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Original Article

OPTIMIZATION AND PHYSICAL CHARACTERIZATION OF QUERCETIN NANOEMULGEL FORMULA AS AN ANTIBACTERIAL AGENT

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ABSTRACT

Objective: This research aims to optimize the quercetin nanoemulgel formula to improve quercetin solubility as an antibacterial agent.

Methods: In this research, quercetin was formulated into nanoemulgel with the factorial design of three independent variables, the concentration of oil phase (virgin coconut oil), surfactant (Tween 80), and co-surfactant (Span 80). The nanoemulgel physical properties (viscosity, spreadability, transmittance value, zeta potential, and particle size) were tested. The data were evaluated using Minitab®18 software; if the p-value<0.05, it is stated that there is a statistically significant difference in the formula, and the use of the response optimizer menu in the Minitab® 18 software determines the optimum formula with multiple responses.

Results: The results showed that the concentration of quercetin used to inhibit the growth of *Staphylococcus aureus* was 2 mg/ml. The size of the particles had a mean of 62.487 nm, the polydispersity index had a mean of 0.365, and the percent transmittance had a mean of 95.533±0.113%. Measurements of the zeta potential had a mean of -26.712±0.154, with the viscosity and spreadability of the preparations made having a mean of 2495±0.250 cps and 4.795±0.028 cm.

Conclusion: This study reported that the VCO amount of 3g, the tween amount of 12g and the span amount of 3.5g were found to be computational recommendations to achieve the optimum conditions only for percent transmittance value response.

Keywords: Quercetin, Nanoemulgel, Virgin coconut oil, Tween 80, Span 80

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INTRODUCTION

Recurrent aphthous stomatitis or thrush is an inflammatory condition of the oral mucosa [1]. One of the bacteria that causes this disease is *Staphylococcus aureus* bacterial infection [2]. *Staphylococcus aureus* is a normal microflora in the oral cavity. When the body's immunity decreases, these bacteria will become pathogens, causing bacteremia and systemic infections in the oral cavity [2, 3]. Thrush medicine as a powerful antibacterial has a relatively high price, and the number is still limited in the market now. Quercetin is a compound that has antibacterial and anti-inflammatory activity [4]. The concentration of quercetin that will be used to inhibit *Staphylococcus aureus* bacteria was determined through the paper disk diffusion method by selecting the lowest concentration capable of inhibiting the growth of the test bacteria by looking at the clear area or zone around the paper disc, which indicated the presence or absence of inhibition of bacterial growth [5]. Quercetin is classified as BCS Class II, which means it has low solubility and high permeability [6].

To overcome this problem, quercetin can be developed using a nanotechnology approach to increase the quercetin field [7]. Quercetin may require a more sophisticated delivery system that ensures a high drug-loading capacity and provides greater skin tackiness to extend drug contact time with the skin [8]. In recent years, nanoparticles have been widely used in pharmaceuticals, significantly increasing drug solubility by reducing the particle size will increase the surface area and the thickness of the protective particle layer [9].

One of the mucosal drug delivery systems is a nanoemulgel preparation. Nanostructured particles for herbal medicines have several advantages over conventional drug delivery systems. Advanced delivery formulations not only help overcome problems such as poor solubility, bioavailability, and stability of quercetin but also overcome the formidable skin barrier in topical delivery [10]. Nanoemulsion is a thermodynamically stable emulsion preparation that undergoes a transparent dispersion derived from oil and water. The nanoemulsion preparation is stabilized by an interfacial film of surfactant molecules with droplet sizes ranging from 20-200 nm [11]. In addition, nanoemulgels reduce the total dose and side effects because they form a non-toxic and non-irritating carrier for skin and mucous membrane delivery and control of release by permeation of the drug through a liquid film, whose hydrophilicity or lipophilicity, as well as thickness, can be controlled precisely [12].

Nanoemulgel consists of oil, water, surfactants, and basis gel [13]. The oil selection used in nanoemulsion formulation is an important factor that the drug will incorporate as a droplet in the oil phase dispersed in the aqueous phase [14]. The oils phase component will help to achieve maximum drug loading in the nanoemulsion system [15]. VCO has been shown to have a high total phenolic and tocopherol content, which is responsible for having high antioxidant properties and normalizing lipids through various pathways, anti-inflammatory and has been found to enhance antithrombotic effects related to inhibition of platelet coagulation [16, 17]. The surfactant added in the formulation must support the formation of the nanoemulsion system from the oily phase and have an excellent dissolving potential for lipophilic drugs. The higher solubility of the drug in the oil phase is important to maintain the drug in dissolved form. The right mix of low and high hydrophilic-lipophilic–balance (HLB) surfactants leads to stable nanoemulsion formulation [15, 18, 19]. This study uses the mixture of tween 80 and Span 80 because these two surfactants possess the same backbone, so they can mix easily, leading to a controlled change in the final [19]. Non-ionic surfactants are less toxic than ionic surfactants and have been widely used in topical products [18, 20]. Based on the material safety data sheet, tween 80 has acute toxicity LD50 oral in rat 34,500 mg/kg and does not cause skin irritation in rabbits [21].

The addition of the nanoemulsion system into the hydrogel matrix can form nanoemulgel preparations. The nanoemulgel dosage form can reduce the surface and interfacial tension of the preparation, where there is an increase in the viscosity of the aqueous phase, which will affect the increase in the stability of the nanoemulsion [22].

The current study aimed to improve quercetin's delivery and skin contact time in the form of nanoemulgel for topical use by optimizing the concentration of VCO, tween 80 and span 80.

MATERIALS AND METHODS

Materials

Quercetin (Merck®), virgin coconut oil (VCO) (Coco Milagro®), tween 80 (Merck®), span 80 (Merck®), sodium alginate, aquadest. The used instruments are a UV-Vis spectrophotometer (Shimadzu UVmini-1240), hotplate dan magnetic stirrer (Thermo Scientific), viscometer Brookfield (Brookfield LVDVE 8673144), particle size analyzer (PSA) (HORIBA Scientific SZ-100), centrifuge, eppendorf tube, ultrasonication (Branson 3800) and pH meter.

Quercetin antibacterial activity test

The inhibitory concentration of quercetin was determined using the paper disk diffusion method. The research was conducted in the Biological Safety Cabinet (BSC). The bacterial test strain (*Staphylococcus aureus*) was grown on Muller Hinton Agar (MHA) for 18–24 h at 37 °C. Then the inoculum of the bacterial strain was transferred into Muller Hinton Broth (MHB) and adjusted to a turbidity standard of 0.5 Mcfarland. 10 mg/ml quercetin stock was prepared in pure DMSO, then the quercetin concentration series was 10 mg/ml; 2 mg/ml; 0.4 mg/ml; 0.08 mg/ml; 0.016 mg/ml. A total of 15 ml of MHA was put into a petri dish and allowed to solidify, then 100 L of *Staphylococcus aureus* bacteria was added to the petri dish and spread using a spreader. Paper discs saturated/dropped with quercetin series and DMSO as negative controls were placed on top of the solidified agar media. A positive control using gentamicin and a sterility control containing only MHA media was also made. All Petri dishes were incubated at 37 °C for 24 h. A clear area or zone around the paper disc indicates bacterial growth [4, 5].

Nanoemulsion preparation

The manufacture of quercetin nanoemulsions was carried out by dripping the quercetin with 70% ethanol until dissolved, then mixing it with virgin coconut oil phase using a stirrer until homogeneously mixed. The mixture was added to the tween-80 and span-80 mixture using a magnetic stirrer for 10 min with a speed of 1000 rpm and a temperature of 80 °C. After 10 min add 100 ml distilled water gradually, and the stirring was increased to 1250 rpm for 10 min. All ingredients that have been mixed are then homogenized using a homogenizer for 2 min and followed by sonication using a sonicator bath for 40 min. Each formula was replicated three times in table 1 [23].

Table 1: Design factorial formulations scheme

Formula	Span 80 (g)	Tween 80 (g)	VCO* (g)	Quercetin (g)	Sodium alginate (g)
1	3	11	3.5	0.2	2
A	4	11	3.5	0.2	2
B	3	12	3.5	0.2	2
AB	4	12	3.5	0.2	2
C	3	11	4	0.2	2
AC	4	11	4	0.2	2
BC	3	12	4	0.2	2
ABC	4	12	4	0.2	2

*VCO-virgin coconut oil

Nanoemulgel preparation

The gelling agent in the form of 2 grams of sodium alginate was developed in 100 ml of nanoemulsion with constant stirring using a mixer until a homogeneous, clear, and transparent mass was formed table 1 [24].

Percent transmittance test

The percent transmittance test was performed by dissolving 1 ml in a 100 ml volumetric flask using distilled water. The solution was measured percent transmittance at a wavelength of 650 nm using a UV-Vis spectrophotometer, and distilled water was used as a blank [23]. The percent transmittance value can be expressed as A =-log %T, where A indicates the absorbance value and shows the percent transmittance [25]. The transmittance percentage value of 90%-100% shows that the nanoemulsion preparation has a transparent and clear appearance [26].

Particle size measurement

Particle size was measured using a particle size analyzer with a dynamic light scattering type. First, the cuvette was cleaned not to affect the analysis results. A sample of 10 ml was taken and put into a cuvette. Then the cuvette was inserted into the sample holder and analyzed [23].

Zeta potential

Zeta potential was measured using a particle size analyzer with measurement type zeta potential. Using 10 ml of the samples dissolved with aquadest in a ratio of 1:1

Organoleptic test

The organoleptic test of nanoemulgel was carried out by physically observing the nanoemulgel preparation with the quercetin, including color, smell, and taste [27].

Nanoemulsion type test

The nanoemulsion type test uses the dilution method by dissolving the sample into the water phase (1:100) and the oil phase (1:100). If the sample is entirely soluble in aquadest, then the nanoemulsion type is oil in water (O/W) type. On the other hand, if the sample dissolves completely in the oil phase, the nanoemulsion type includes the water in the oil (W/O) type [23].

Viscosity

The viscosity measurement of the preparation was carried out using a Brookfield viscometer (Brookfield LVDVE 8673144) by preparing 500 ml nanoemulgel in a glass beaker and then selecting the appropriate spindle number. Measurements were carried out on all replications of the preparations made.

Spreadability

The spreadability of nanoemulgel was measured 24 h after preparation. In this test, 1 gram of gel was placed in the middle of a glass plate and given a weight of 150 grams combined, allowed to stand for 1 min, then the diameter of the distribution was measured with a caliper [28, 29].

Accelerated stability testing

The stability test of the formula was carried out with two tests using the centrifugation method and the freeze-thaw cycle stability test. The centrifugation test was carried out by inserting the sample into an eppendorf tube and centrifuging for 5 min at a speed of 5000 rpm. The sample's stability is seen by whether there is a phase separation [30]. The sample stability parameters included phase separation, precipitation, creaming, and cacking.

The freeze-thaw cycle test is this carried out in three cycles by storing the preparation at a temperature of-15 °C for 48 h and transferring it to a temperature of 25 °C for 48 h for each cycle. The stability parameters measured were sample pH and particle size using the transmittance value. The freeze-thaw cycle test was repeated for three cycles [30].

Data analysis

The results to be obtained in this study are the physical properties of nanoemulgel, including percent transmittance, zeta potential, particle

size, viscosity, and spreadability. The optimum area was obtained using the Minitab® 18 application, which previously had a pure experimental design using the factorial design method. Normally distributed and homogeneous data can be analyzed using a three-way Analysis of Variance (ANOVA) with a 95% confidence level. The p-value<0.05 indicates a significant difference in the physical properties of the nanoemulsion. This was followed by looking for a contour plot and computational optimizer response to determine the optimum area of the oil phase: virgin coconut oil and surfactant combination tween-80 and span-80 using DOE (Design of Experiment).

RESULTS AND DISCUSSION

Quercetin antibacterial activity

Quercetin is a compound that can provide activity as an antibacterial [4]. The antibacterial activity test of quercetin was carried out to determine the presence or absence of antibacterial activity on quercetin against *Staphylococcus aureus* bacteria. Antibacterial testing of quercetin was carried out to determine the quercetin dosage for nanoemulgel formulations. Antibacterial activity testing was carried out using the paper disk method. In testing the antibacterial activity of quercetin, sterility control was made to ensure that the test was carried out aseptically. The study's results showed that the sterility control was clear, and no *Staphylococcus aureus* bacteria were growing.

They tested the potential antibacterial activity of quercetin dissolved with DMSO (Dimethyl sulfoxide). The choice of DMSO as a solvent is because DMSO can dissolve quercetin and does not have antibacterial activity, so the antibacterial activity results obtained are purely derived from quercetin [31]. Testing the antibacterial activity of quercetin showed the presence of inhibition against *Staphylococcus aureus* bacteria. The company can see the inhibition zone on the test results of a clear area around the paper disk saturated with quercetin solution. The following results of testing the antibacterial activity of *Staphylococcus aureus* can be seen in table 2. The results of the measurement of the inhibition zone showed that the antibacterial activity of quercetin was in the moderate category because the average concentration of each concentration was 6-10 mm, while the positive control, namely gentamicin, had an average inhibition zone of 20.9 mm which was in the strong category [32]. The negative control used was DMSO with an average inhibition zone of 0 mm, which indicated that DMSO had no antibacterial activity and pure antibacterial activity of quercetin.

Table 2: Diameter of quercetin inhibition zone against *Staphylococcus aureus*

Quercetin concentration (mg/ml)	Inhibition zone (mm)			Zone of inhibition (mm)*	Antibacterial strength criteria
	R 1	R 2	R 3		
10	10.40	9.90	9.45	9.92±0.475	Moderate
2	9.80	10.60	9.70	10.03±0.493	Moderate
0.4	9.40	10.25	8.20	9.28±1.030	Moderate
0.08	8.50	10.50	8.30	9.10±1.217	Moderate
0.016	8.70	9.40	8.15	8.75±0.626	Moderate
Positive control (Gentamicin)	27.00	27.00	27.00	27.00±0.000	Strong
Negative control (DMSO)	0	0	0	0±0.000	No inhibition zone

*mean±SD, n=3

Based on the results of testing the antibacterial activity of quercetin with various concentrations, it can be concluded that the concentration of quercetin used for nanoemulgel preparations is 2 mg/ml. This is because 2 mg/ml concentration produces the most significant inhibition power among other concentrations. This result can be strengthened by Jaisinghani *et al.* (2017) [4], who state that quercetin with a concentration of 20 mcg/ml can inhibit the growth of *Staphylococcus aureus* bacteria.

Physical properties and stability tests of quercetin nanoemulgel

Organoleptic

Fig. 1 shows the nanoemulsion formed from the obtained nanoemulsion, which is yellowish and transparent. The combination of tween-80 and span-80 is used as a surfactant that can unite the oil and water phases due to forming a film layer around the droplets. The HLB value of the mixture from the combination of tween-80 and span-80 ranged from 12–13. In addition, quercetin was used as an antibacterial agent, 70% ethanol to dissolve quercetin, and aquadest as a solvent.

When mixing using a magnetic stirrer, a temperature of 80 °C is given to help the evaporation process of 70% ethanol, where 70% ethanol has a low boiling point of 78 °C, so it evaporates faster. It does not cause poisoning when using the formula [33].

The nanoemulgel preparation made has a yellowish color and is slightly cloudy when used. It has a cooling effect because the most significant content of the formula is water.

Nanoemulsion type test

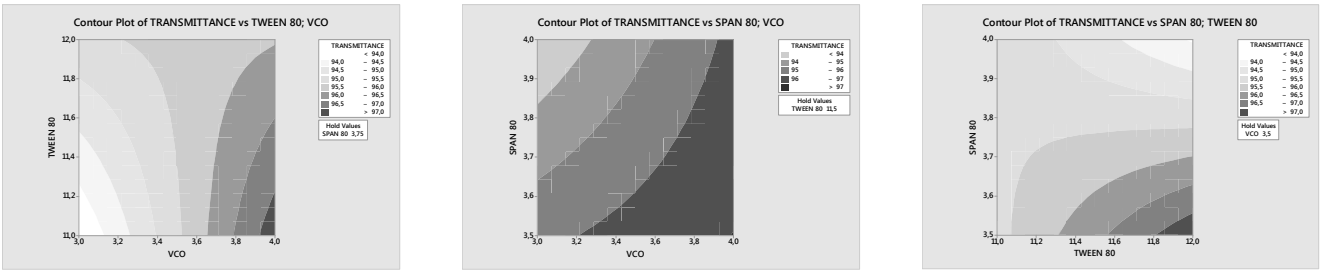
The nanoemulsion type test was carried out to determine the type of nanoemulsion formed by dissolving the nanoemulsion sample into the water phase and the oil phase in a ratio of 1: 100. The test results for eight formula nanoemulsion determined the nanoemulsion types were found to be of the oil-in-water (O/W). The nanoemulsion preparation is dispersed in the aqueous phase (aquadest). These results are in line with expectations where the HLB value of the mixture ranging from 12–13 can produce an oil-in-water (O/W) nanoemulsion type. Oil in water emulsion can be improved lipophilic drug delivery and improvement patient comfort [15].

Transmittance value

The percent transmittance test (%T) was carried out to ensure that the size of the nanoemulsion formed was in the nanometer range. The percentage transmittance value of 90%-100% indicates that the preparation has a clear and transparent appearance [26]. The percent transmittance was measured at a wavelength of 650 nm with a UV-vis spectrophotometer. The percentage of transmittance above 90% indicated the formation of nano-sized particles (<200 nm) [34]. The results of the percent transmittance measurement of the eight quercetin nanoemulsion formulas obtained were found to be in the range of 93%-97% table 3. From the results, it was found that formulas B, AC, AB, and A had the highest percent transmittance, respectively 97.8%±0.404, 97.6%±0.115, 97%±0.556 and 97%±0.057. The percent transmittance is getting closer to 100% the color will be clearer and more transparent.

The statistical analysis found that the percent transmittance measurement obtained had a significant model, indicated by a p-value<0.05 (0.000). In comparison, the factor that most influences the results of the percent transmittance measurement is VCO, which is indicated by the highest f-value, 279.90 table 4. The appearance of the contour plot of percent transmittance versus tween 80, span 80, and VCO is depicted in fig. 2. From the contour plot formed, it can be seen that all areas included are acceptable because the quercetin nanoemulsion has the percent transmittance of nanoparticles, which ranges from 90-100%

Fig. 1: Nanoemulsion preparation



a. **b.** **c.**

Fig. 2: Contour plot response transmittance versus tween 80 and VCO (a) Contour plot response transmittance versus span 80 and VCO (b) Contour plot response transmittance versus span 80 and tween 80 (c)

Table 3: Results of physical properties of nanoemulsion and nanoemulgel quercetin

Formula	*Spreadability (cm)	*Viscosity (cps)	*Transmittance value (%)	*Zeta potential (mV)
1	4.7±0.152	2498.7±0.577	93.7%±0.173	-28.4±0.100
A	4.75±0.208	2498.3±0.577	97%±0.057	-25.7±0.500
B	4.95±0.115	2494.7±0.577	97.8%±0.404	-29.5±0.200
AB	4.95±0.058	2498±0.577	97%±0.556	-30±0.929
C	4.95±0.100	2499±0	93.3%±0.152	-29.7±0.351
AC	4.425±0.100	2489±0.677	97.6%±0.115	-23.9±0.901
BC	4.9±0.153	2498±0.577	92.9%±0.288	-21.4±0.954
ABC	5±0.100	2489.7±0.577	94.9%±0.451	-25.1±0.737

*mean±SD, n=3

Table 4: Statistical analysis of physical properties

	Transmittance (%)		Zeta potential (mV)		Viscosity (cps)		Spreadability (cm)	
	[a]P value	[b]F value	[a]P value	[b]F value	[a]P value	[b]F value	[a]P value	[b]F value
Model	0.000	119.15	0.000	67.75	0.000	179.24	0.001	6.40
VCO	0.000	279.90	0.000	15.53	0.000	289.29	0.312	1.09
Tween 80	0.075	3.61	0.169	2.07	0.000	17.29	0.002	13.89
Span 80	0.000	163.87	0.000	155.39	0.001	240.14	0.467	0.56
VC0*Tween 80	0.000	144.98	0.000	137.61	0.000	32.14	0.022	6.42
VCO*Span 80	0.000	48.59	0.928	0.01	0.000	641.29	0.022	6.42
Tween 80*Span 80	0.000	180.50	0.000	130.52	0.001	17.29	0.661	0.20
VCO*Tween 80*Span80	0.003	12.59	0.000	33.51	0.001	17.29	0.001	16.20

[a]P value determined the significant values, three-way ANOVA, [b]F value determined the variation has the significant impact, three-way ANOVA

Table 5: Particle size measurement results

Formula	Particle size (nm)	Polydispersity index
1	68.6	0.444
A	49.2	0.447
B	19.9	0.226
AB	21.1	0.301
C	124.3	0.294
AC	27.9	0.427
BC	118.6	0.285
ABC	70.3	0.495

Particle size measurement

Particle size measurement is done by the dynamic light scattering method. The particle size measurements of the eight quercetin nanoemulsion formulas obtained were found to be in the range of 19.9-118.6 nm table 5. These results show that formulas B, AB, and AC have the smallest particle sizes, 19.9 nm, 21.1 nm, and 27.9 nm. The transmittance percentage is getting closer to 100% the color

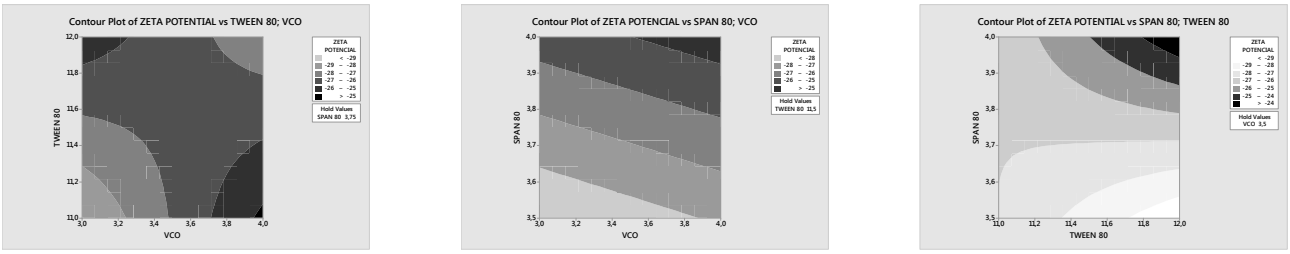
will be clearer and more transparent [34]. The eight formulas obtained had transmittance percentages below 200 nm.

The polydispersity index (PDI) describes the uniformity of globule size in the preparation and determines the presence or absence of aggregation. The PDI can range from 0 to 1, where 0 (zero) stands for a monodisperse system and 1 for a polydisperse particle dispersion [35]. However, the PDI is lower than 0.5, indicating narrow and favorable particle size distribution [26, 36]. The PDI results in the range of 0.226–0.495 so that the nanoemulsion formed has a uniform particle size.

Zeta potential

Zeta potential is a charge parameter of electricity between colloidal particles and is very important in determining the stability and aggregation of colloidal nanoparticles. This analysis involves electrostatic interactions between the charged nanoparticle surface and the opposite charge ions in the solution. A good Zeta potential has a value that ranges from +30 mV to-30 mV [37]. The magnitude of this zeta potential indicates the stability of the colloidal system. From the measurement results, the zeta potential value of the eight formulas is-21.4 mV to-30 mV, which fulfills a good zeta potential value. The formula with the highest zeta potential was formula BC (-21.4±0.954), and the lowest zeta potential was formula AB (-30±0.929) table 3.

The statistical analysis found that the zeta potential measurement obtained had a significant model, indicated by a p-value<0.05 (0.000). In comparison, the factor that most influences the results of the percent transmittance measurement is span 80, which is indicated by the highest f-value, 155.39 table 4.



a. **b.** **c.**

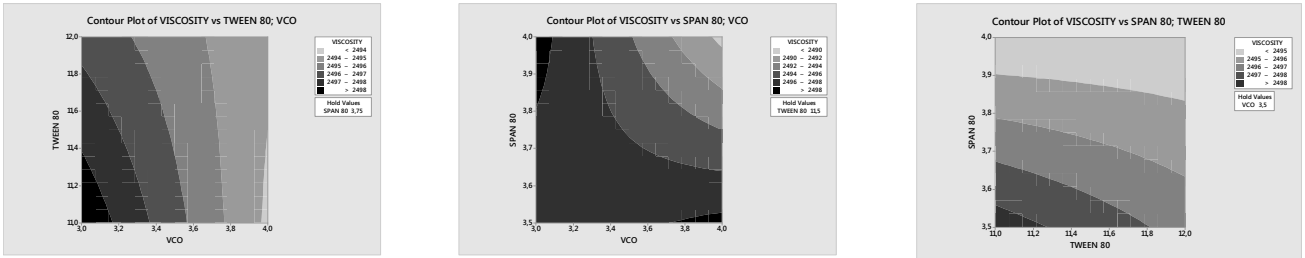
Fig. 3: Contour plot response zeta potential versus tween 80 and VCO (a) Contour plot response zeta potential versus span 80 and VCO (b) Contour plot response zeta potential versus span 80 and tween 80 (c)

Fig. 3 is a contour plot showing the interaction results between tween-80, span-80, and VCO on the zeta potential response in 2D. Based on the resulting plot, it can be concluded that the entire area formed is the optimum area for the concentration of VCO, tween-80, and span-80 to the zeta potential response. This is because the area meets the desired zeta potential criteria of+30 mV to-30 mV [37].

Viscosity

The results of the quercetin nanoemulgel viscosity test performed with a Brookfield viscometer. Viscosity testing aims to determine the value of the viscosity of a substance. The higher the viscosity value, the higher the viscosity level of the substance. The viscosity value of a good gel preparation is 2000-4000 cps [38]. From the test results, eight nanoemulgel formulas have a viscosity of 2489-2499, which is still in the good gel viscosity range table 3.

The statistical analysis showed that the significantly formed model was stated with a p-value of<0.05 (0.000). The factor that most affected the viscosity was VCO which had the highest f-value of 289.29 table 4.



a. **b.** **c.**

Fig. 4: Contour plot response viscosity versus tween 80 and VCO (a) Contour plot response viscosity versus span 80 and VCO (b) Contour plot response viscosity versus span 80 and tween 80 (c)

Fig. 4 is a contour plot showing the interaction results between tween-80, span-80, and VCO on the 2D viscosity response. Based on the resulting plot, it can be concluded that the entire area formed is the optimum area for the concentration of VCO, tween-80, and span-80 on the nanoemulgel viscosity response, and this is because the area meets the criteria for a suitable gel viscosity of 2000-4000 cps.

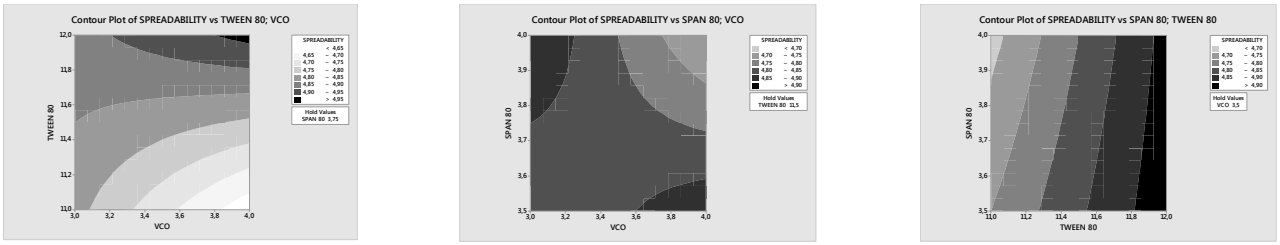
Spreadability

The spreadability test was carried out for guarantees even distribution of gel when applied to the skin performed as soon as the gel was made. Good spreadability of gel preparations between 5-7 cm [38]. The results of the spreadability test are not good because it has a diameter of 4.7-5 cm. there is only one formula that meets the criteria for good spreadability, namely the ABC formula (5±0.1) table 3. The statistical analysis results for the dispersion response show that the model form is significant, as stated by the p-value<0.05 (0.001). The factors that most influence the response are tween 80, which had the highest f-value of 13.89 table 4.

Fig. 5 is a contour plot showing the interaction results between tween-80, span-80, and VCO on the 2D spreadability measurement response. Based on the resulting plot, it can be concluded that the area that shows the optimum concentration of VCO, tween-80, and span-80 on the dispersion response is only the black area.

Accelerated stability testing and optimization

After evaluating the nanoemulsion formulation, the test accelerated stability was continued, indicating no phase separation, and flocculation was observed, proving its stable nature.



a. **b.** **c.**

Fig. 5: Contour plot response spreadability versus tween 80 and VCO (a) Contour plot response spreadability versus span 80 and VCO (b) Contour plot response spreadability versus span 80 and tween 80 (c)

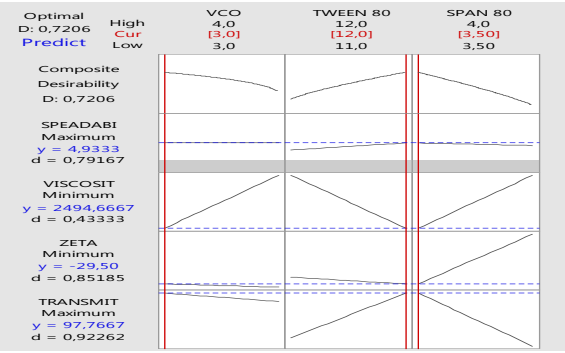


Fig. 6: Optimizer plot for response spreadability, viscosity, zeta potential, and transmittance

Optimization targets were set for the response of dispersion, viscosity, zeta potential, and percent transmittance. The response optimizer menu in the Minitab® 18 software determines the optimum formula with multiple responses [39]. The results of processing and computational recommendations to achieve the optimum conditions were found at the VCO amount of 3g, the tween amount of 12g, and the span amount of 3.5g was found as computational recommendations to achieve the optimum conditions. But this concentration is predicted to produce a composite desirability value of all responses of 0.7206. This is not considered good because a desirability value close to 1 indicates a high model's ability to produce the expected response value fig. 6 [39]. From the results obtained, only the percent transmittance has a desirability close to 1, which is 0.922, so the optimum response for the formula is only the transmittance percent.

CONCLUSION

Based on the results obtained in this study, the concentration of the quercetin used to inhibit the growth of *Staphylococcus aureus* bacteria that causes canker sores is 2 mg/ml because, at that concentration, it produces the most significant inhibitory power. The optimum VCO, tween-80, and span-80 were found at the VCO amount of 3g, the tween amount of 12g, and the span amount of 3.5g. Still, it is predicted to produce a composite desirability value of all responses of 0.7206, which is not considered good. From the results obtained, only the percent transmittance has a desirability close to 1, which is 0.922, so the optimum response for the formula is only the transmittance percent. This study also reported that the effect variation concentration of VCO, tween 80 and span 80, has significantly impacted the physical properties (viscosity, spreadability, transmittance value, and zeta potential) of the quercetin nanoemulgel.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally to this research article.

CONFLICT OF INTERESTS

The authors declared that no conflict of interest should arise concerning the authorship of this research article.

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