a temperature of 60°C. Next, the oil phase was added drop by drop using a drop pipette into the water phase on a magnetic stirrer at a constant speed of 3000 rpm for 15 minutes. This was done based on research by Shena and Kumar (2022) which aims to obtain homogeneous nanoemulsions. gradually. Then proceed with ultra turrax (IKA-300®) to reduce the droplet size at a speed of 7800 rpm for 15 minutes. The nanoemulsion which has reduced its droplet size, is then subjected to a sonication process using a bath sonicator (Polar-FALC®) for one hour which aims to increase the stability of the nanoemulsion preparation by helping to reduce the droplet size and making the nanoemulsion preparation clear or transparent (Nirmala et al., 2020).

2.2.4 Purification and Determination of Percentage of Encapsulation (%EE)

A 0.5 mL emulsion sample was centrifuged at 12,000 rpm for 30 minutes at 4°C using a centrifuge (Hettic-100®) to obtain 2 phases, namely the adsorbed phase and the non-adsorbed phase. Separate the non-adsorbed phase, then add ethanol to the 0.5 mL into the adsorbed phase, and centrifuge again. This treatment was carried out three times to obtain a solution of erythromycin particles with a non-adsorbed phase. The non-adsorbed phase was then sampled and the absorbance value was measured using UV-Vis spectrophotometry (Biobase®) at the erythromycin wavelength. The level of erythromycin that was not adsorbed was calculated using the regression equation ($y = a + bx$) that was previously obtained, with y as absorbance and x as concentration (Mardiyanto et al., 2022). After measuring the absorbance of erythromycin, the free drug level was then determined, then the percent EE was determined using the formula in Equation (1).

$$\%EE = \frac{\Sigma \text{ Amount of Formula} - \Sigma \text{ Amount Supernatant}}{\Sigma \text{ Amount of Formula}} \times 100\% \quad (1)$$

2.2.5 Organoleptic Test

The color, transparency, and odor of all erythromycin nanoemulsion formulations were observed using the senses of sight and smell to meet the requirements for the hedonic test.

2.2.6 Determination of Percent Transmittance

Erythromycin nanoemulsion was diluted 100 times with distilled water. A total of 3 mL of sample was placed in a cuvette, transmittance was measured at 650 nm using a UV-Vis spectrophotometer, and distilled water was used as a blank. The percentage of transmittance describes the uniformity of droplet size, the closer it is to 100%, the clearer the nanoemulsion is, and the resulting nanoemulsion droplets are more uniform in size.

2.2.7 Determination of Viscosity

The viscosity of the nanoemulsion was determined using a viscometer (Rion®). The nanoemulsion sample is inserted into the viscometer cup and then the cup is raised until the spindle is immersed in the nanoemulsion sample. The shear speed used was 100 rpm at a temperature of 25°C. Measurements

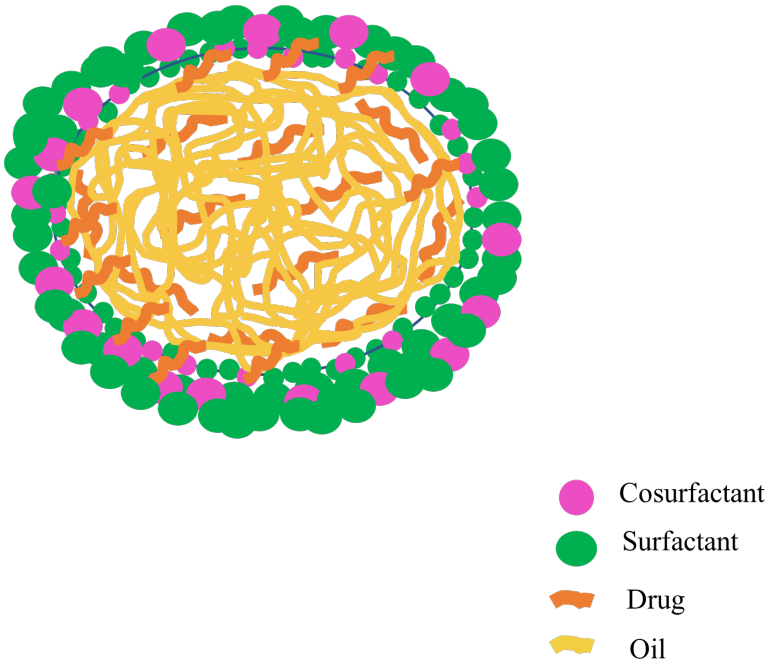

Figure 2. Illustration Structure of Nanoemulsion

Table 2. Organoleptic Results

Results			
F1	F2	F3	F4
White Turbid Odorless	White Slightly Turbid odorless	White Slightly Turbid odorless	Transparent CLear odorless

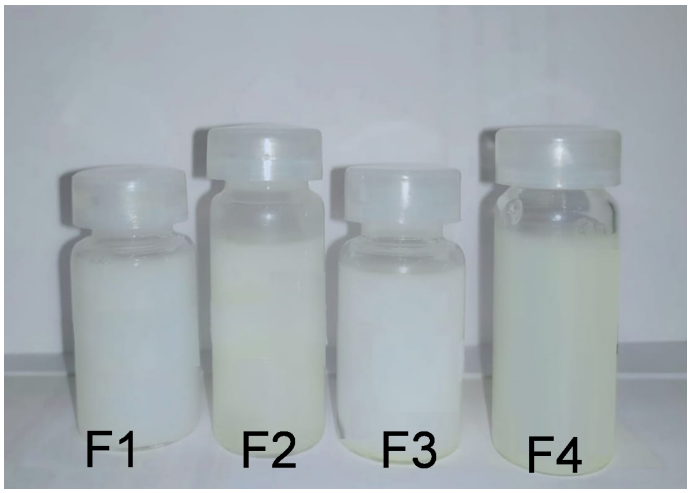

Figure 3. Product of Four Formula (F1, F2, F3, and F4)

were carried out 3 times (Nita et al., 2019; Rahmadevi et al., 2020; Gul et al., 2022).

2.2.8 Determination of pH

The pH of the sample was measured using a calibrated pH meter (Levion®), by immersing the instrument bulb in 30 mL of each formula (Tarik Alhamdany et al., 2021).

2.2.9 Stability Test

Evaluation of nanoemulsion stability using the Cycling Test method was carried out by storing the nanoemulsion at low temperature (4±2°C) in the refrigerator (LG®) for 24 hours, then storing it again at high temperature (40±2°C) in the oven (Oberhaus®) for 24 hours (one cycle) (Ali et al., 2014). The storage time at two different temperatures is considered as one cycle. This test was carried out in 6 cycles, and observed in the 0th to 6th cycles, for 12 days. Based on this, the Cycling Test is a type of thermodynamic stability test. The observations carried out were in the form of organoleptic and pH observations of the nanoemulsion preparation.

This centrifugation test is called a mechanical stability test. The nanoemulsion centrifugation test was carried out using a centrifuge at a speed of 3750 rpm for 1 hour. Then, instability parameters were observed which included phase separation, deposition, creaming, and cracking.

Table 3. The %EE of Nanoemulsion

Formula	Tween 80 (mL)	PEG 400 (mL)	%EE±SD(%)	%CV
F1	15	15	68.813±0.22	0.003198508
F2	25	15	80.701±0.13	0.002640438
F3	15	25	80.821±0.218	0.002698385
F4	25	25	82.732±0.295	0.003567908

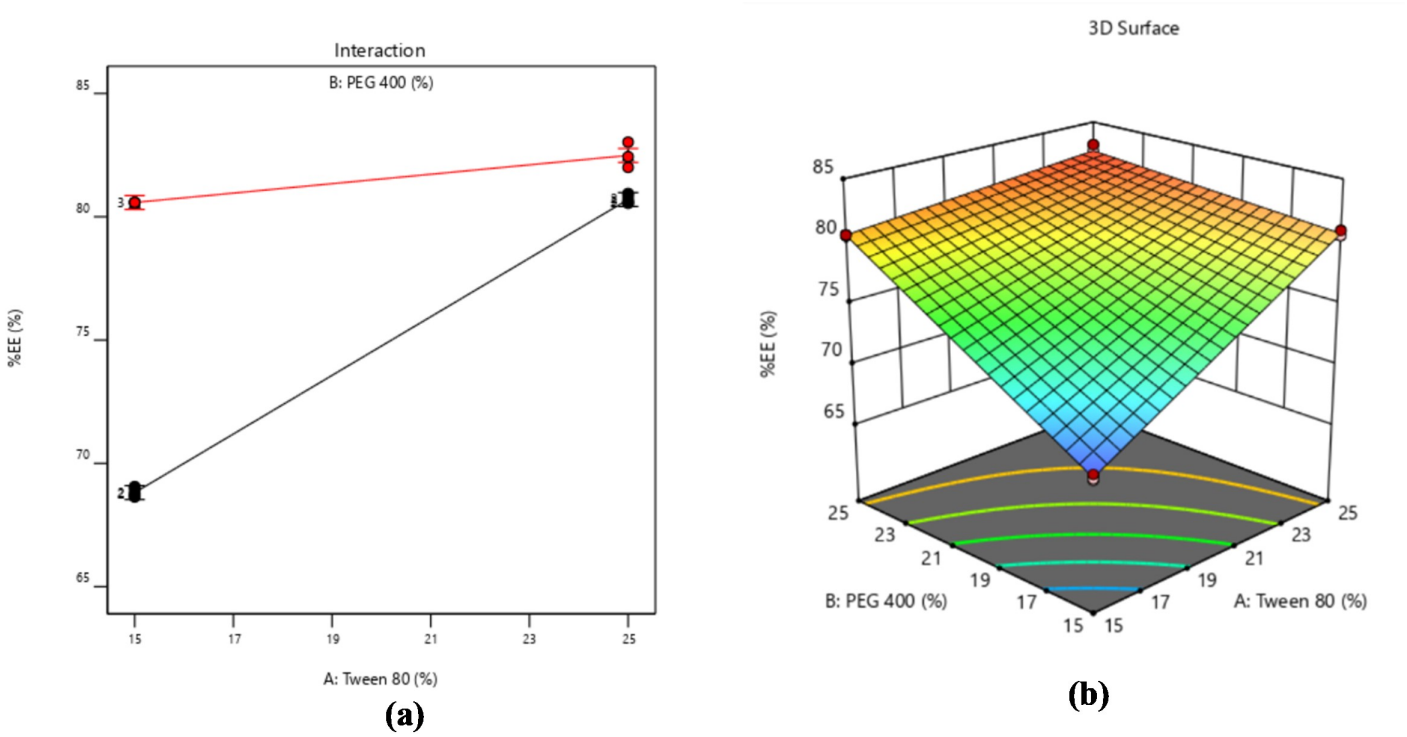

Figure 4. (a) Interaction Curve, (b) 3D Surface %EE

Table 4. Analysis Respond %EE by Factorial design

Parameter	Value
p value	<0.0001*
Adjusted R^2	0.9972
Predicted R^2	0.9955
Adjusted R^2 dan Predicted R^2	0.0017
Adequate Precision	79.0454

*p-value<0.05 (significant differences)

2.2.10 Determination of Optimum Formula

Determining the optimum formula was carried out using the Two-Level Factorial design method using the Design-Expert 12 application. Four formulas were created according to the concentration variations determined by the application. Next, organoleptic testing, pH, specific gravity, viscosity, and percent transmittance were carried out on the four formulas. According to Gurpreet and Singh (2018), test results that meet the requirements consist of organoleptic preparations that appear transparent, the pH of the preparation is close to skin pH in the range of 4.5-6.5, low viscosity which indicates the type (O/A).

The test results are entered as response parameters to select the optimum formula. The optimal formula is the formula that has the highest desirability value. The closer the desirability value is to 1, the closer the response value is to the target (Iskandar et al., 2024).

2.2.11 Characteristics of Optimum Formula Nanoemulsion

Particle size, PDI, and Zeta potential were measured using a particle size analyzer with dynamic light scattering type (Zetasizer ZS90, Nanoseries, Malvern®, UK). All samples were diluted 1000 times with demineralized water before testing and then placed in a quartz cuvette (Mao et al., 2022). Analysis was carried out at a temperature of 25°C at an angle of 90°. Measurements were carried out three times in replication and the average value was calculated. For the topology of emulsion globules, measurement was conducted in the following steps.

Table 5. %Transmittance of Nanoemulsion

Formula	Tween 80 (mL)	PEG 400 (mL)	% Transmittance±SD(%)	%CV
F1	15	15	82.867±0.057	0.000697
F2	25	15	75.200±0.100	0.00133
F3	15	25	90.167±0.057	0.00064
F4	25	25	99.233±0.208	0.002098

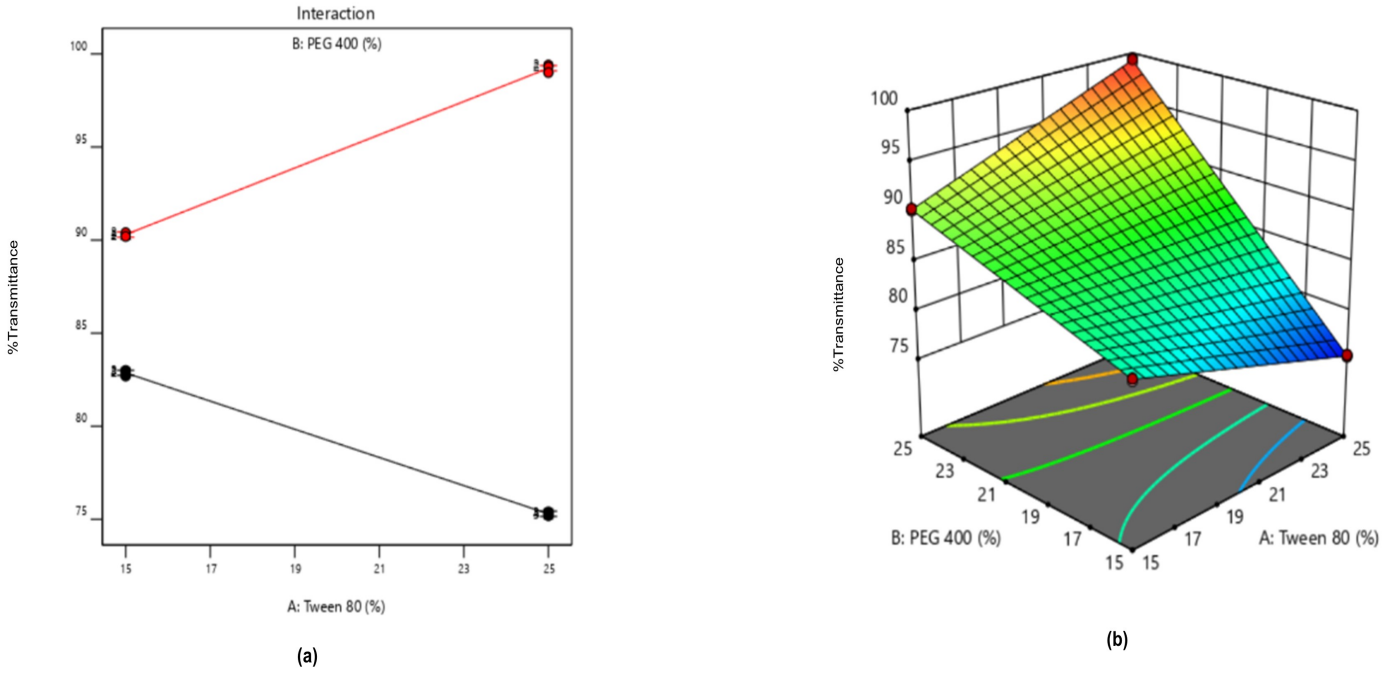

Figure 5. (a) Interaction Curve, (b) 3D Surface %Transmittance

Table 6. Analysis Respond %Transmittance by Factorial Design

Parameter	Value
p value	<0.0001*
Adjusted R^2	0.9997
Predicted R^2	0.9996
Adjusted R^2 dan Predicted R^2	0.0001
Adequate Precision	281.6228

p-value<0.05 (significant differences)

The AFM mica plate is cleaned with tips so that the surface forms a new layer. 100 microliters of sample are dropped on a clean mica plate and dried with an air gun at low speed for 15 minutes and can also be dried under ambient conditions. The sample was placed on the AFM (Bruker®)nanoscope cantilever holder with the help of a monitor so as not to damage the cantilever. The controller is run over an area of 0.5 cm with a movement of 40 N/m and a resonant frequency of 250 kHz.

3. RESULTS AND DISCUSSION

3.1 Formula Design

Drugs with low solubility in water can result in slower penetration compared to drugs with high solubility. This can affect the level of absorption and effectiveness (Momoh et al., 2019), therefore special formulations are required to solve this deficiency.

According to Dhillon et al. (2019), nanoemulsion formulation is one of the newest drug delivery systems that has several advantages. These advantages include increasing the solubility capacity of the active substance, making it very suitable for application to erythromycin which has a low solubility level. In addition, nanoemulsions that have a droplet size of <500 nm can increase topical penetration, by being able to penetrate the stratum corneum. The stratum corneum is approximately 15-20 μm thick and consists of layers of keratin cells that form a strong and flexible membrane. This layer contains a lipophilic protein matrix. So the smaller the droplet size, the drug can penetrate well through the stratum corneum. Droplets carrying erythromycin that have penetrated the stratum corneum then enter through the gaps between dense epidermal cells, until

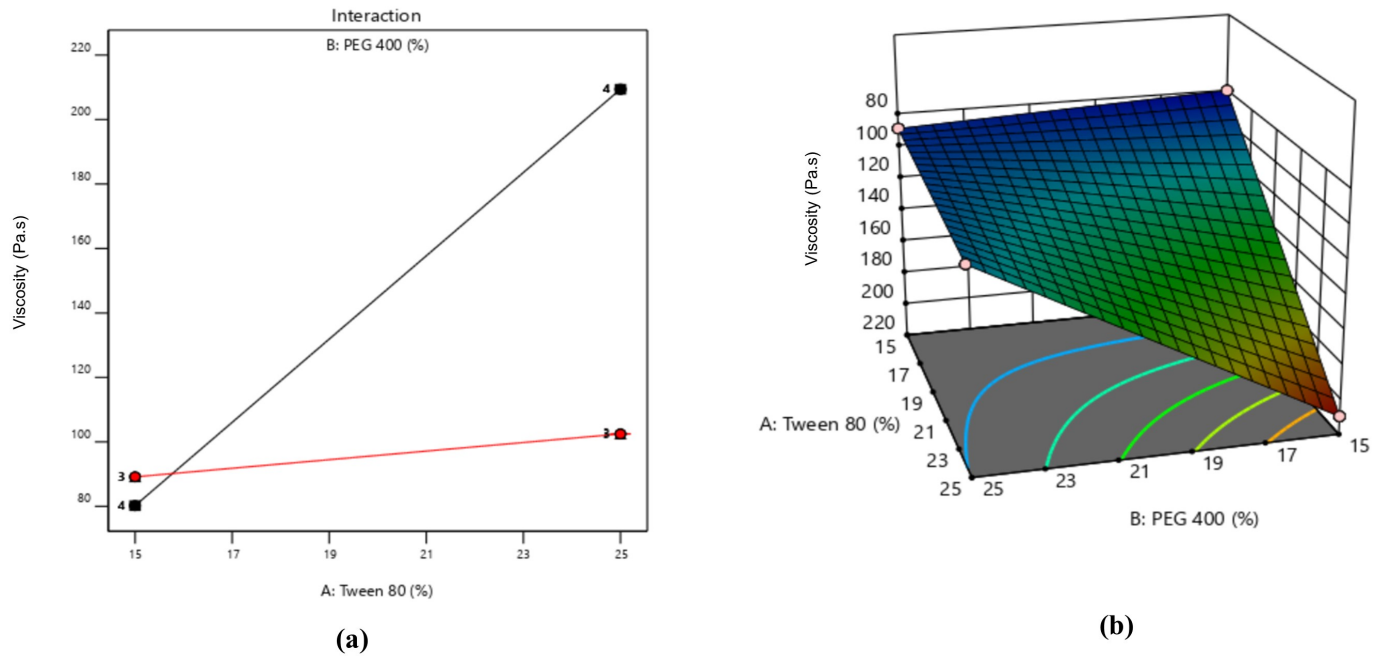

Figure 6. (a) Interaction Curve, (b) 3D Surface Viscosity

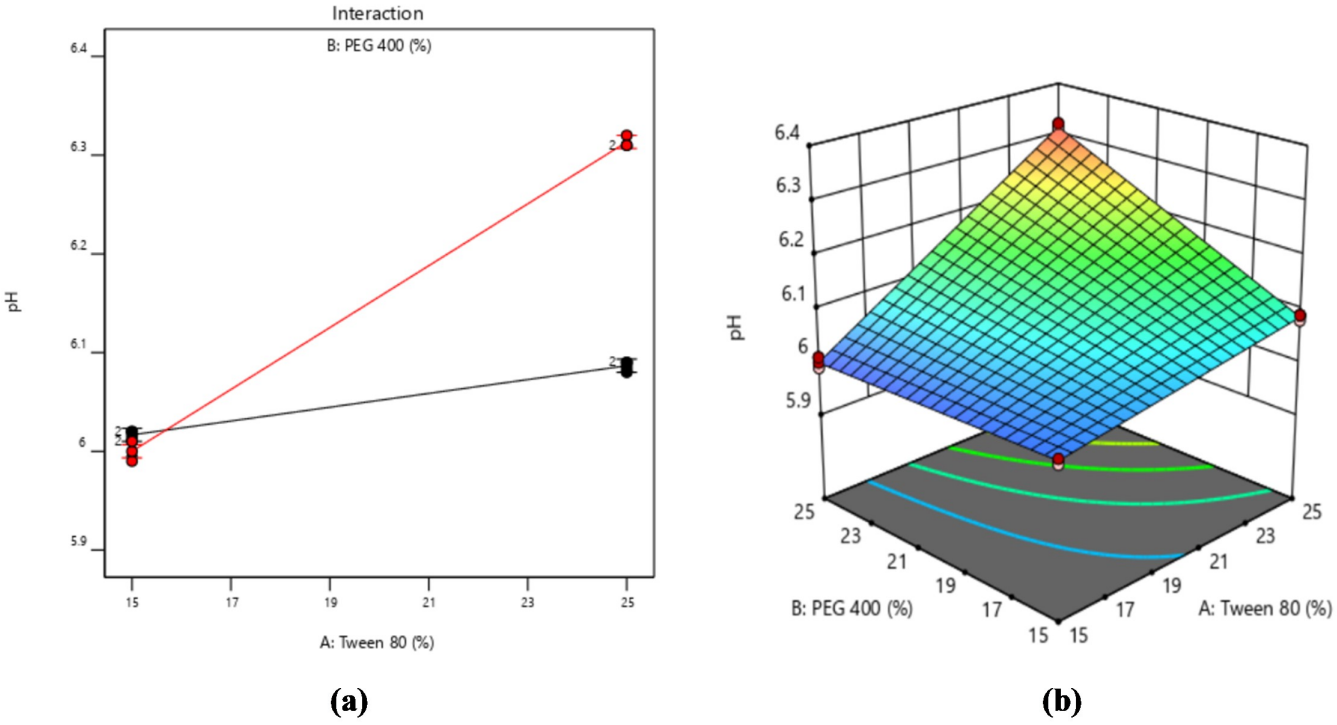

Figure 7. a) Interaction Curve, (b) 3D Surface of pH of Nanoemulsion

they reach the dermis, which is where acne-causing bacteria exist. Drugs that have entered the dermis then attack bacteria according to their mechanism of action in killing bacteria (Nastiti et al., 2017).

Forming the nanoemulsion containing erythromycin (illustrated in Figure 2) was carried out using the PIT or Phase Tran-

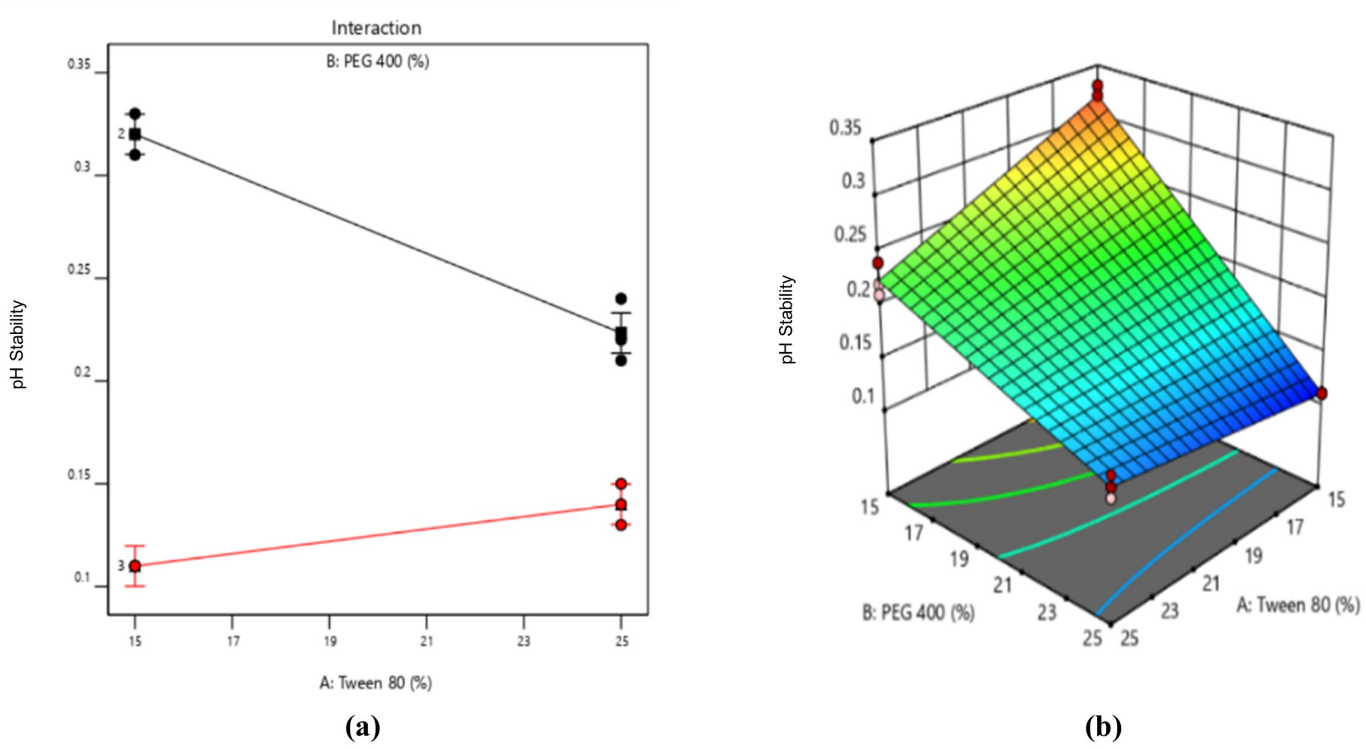

Figure 8. (a) Interaction Curve, (b) 3D Surface pH Stability

Table 7. Viscosity of Nanoemulsion

Formula	Tween 80 (mL)	PEG 400 (mL)	Value of Viscosity (Cp.s)±SD	%CV
F1	15	15	80.216±0.028688	0.000358
F2	25	15	209.389±0.097746	0.000467
F3	15	25	89.154±0.014572	0.000163
F4	25	25	102.469±0.056012	0.000547

Table 8. Respond of Viscosity

Parameter	Value
p value	<0.0001*
Adjusted R^2	1.000
Predicted R^2	1.000
Adjusted R^2dan Predicted R^2	0
Adequate Precision	3819.2386

*p-value<0.05 (significant differences)

sition Temperature method combined with the high-speed stirring method. The PIT method is a development of the spontaneous emulsification method and Low-energy methods. The main parameter of the PIT method in making nanoemulsions is temperature, so in making erythromycin nanoemulsions using a magnetic stirrer to mix each phase, at a temperature of 60oC for 15 minutes. This PIT method is very suitable for forming oil in water (O/W) type nanoemulsions, because it can produce perfect solubility of the oil phase in bicontinuous nanoemulsions which can produce nanoemulsions with small droplet sizes. The PIT method also shows high emulsification efficiency and produces a small PDI (Safaya and Rotliwala, 2019).

In the forming of nanoemulsions, high-speed stirring is required. The high-speed stirring method for making erythromycin nanoemulsion uses Ultraturrax at a speed of 7800 rpm for 15 minutes. This method can help the process of reducing the size of nanoemulsion droplets. This is because, forming nanoemulsions to obtain nano-sized droplets, not only uses mechanical tools but also needs homogenizers. Characteristics of the erythromycin nanoemulsion product (Figure 3) and the organoleptic (Table 2) were shown in following data.

There are differences in the concentrations of Tween 80 and PEG 400 as surfactants and cosurfactants in the nanoemulsion preparations. Making nanoemulsion preparations was carried out by dispersing the oil phase into the water phase

Table 9. Results of pH Evaluation of Nanoemulsion

Formula	Tween 80 (mL)	PEG 400 (mL)	pH±SD	%CV
F1	15	15	6.01667±0.00577	0.00096
F2	25	15	6.08667 ±0.00577	0.00095
F3	15	25	6.00000 ±0.01000	0.00167
F4	25	25	6.31333 ±0.00577	0.00091

Table 10. Respond of pH Evaluation

Parameter	Value
p value	<0.0001*
Adjusted R^2	0.9971
Predicted R^2	0.9952
Adjusted R^2dan Predicted R^2	0.0019
Adequate Precision	76.7505

*p-value<0.05 (significant differences)

Table 11. Respond of pH Stability Test

Parameter	Value
p value	<0.0001*
Adjusted R^2	0.9852
Predicted R^2	0.9759
Adjusted R^2dan Predicted R^2	0.0093
Adequate Precision	34.9461

in a drop-by-drop manner assisted by stirring on a magnetic stirrer at a speed of 3500 rpm at a temperature of 60°C. The drop-by-drop process can avoid agglomeration in nanoemulsion droplets so that the mixture of the two liquid phases in nanoemulsion can be homogeneous with uniform droplet sizes.

The encapsulation efficiency results (Table 3) were obtained by determining the maximum wavelength first and then determining the standard curve equation for the erythromycin mother solution. Based on the results of the wavelength measurements carried out, the maximum wavelength of erythromycin was obtained at 207 nm. Measurement of %EE was conducted using the indirect method. The indirect method is carried out by measuring erythromycin that is not absorbed in the supernatant after centrifugation. Based on the calculation of the %EE of the four formulas, the EE percent value of formula 4 shows better results than the other formulas. The percent EE results of formula 4 with the amount of Tween-80 of 25 mL and PEG-400 of 25 mL resulted an EE value of 82.732±0.295%. This result follows the theory that states that the higher the %EE value or the closer it is to 100%, the better the preparation of erythromycin nanoemulsion, because the more erythromycin levels are encapsulated by the dispersed phase used, the greater the drug content obtained.

The results of the response analysis of percent entrapment efficiency are shown above in Table 4. The Adjusted R^2 parameter was used in this study because there was more than one independent variable selected, namely tween 80 and PEG 400. The Adjusted R^2value describes the resulting value of the response which represents the population of the sample. The Adjusted R^2 result obtained was 0.9972, which can be interpreted as the data obtained representing 99.72% of the population and being able to explain a good linear relationship between the dependent variable in the form of entrapment efficiency. The interaction curve shows the high and low interactions of the independent variables does not affect the response. This is indicated by two lines that do not intersect on the Interaction curve in Figure 4(a). The red line represents high concentration PEG 400 while the black line represents low concentration PEG 400. At high concentrations of PEG 400, as the use of Tween 80 increases, the adsorption efficiency will increase, but at low concentrations of PEG 400, as the use of Tween 80 increases, the adsorption efficiency decreases. High-concentration PEG 400 has a greater influence than low-concentration PEG 400 because it has a higher slope value. The results of the Interaction curve are also supported by the 3D Surface graph in Figure 4(b) which depicts the increasingly orange-colored area indicating the best adsorption efficiency.

Table 12. Evaluation of Physical Properties of Nanoemulsion

Physical Properties	Results±SD	%CV
Size of Droplet	170.6±12.8594 nm	0.07536
PDI	0.403±0.04406	0.10924
Zeta Potential	-8.8667±0.25697 mV	-0.00027

The results of the analysis indicated that tween 80 has a contribution to entrapment efficiency. The regression equation obtained from the analysis results is $y = 78.14 + 3.45A + 3.39B - 2.49AB$. In the regression equation, it can be seen that the coefficients of variables A and B are positive, which indicates that the resulting response will increase in line with increasing concentration of the factors used.

The results of the analysis of the percent transmittance response are shown above in Table 5. The Adjusted R^2 parameter (Table 6) is used in this research because there is more than one independent variable selected, namely tween 80 and PEG 400. The Adjusted R^2 value describes the resulting value of the response which represents the population of the sample. The Adjusted R^2 result obtained was 0.9997, which can be interpreted as the data obtained representing 99.97% of the population and being able to explain a good linear relationship between the dependent variable in the form of percent

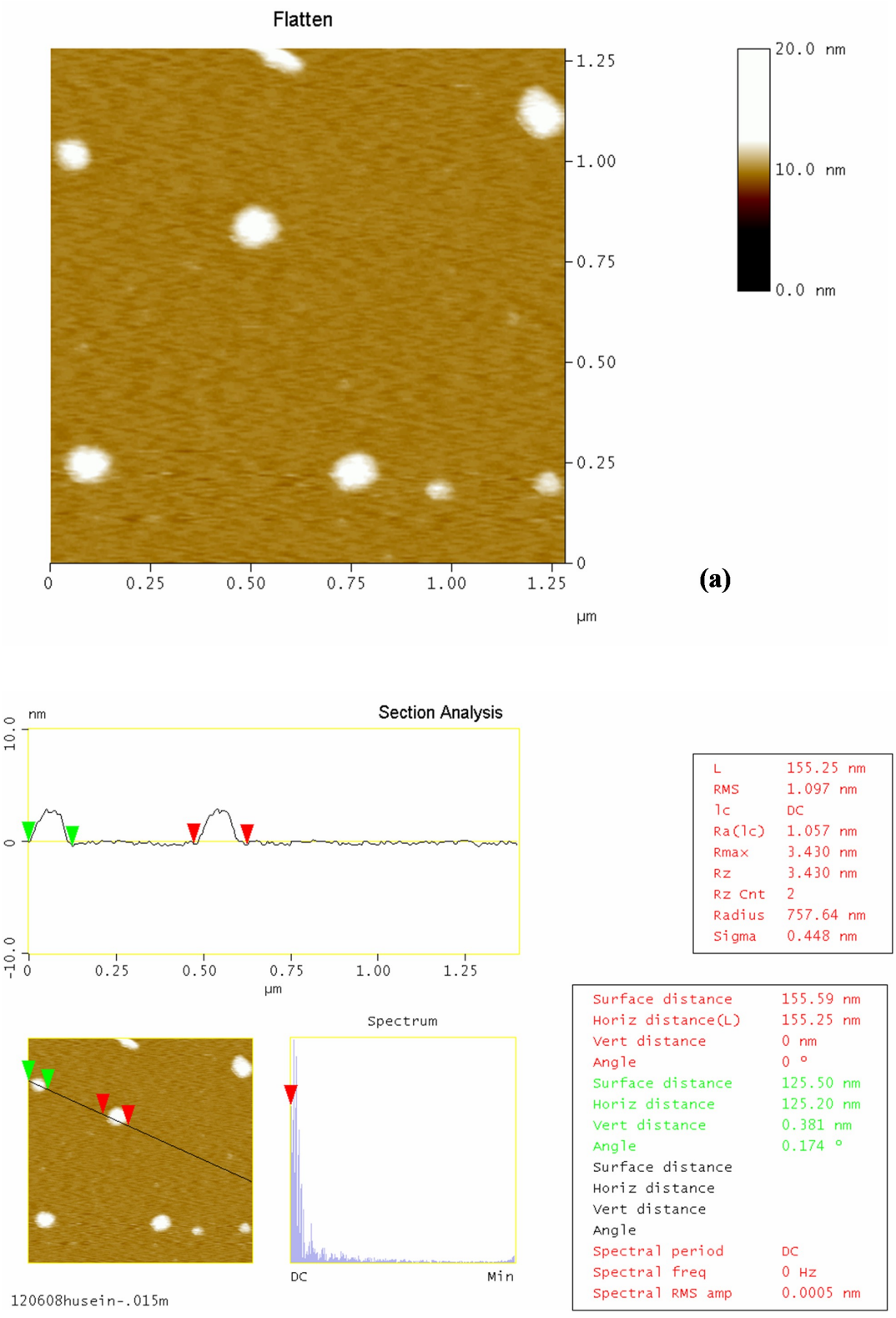

Figure 9. The AFM Image (a) and Section Analysis (b) of Nanoemulsion Containing Erythromycin

transmittance.

The interaction curve shows that the interaction between high and low independent variables does not affect the response. This is indicated by two lines that do not intersect on the Interaction curve in Figure 5(a). The red line represents high concentration PEG 400 while the black line represents low concentration PEG 400. At high concentrations of PEG 400, as the use of Tween 80 increases, the transmittance percentage will increase, but at low concentrations of PEG 400, as the use of Tween 80 increases, the transmittance percentage will decrease. High-concentration PEG 400 has a greater influence than low-concentration PEG 400 because it has a higher slope value. The results of the Interaction curve are also supported by the 3D Surface graph in Figure 5(b) depicting the increasingly red area indicating the best percent transmittance.

The regression equation obtained from the analysis results is $y = 86.93 + 0.3417A + 7.84B + 4.13AB$, based on the regression equation it can be seen that the coefficient of Tween 80, PEG 400, and the interaction between the two has a impact to the formula. From the data (Table 7) it is known that the Adjusted R^2value was 1.0000 (Table 8), which indicated that the regression obtained represents 100% of the population and can explain the linear relationship between two variables that both contribute to the formula.

The interaction curve in Figure 6(a) illustrates the existence of high and low interactions between the two independent variables contributing or having an effect on the viscosity response, this is because in the graph you can see the red line and black line intersecting. The black line illustrates PEG 400 at a low concentration, while the red line depicts a high concentration. At low concentrations of PEG 400, as Tween 80 increases, the viscosity value becomes higher. The reverse condition occurs at PEG 400 with a high concentration, as Tween 80 increases, the viscosity value becomes smaller. The results of this Interaction curve can also be supported by the 3D Surface graph in Figure 6(b), where the blue area shows the lowest viscosity value and the red area shows an increasingly higher viscosity value.

The viscosity response as the following regression equation was $y = 120.31 + 35.62A - 24.50B - 28.96AB$. Through the regression equation, it can be seen that tween 80 has a positive coefficient, which means the response will be directly proportional as tween 80 increases. This is also supported in Figure 6(a), the tween 80 point is on the right, and furthest away from the line on the Normal Plot which means it has the highest percent contribution value. In Figure 6(b) the Pareto Chart at tween 80 has a yellow color which means it has a positive interaction with the viscosity response.

The pH analysis results, as listed in Table 9, revealed several assessment parameters. The predicted R^2value (Table 10) reflected the extent to which the model that has been created matches the response results compared to the system predictions. Based on Figure 7 a R^2 value of 0.9952, this showed that the level of agreement between the regression response results and the regression estimated by the system is around 99.52%. The adjusted R^2parameter is used in this context because the model involves more than one independent variable, namely tween 80 and PEG 400. The adjusted R^2value describes the extent to which the response results represent the entire population and sample used. The adjusted R^2result of 0.9971 indicates that the model created can cover around 99.71% of the relevant population and well explains the linear relationship between the independent variables between Tween 80 and PEG 400, with the dependent variable, pH The regression equation becomes $y = 6.10 + 0.0958A + 0.0525B + 0.0608AB$. Tween 80, PEG 400, and the interaction between the two have positive coefficients, which means the response will be directly proportional to the increase in tween 80 and PEG 400 and the high interaction between parameter.

During the stability test, the change of pH was observed. Based on the data, it was known that the regression equation obtained based on determination of stability (Table 11) was $y = 0.1983 - 0.0167A - 0.0733B + 0.0317AB$. It indicated that tween 80 and PEG 400 have a negative coefficient, while the interaction between the two has a positive coefficient. A positive coefficient on the interaction variable between the two can mean that the response to a decrease or change in pH will be higher with an increase in the interaction of tween 80 and PEG 400. On the other hand (Figure 8), a negative coefficient for tween 80 and PEG 400 can be interpreted as a response to a decrease or change in pH that will be lower with an increase in the concentration of tween 80 and PEG 400. PEG 400. This is following the theory which states that Tween 80 and PEG 400 can increase the stability of nanoemulsion preparations so that the pH does not decrease or change very much.

3.2 Determination of Optimum Formula

Based on the results of the characterization of erythromycin nanoemulsion preparations such as percent EE, percent transmittance, viscosity, specific gravity, and pH and stability testing has also been carried out on the four erythromycin nanoemulsion formulas, formula 4 can be categorized as the best formula or optimum formula.

3.3 Characterization Results of Droplet Size, Polydispersity Index, and Zeta Potential of Optimum Erythromycin Nanoemulsion Formula

Regarding the data of physical properties evaluation (Table 12) droplet diameter measurement results for the optimum erythromycin nanoemulsion formula were 170.6±12.8594 nm. These results indicate that the nanoemulsion droplet size meets the requirements. According to Singh et al. (2017), the average droplet size obtained in nanoemulsions is bellow than 500 nm. The droplet size is small and meets these requirements because Tween 80 and PEG 400 in the optimum formula have the appropriate concentration.

Droplet size distribution parameters can be seen through calculating the polydispersity index (PDI) which can be used as a reference for the level of droplet size uniformity. The PDI value produced by the optimum erythromycin nanoemulsion

formula has met the requirements, namely 0.403 or < 0.5, which indicates that there are 59.70% of droplets that have a uniform size and are classified as a monodisperse disperse type.

The zeta potential value of the optimum erythromycin nanoemulsion formula was -8.8667±0.25697 mV which is classified as a droplet that rapidly coagulates or flocculates. According to Revathi and Dhanaraju (2019), this falls into the zeta potential range of 0 to ±5. A small zeta potential value can create a larger attractive force between droplets, which can cause agglomeration The zeta potential results of the erythromycin nanoemulsion formulation have a negative charge. The negative charge in the optimum formula for erythromycin nanoemulsion is obtained due to the presence of VCO lipids which adsorb erythromycin. According to Dayrit (2014), VCO contains lauric acid which has a carboxylic acid (COOH) functional group. The COOH group in VCO can be hydrolyzed into its ionized form, namely COO^-, which has a negative charge when dispersed in the water phase (aquadest). This resulted the negative charge on the VCO being measurable and detected by the Zetasizer instrument when measuring or reading the zeta potential value.

The results of AFM in the form of topographic image (Figure 9) and data processing with the analysis section show that the globule size was 155.59 nm not that different from measurements of water conditions as carried out with DLS. This data indicates solving the problem of imaging globules which is difficult to do with SEM and TEM.

4. CONCLUSIONS

Based on research data, it was known that the optimum formula was Formula 4 (F4) a nanoemulsion containing Tween-80 of 25% and PEG-400 of 25%. These two parameters had a desirability value <1. The results of the physical property characterization of F4 showed that the particle size was 170.6±12.8594 nm, polydispersity index (PDI) 0.403±0.04406, and zeta potential -8.8667±0.25697 mV respectively.

5. ACKNOWLEDGMENT

This research was funded by PNBP Sriwijaya University for Kompetitif schematic research of 2024. The gratitude towards the Pharmaceutical Technology Laboratory and group of Pharmaceutical Science Sriwijaya University and PT DKSH for the contribution in equipment and discussion of research data.

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