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Response surface methodology for optimization of turmeric essential oil-loaded nanoemulgel

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ABSTRACT: Turmeric essential oil has antioxidant and anti-inflammatory activities in a topical dosage form. Nanoemulgels are one of them. Using emulsifiers and gelling agents in the formula affects the nanoemulgels (Negs) characteristics and stability. This study focuses on the systematic development, optimization, and characterization of Turmeric Essential Oil-loaded Nanoemulgels (TEO-loaded Negs), having promising topical applications. The TEO-loaded Negs were prepared by the high-speed homogenization method. The formula design used Central Composite Design from Response Surface Methodology (CCD-RSM) to obtain the optimal TEO-loaded Negs formula. The optimization variables of the TEO-loaded Negs formula include the concentrations of Span 80-Tween 80 (X1) and Carbopol 980 (X2) with the response of particle size (PS) (Y1), polydispersity index (PDI) (Y2), zeta potential (ZP) (Y3), pH (Y4), spreadability (Y5) and adhesion (Y6). The actual TEO-loaded Negs responses were compared with the CCD-RSM predictions. In addition, other physical evaluations such as homogeneity observations, phase separation tests, viscosity, and flow properties were carried out. The optimal TEO-loaded Negs developed were made with 8.68% Span 80-Tween 80 and 1.18% Carbopol 980. The evaluation results showed the optimal TEO-loaded Negs nano-metric size (182.3 ± 5.5 nm) with low PDI (0.242 ± 0.003), good ZP (-57.23 ± 2.91 mV), pH (4.51 ± 0.02), spreadability (6.0 ± 0.2 cm), and adhesion (6.45 ± 0.19). TEO-loaded Negs have a good appearance and do not undergo phase separation at extreme temperature storage. Thus, the developed TEO-loaded Negs can be a potential delivery system and a promising suitable approach for topical preparations.

KEYWORDS: Central composite design; nanoemulgels; response surface methodology; turmeric essential oil; topical delivery.

1. INTRODUCTION

Most water-based liquid or semisolid systems have limitations for delivering lipophilic drugs (1). Nanoemulsions are an established alternative for delivering lipophilic drugs by increasing topical absorption (2). The main advantage of topically administered nanoemulsions is the ability to increase penetration and permeation of active ingredients through the skin without adding chemical enhancers and non-friendly solvents to the formula, which can cause skin irritation, especially with long-term usage (2,3). However, this system has problems with low viscosity due to poor spreadability and retention in the skin (4). A combination of nanoemulsion and hydrogel was made to improve the characteristics, called nanoemulgels (Negs) (2,3,5).

Negs consist of two systems; an emulsion containing nano-sized globules and a hydrogel system. The emulsion acts as a drug delivery platform and is stabilized by an emulsifier in the form of surfactants and cosurfactants (2). Surfactants reduce the interfacial tension of immiscible liquids and change the entropy of the dispersion, thereby stabilizing a thermodynamically unstable system; cosurfactants are combined with surfactants in the emulsification process by disrupting the surface layer (6). The emulsifier plays a role in the emulsification process to increase stability when the product is stored for a long time. On the other side, gels are made from polymers, a gelling agent, that expand after absorbing a liquid (7). Gelling agents increase the viscosity of the formula and can react with surfactants to change the viscosity (8). In addition, topical Negs can improve patient compliance due to their non-greasy, non-irritating properties and better drug release (9).

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Turmeric essential oil (TEO) is extracted from the turmeric rhizome of Curcuma Longa L. (Zingiberaceae) by steam distillation (10). Chemical constituents with the most significant proportion were oxygenated monoterpenes, and sesquiterpenes include ar-turmerone, α-turmerone, and β-turmerone (11–13). The pharmacological activities of TEO have been reported in the form of antioxidants, anti-inflammatory, antinociceptive, antidermatophytic, antifungal, and antibacterial activities (10,14–17). Like other essential oil, TEO has limited use due to its volatility, instability under certain conditions, lipophilicity, and low aqueous solubility (18,19). Many recent studies are oriented toward solving these limitations, so that efficacy of the essential oil lasts longer and increases. Previously, TEO has been developed for cream as a conventional drug delivery system; patch and nanoemulsions as drug delivery systems (15,20–22). However, the available methods for manufacturing Negs exhibit various limitations, which directly or indirectly affect the quality of the Negs formulations. Currently, the principle of quality by design is adopted to ensure the quality of drugs, their safety, and efficacy (23). The quality by design (QbD) trend is used to design, optimize, and examine the relationship between certain factors and their associated responses to obtain the most optimal formula (24). Central Composite Design (CCD) on Response Surface Methodology (RSM) can be used to select optimal formulas and predict models that rely on statistical analysis (ANOVA) and exact equations (25,26). In this aspect, we intended to develop a TEO-Negs formulation containing turmeric oil that would be optimized using a complete 22 factorial design and determine the independent factors' precise influence on the investigated dependent variables. The choice and procedure factors were Span 80-Tween 80 (X1) and Carbopol 980 (X2). The process and formulation factors on particle size (PS) (Y1), polydispersity index (PDI) (Y2), zeta potential (ZP) (Y3), pH (Y4), spreadability (Y5), and adhesion (Y6) were investigated. This work is the first step in developing an optimized turmeric oil preparation suitable for transdermal drug delivery for topical application.

2. RESULTS AND DISCUSSION

2.1. Preparation and Optimization of TEO-loaded Negs

TEO-loaded Negs are produced using the high-energy method efficiently. Variations in the emulsifier and gelling agent concentration in different compositions and formulations of Negs have been produced. A total of 14 formulas were prepared and optimized using Design-Expert®, version 13 software. The optimized Negs are selected based on the minimum of PS, PDI, maximum ZP, pH range, spreadability, and adhesion values. The analysis results show that the optimal formula is obtained with the composition of Span 80-Tween 80 of 8.68% and Carbopol 980 of 1.18% based on minimum PS, PDI, maximum ZP, the desired range of pH, dispersion, and adhesion that are suitable for the application transdermally.

2.2. Central Composite Design (CCD)

TEO-loaded Negs were optimized based on CCD in the RSM. CCD was applied to determine the optimum emulsifier concentration (X1) and gelling agent concentration (X2) as the main factors influencing the dependent response. Prediction of the factorial axial design and the possible curvature in the response can be obtained from the optimization process with an effective second-level design (27). In developing TEO-loaded Negs, prediction of the main effect of the independent variable on the dependent variable is essential. Two factors were selected as independent variables based on the literature survey, and six responses were decided as the dependent variable with the most significant effect on Negs. The independent variables with their levels and the observed response variables are shown in Table 1.

The six responses show different models in their application, depending on the most considerable R-squared value and the smallest residual predictive sum of squares value. The selected model showed a non-statistically significant lack of fit, and model validation was confirmed by the residual plot test of the regression model indicated from supplementary information for all responses. The quadratic model describes the effects of various factors, including individual factors, interactions, and quadratic effects on responses. Table 2 shows the statistical analysis of the quadratic model for the response of the PDI, the 2FI model for the ZP response, and linear models for the response of PS, pH, spreadability, and adhesion. This table selects factors with p-values below the pre-defined threshold (here 0.05, with a 95% confidence level) as influential factors.

Particle size parameters are often used to characterize nanoparticles. The mean TEO-loaded Negs globule diameter (Y1) was adjusted from 160.8 nm (STD#10) to 457.0 nm (STD#2). As shown in Table 2,

statistical analysis revealed that the most critical factor affecting the PS of Negs globules was the emulsifier concentration because the p-value was more significant than other factors (p-value <0.01). The positive coefficient has a synergistic effect on the response. In contrast, the negative coefficient has an antagonistic effect which concludes the inverse relationship of the independent variable with the response (27,28). In addition, this factor has a more significant coefficient. It directly affects the PS, which means that increasing the emulsifier concentration causes a decrease in the diameter of the globule PS in the TEO-loaded Negs. High surfactant concentrations (above 5.5%) resulted in globules measuring below 200 nm and low surfactant concentrations (below 5.5%) producing globules above 200 nm. That fact is in line with other studies that increasing surfactant concentrations can reduce droplet PS in Negs (29,30). The surfactant lowered the interfacial tension between the oil and water phases, reducing the free energy required to disrupt or break the droplets and resulting in a smaller droplet diameter. It can also form a protective layer around the droplets and prevent them from coalescing with others. However, the emulsifier must absorb quickly enough around the droplet to form this protective layer (30,31).

In Table 1, the polydispersity index of TEO-loaded Negs (Y2) varied from 0.000 (STD#2 and #4) to 0.571 (STD#1, #5, #6, #8, #9, #11 to #14). From the analysis results, no single factor significantly affects the PDI because all p-values are > 0.05. In addition, no factors directly affect the PDI of the Negs. The PDI measures the distribution of molecular mass in a sample. The smaller the PDI value (close to 0), the more stable the formula from Negs is because the greater the PDI value indicates the particles formed are not uniform, so that the formula will flocculate quickly. An index value less than 0.05 is included in monodisperse, while an index greater than 0.7 indicates that the sample has a broad particle size distribution. A-0.2 and below are considered acceptable for nanoparticle preparations (32).

The zeta potential of TEO-loaded Negs (Y3) was in the range of 13.90 mV (STD#2) to 46.72 mV (STD#10) (Table 1). According to statistical analysis on CCD, the emulsifier concentration factor significantly affected ZP compared to other factors. ZP represents the electric charge between the shear plane of the final outer layer and the bulk solution, which significantly affects the stability of the dispersion (33). This factor is strongly influenced by the composition of the Negs and its electrical phenomena. TEO-loaded Negs, which have positive zeta potential, show good interaction with negatively charged skin (34). ZP is the scientific term for the electrokinetic potential in colloidal systems. The high electric charge on the nanoparticle surface will prevent the aggregation of the nanoparticles because of the strong repulsion between the particles. ZP is usually influenced by the physicochemical properties of the drug, polymer, carrier, presence of electrolytes, and their adsorption (35). The ZP requirement is above ± 30 mV. The higher the ZP value, the slower the aggregation formed to prevent separation (36).

The pH test is carried out to measure the level of acidity or alkalinity of Negs. The pH values (Y4) were in the range of 4.57 (STD#13) to 6.39 (STD#14) (Table 1). In this response, the gelling agent concentration factor has a significant effect compared to other factors. The pH requirement of a topical preparation is the same as the skin pH. Preparations that are too acidic can irritate the skin and cause a stinging sensation, while preparations that are too alkaline can cause dry and itchy skin. The results of the pH test carried out on 14 formulas were eligible, where compatible with the skin (4.5-6.5) (37). The increase influenced the decrease in the pH value obtained from this study in the concentration of Carbopol. That is caused by the reaction between the carboxylate group in Carbopol with water so that more H_3O^+ (acid) is formed and makes the preparation more acidic.

On the spreadability response (Y5), the results were obtained in the range of 4.90 (STD#11) to 6.25 (STD#10). In statistical analysis, the emulsifier concentration factor significantly affects the dispersion of Negs. The spreadability is carried out to see the dispersion of Negs on the skin. Terms of good dispersion are 5-7 cm. If the dispersion is too small, it is relatively difficult to spread when applied to the skin, while the dispersion tends to spread too quickly when applied so that it will cause an uncomfortable feeling when used (38). Based on the results of the spreadability test, only one Negs did not meet the requirements, namely F11, ie. 4.9 cm. The smaller the concentration of Carbopol 980, the greater the dispersion obtained. The higher the concentration of Carbopol in preparation, the more the preparation's viscosity. Adhesion and spreadability have the opposite results. The higher the viscosity of preparation, the higher the adhesive power produced, while the smaller the dispersion power (39).

Finally, the adhesion value is from 4.22 (STD#14) to 7.08 (STD#13) in Table 1. Statistically, this adhesion was significantly affected by the concentration of the gelling agent. They met the requirements based on the adhesion test results carried out on 14 formulas. An adhesion test is carried out to see how long a preparation can be attached to the skin. The stickiness requirement is more than 4 seconds. The longer a

preparation can be attached to the skin, the better, where it is expected that more active substances can be absorbed due to the time the preparation is in contact with the skin (40). This result also shows that the smaller the concentration of Carbopol 980, the lower the adhesive power obtained. In the end, the emulsifier concentration factor significantly affected the response of PS, ZP, and spreadability of TEO-loaded Negs. At the same time, the concentration factor of the gelling agent affects the pH and adhesion.

2.3. Optimized TEO-loaded Negs

The analysis was carried out using CCD-RSM to determine the optimal conditions for the concentrations of Span 80-Tween 80 and Carbopol 980 for manufacturing Negs. The optimal value determines each response, and the results are used to perform optimization. Variables and response measurements will be used by the program to perform optimization. The effect of variables in this study were the concentrations of Span 80-Tween 80 and Carbopol 980 that responded to PS, PDI, ZP, pH, spreadability, and adhesion. The formula with the maximum desirability value is the optimal formula generated from the optimization phase of the program (41).

The optimization value formed is indicated by the desirability value close to one. The desirability value range is 0-1. Figure 1 describes the optimization results in the form of a 2D contour. *Contour* is a two-dimensional response image that is presented using a predictive model for the response values of PS, PDI, ZP, pH, spreadability, and adhesion. The contour graph shows the desirability value of 0.801, which is the closest value to 1 compared to the other points. Figure 2 shows the projection in the form of a 3D surface; the low area shows low desirability while the high area shows high desirability and is getting closer to 1. 25, ZP 56.30 mV, pH 4.5, adhesion 6.98 seconds, and spreadability 6.07 cm.

2.4. Evaluation of TEO-loaded Negs

Optimal TEO-loaded Negs evaluations were carried out, including organoleptic, homogeneity, freeze-thaw, PS, PDI, ZP, pH, adhesion and spreadability, viscosity, and flow properties be seen in Tables 4 and 5. In addition, Table 3 contains the actual and predicted values of the optimized Negs for each response-dependent variable. The optimum formula results from the predictions of the RSM program using the Central Composite Design (CCD) model are the concentrations of Span 80-Tween 80 of 8.68% and Carbopol 980 of 1.18%.

The particle size of the optimum formula is 182.3 nm; this result is almost close to the RSM prediction's PS, 180.2 nm. The PDI value of the optimum formula obtained is 0.242; this result is close to the PDI result of the RSM prediction, which is 0.250. The ZP of the optimum formula is 57.23 mV; this result is close to the ZP of the RSM prediction, which is 56.30 mV. The pH value obtained from the optimum formula is 4.51; this result is close to the pH value predicted by RSM, which is 4.5. The adhesion of the optimum formula is 6.45 seconds; this result is close to the result of RSM prediction, which is 6.98 seconds. The spreadability obtained from the optimum formula is 6.00 cm; this result is close to the dispersion from the RSM prediction, which is 6.07 cm.

Based on Table 4, the organoleptic results of TEO-loaded Negs have a distinctive turmeric odor, are white, and are semi-solid. The homogeneity test results of the optimum TEO-loaded Negs formula showed a homogeneous preparation, as evidenced by the absence of coarse grains on the object glass. The results of the Freeze-Thaw test on the optimum formula for six cycles showed promising results; namely, there was no separation. It aims to see the separation of the water and oil phases due to the influence of extreme temperatures (42). Viscosity testing of the optimal formula for TEO-loaded Negs was carried out using a Brookfield digital RV DV-E spindle seven at a speed of 50 rpm. The viscosity of the TEO-loaded Negs preparation was 32240 cP, indicating reasonably high viscosity. That is due to the neutralization of the gel base with the addition of triethanolamine. Carbopol is dispersed in water to form an acidic colloidal solution with low viscosity. Neutralizing the gel base will increase the viscosity of the preparation because the gelling agent expands well. Judging from the dispersion and adhesion produced, although the TEO-loaded Negs have a reasonably high viscosity, the spreadability follows the requirements. In contrast, the adhesion power is relatively short despite meeting the requirements.

The results of the flow properties test showed that the optimal formula made was a thixotropic plastic flow type. *Thixotropic* is a flow property expected in pharmaceutical preparations because it has high consistency in the container but can be poured and dispersed easily. That can be seen from the graph results,

which show that the descending curve is to the left of the ascending curve. The plastic flow curve does not pass through the point (0,0) but intersects the shearing stress axis (or will intersect if the straight section of the curve is extrapolated to the axis) at a certain point called the yield value. The yield value is the amount of force or shear stress that must be exceeded so that the preparation can flow. The larger the flocculated substance in the preparation, the greater the yield value (43). From the curve obtained, the yield value is 224,276 dyne/cm^3.

4. CONCLUSION

Based on the results of the RSM analysis, the optimum concentration of Span 80-Tween 80 as a surfactant was 8.68%, and Carbopol 980 as a gelling agent was 1.18%. The resulting response is a PS of 182.3 nm, PDI 0.242, ZP 57.23 mV, pH 4.51, adhesion of 6.45 seconds, and spreadability of 6 cm. The viscosity of the optimum formula is 32240 cP with thixotropic plastic flow properties. Thus, the developed TEO-Negs can be a potential delivery system and a promising suitable approach for topical preparations.

5. MATERIALS AND METHODS

5.1. Materials

TEO (Curcuma longa) purchased from Darjeeling Sembrani Aroma (Indonesia), Sorbilene O E/P from Lamberti (Italy), Span 80 from Croda (Singapura), Propylene glycol from Dow Chemical Pacific (Singapura), Carbopol 980 NF from Lubrizol AM (Cleveland), Nipagin M from Clariant Produkte (Deutschland), Propyl Paraben from Alpha Chemika (India), and Triethanolamine from Dow Chemical Pacific (Switzerland).

5.2. Methods

5.2.1. Preparation of TEO-loaded Negs

TEO-loaded Negs are made using a high-energy method, which uses a mechanical device to produce a highly disruptive force to break up the oil and water phases to obtain nano-sized globules (2). The oil phase (M1) was prepared by mixing Span 80 with 5% turmeric oil using a magnetic stirrer (WiseStir Wisd) at 1500 rpm for 20 min. A total of 0.18% methylparaben and 0.02% propylparaben dissolved in 15% propylene glycol (M2). Then the distilled water was stirred with tween 80, and M2 was added gradually until homogeneous at 1500 rpm for 20 min (M3). M1 was stirred with M3 until homogeneous at 1500 rpm for 40 min to form a clear and transparent nanoemulsion, then let left for 24 hours. The gel base was prepared by mixing Carbopol 980 NF with distilled water at 70°C and left for 24 hours. Then add gradually 1% TEA to form a gel mass. Nanoemulsion was added slowly into the gel base while homogenized using a homogenizer (AEG) at 2000 rpm for 10 min.

5.2.2. Experimental design (CCD-RSM)

This study selects the CCD-RSM method to develop the TEO-loaded Negs formulation. A 2-factor CCD-RSM at two levels (high and low) was used for preliminary screening on PS, PDI, ZP, pH, spreadability, and adhesion. Based on previous experiments and literature reviews, high and low levels of variables were determined. CCD of the statistical package Design-Expert® version 13 software (Stat-Ease Inc., Minneapolis, MN) was used to assess the effect of the selected independent variable on the response variable to obtain the optimal formula for TEO-loaded Negs. CCD planned 14 experiment runs under controlled circumstances (Table 5). The independent variables were Span 80-Tween 80 concentration (X1) and Carbopol 980 concentration (X2). The observed response of the dependent variables was PS (Y1), PDI (Y2), ZP (Y3), pH (Y4), spreadability (Y5), and adhesion (Y6).

5.2.3. Determination of PS, PDI, and ZP of Negs

PS and PDI of Negs were determined by dynamic light scattering (DLS) technique or photo correlation spectroscopy using the Delsa Max Pro Particle Size Analyzer LS 100Q (Beckman Coulter, USA) at 25°C. For analysis, 1 mL of sample was dispersed in 9 mL aqua pro injection. Into the cuvette, 1 mL of suspension and 5 mL of aqua pro injection were added as a diluent, and the results were read on the instrument. All

measurements were made at a scattering angle of 90°. ZP was determined by particle size analyzer through mobility and conductivity measurements. The temperature and the mean electric field were applied at 25°C and 16 V/cm, respectively (44). The mean of the three repeated measurements of each sample is reported as the final result.

5.2.4. Determination of pH, spreadability, and adhesion

pH was measured at a temperature of 25°C using a pH meter that had been previously calibrated with buffer solutions of pH 4 and 7. The calibration process was completed when the pH value indicated on the screen matched the expected pH value and was stable. Afterward, the electrode was dipped in Negs and recorded the value shown on the screen (45). Spreadability was measured by adding 0.5 g of Negs in the center of a glass covered with another glass. Measurement of the diameter of the preparation distribution longitudinally and transversely, and every minute of adding 50 g to a total weight of 150 g (46). Adhesiveness was determined by placing 0.5 g of Negs on a slide, then covering it with another slide, and being given a load of 1 kg for 3 minutes. The glass object was mounted on the test apparatus, and 80 g of the load was released until both glass objects were released, and the time was recorded (46).

5.2.5. Visual observation and homogeneity test

Negs are placed in a glass object and directly observed for color, smell, and shape (47). A-0.1 g Negs were spread over the slide, and homogeneity was observed. If there are no coarse grains, the test preparation is declared homogeneous (48).

5.2.6. Viscosity and reological flow

Viscosity and rheology were determined with a Brookfield RV DV-E Viscometer with appropriate spindle and speed. Negs were put into a beaker glass until it reached 500 mL; the spindle was installed, and the measured value was recorded as viscosity Negs. In this study, the spindle used is spindle no. 7. Flow properties are determined by measuring the viscosity using a suitable spindle from low to high rotational speed and vice versa (45).

5.2.7. Freeze-thaw test

Negs were stored at 4 ± 2°C, then transferred to 40 ± 2°C for 48 hours (1 cycle), then repeated for 6 cycles. Phase separation was observed in each cycle (45).

5.2.8. Statisical analysis

Statistical analysis was performed using the Design-Expert® version 13 software (Stat-Ease Inc., Minneapolis, MN). All measurements were repeated three times, and the analysis was performed at a sig. $p < 0.05$.

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REFERENCES

[1] Kirtane AR, Karavasili C, Wahane A, Freitas D, Collins J, Tamang S, et al. Development of oil-based gels as versatile drug delivery systems for pediatric applications. Sci Adv. 2022;8(21):1–19. [CrossRef]

[2] Sengupta P, Chatterjee B. Potential and future scope of nanoemulgel formulation for topical delivery of lipophilic drugs. Int J Pharm. 2017;526(1–2):353–65. [CrossRef]

[3] Eid AM, El-Enshasy HA, Aziz R, Elmarzugi NA. Preparation, characterization and anti-inflammatory activity of Swietenia macrophylla nanoemulgel. J Nanomedicine Nanotechnol. 2014;5(2). [CrossRef]

[4] Khurana S, Jain NK, Bedi PMS. Nanoemulsion based gel for transdermal delivery of meloxicam: Physico-chemical, mechanistic investigation. Life Sci. 2013;92(6–7):383–92. [CrossRef]

[5] Talat M, Zaman M, Khan R, Jamshaid M, Akhtar M, Mirza AZ. Emulgel: an effective drug delivery system. Drug Dev Ind Pharm. 2021;47(8):1193–9. [CrossRef]

[6] Sultana N, Akhtar J, Badruddeen, Khan MI, Ahmad U, Arif M, et al. Drug Development Life Cycle. In: Intech. IntechOpen; 2022.

[7] Ashara KC, Paun JS, Soniwala MM, Chavda JR, Mendapara VP, Mori NM. Microemulgel: an overwhelming approach to improve therapeutic action of drug moiety. Saudi Pharm J. 2016;24(4):452–7. [CrossRef]

[8] Dhawan B, Aggarwal G, Harikumar S. Enhanced transdermal permeability of piroxicam through novel nanoemulgel formulation. Int J Pharm Investig. 2014;4(2):65–76. [CrossRef]

[9] Ahmad MZ, Ahmad J, Alasmary MY, Akhter S, Aslam M, Pathak K, Jamil P, Abdullah MM. Nanoemulgel as an approach to improve the biopharmaceutical performance of lipophilic drugs: Contemporary research and application. J Drug Deliv Sci Technol. 2022;72(May 11):103420. [CrossRef]

[10] Liju VB, Jeena K, Kuttan R. An evaluation of antioxidant, anti-inflammatory, and antinociceptive activities of essential oil from Curcuma longa. L. Indian J Pharmacol. 2011;43(5):526–31. [CrossRef]

[11] Devkota L, Rajbhandari M. Composition of essential oils in turmeric rhizome. Nepal J Sci Technol. 2016;16(1):87–94. [CrossRef]

[12] Guimarãesa AF, Vinhas ACA, Gomes, Angélica Ferraz Souza LH, Krepsky PB. Essential oil of Curcuma longa L. rhizomes chemical composition, yield variation and stability. Quim Nov. 2020;43(7):909–13. [CrossRef]

[13] Islamadina R, Can A, Rohman A. Chemometrics application for grouping and determinating volatile compound which related to antioxidant activity of turmeric essential oil (Curcuma longa L). J Food Pharm Sci. 2020;8(2):1. [CrossRef]

[14] Dosoky NS, Setzer WN. Chemical composition and biological activities of essential oils of curcuma species. Nutrients. 2018;10(9):10–7. [CrossRef]

[15] Jankasem M, Wuthi-udomlert M, Gritsanapan W. Antidermatophytic properties of ar -turmerone, turmeric oil, and curcuma longa preparations. ISRN Dermatol. 2013;2013:1–3. [CrossRef]

[16] Mishra R, Gupta AK, Kumar A, Lal RK, Saikia D, Chanotiya CS. Genetic diversity, essential oil composition, and in vitro antioxidant and antimicrobial activity of Curcuma longa L. germplasm collections. J Appl Res Med Aromat Plants. 2018;10(December 2017):75–84. [CrossRef]

[17] Sharifi-Rad J, Rayess Y El, Rizk AA, Sadaka C, Zgheib R, Zam W, et al. Turmeric and ıts major compound curcumin on health: Bioactive effects and safety profiles for food, pharmaceutical, biotechnological and medicinal applications. Front Pharmacol. 2020;11(September):1–23. [CrossRef]

[18] Ibáñez MD, Blázquez MA. Curcuma longa l. Rhizome essential oil from extraction to its agri-food applications. A review. Plants. 2021;10(1):1–31. [CrossRef]

[19] Yan W, Bowen WD, Hopson R, Mathew AE, Jacob JN. Biological studies of turmeric oil, part 2: Isolation and characterization of turmeric oil components; cytotoxic activity against pancreatic cancer cells. Nat Prod Commun. 2013;8(6):811–4. [CrossRef]

[20] Ali MS, Alam MS, Imam F, Siddiqui MR. Topical nanoemulsion of turmeric oil for psoriasis: Characterization, ex vivo and in vivo assessment. Int J Drug Deliv. 2012;4(2):184–97. [CrossRef]

[21] Wang X, Gu Y, He Y, Sang L, Dai Y, & Wang D. Preparation and optimization formulation of zedoary turmeric oil nanoemulsion based thermo-sensitive gel for improved application in ophthalmology. J Drug Deliv Sci Technol. 2021;65(June):102682. [CrossRef]

[22] Vishwakarma AK, Maurya OP, Nimisha, Srivastava D. Formulation and evaluation of transdermal patch containing turmeric oil. Int J Pharm Pharm Sci. 2012;4(SUPPL. 5):358–61.

[23] ICH. ICH Guideline Q9 on Quality Risk Management. Vol. 44, Regulatory ICH. 2005. 1–20 p.

[24] Abdallah MH, Elsewedy HS, Abulila AS, Almansour K, Unissa R, Elghamry HA, et al. Quality by design for optimizing a novel liposomal jojoba oil-based emulgel to ameliorate the anti-inflammatory effect of brucine. Gels. 2021;7(219):1–18. [CrossRef]

[25] Sindi AM, Hosny KM, Alharbi WS. Lyophilized composite loaded with meloxicam-peppermint oil nanoemulsion for periodontal pain. Polymers (Basel). 2021;13(14). [CrossRef]

[26] Yalçinkaya SE, Yildiz N, Ak MS, Çalimli A. Preparation of polystyrene/montmorillonite nanocomposites: Optimization by response surface methodology (RSM). Turkish J Chem. 2010;34(4):581–92. [CrossRef]

[27] Ye Q, Li J, Li T, Ruan J, Wang H, Wang F, et al. Development and evaluation of puerarin-loaded controlled release nanostructured lipid carries by central composite design. Drug Dev Ind Pharm. 2021;47(1):113–25. [CrossRef]

[28] How CW, Rasedee A, Abbasalipourkabir R. Characterization and cytotoxicity of nanostructured lipid carriers formulated with olive oil, hydrogenated palm oil, and polysorbate 80. IEEE Trans Nanobioscience. 2013;12(2):72–8. [CrossRef]

[29] Chuacharoen T, Prasongsuk S, Sabliov CM. Effect of surfactant concentrations on physicochemical properties and functionality of curcumin nanoemulsions under conditions relevant to commercial utilization. Molecules. 2019;24(15):1–12. [CrossRef]

[30] Sarheed O, Dibi M, Ramesh KVRNS. Studies on the effect of oil and surfactant on the formation of alginate-based O/W lidocaine nanocarriers using nanoemulsion template. Pharmaceutics. 2020;12(12):1–21. [CrossRef]

[31] McClements DJ. Food Emulsions: Principles, Practices, and Techniques. Third Edition. New York: CRC Press; 2016. 719 p.

[32] Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, et al. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics. 2018;10(2):1–17. [CrossRef]

[33] Choi AJ, Kim CJ, Cho YJ, Hwang JK, Kim CT. Characterization of capsaicin-loaded nanoemulsions stabilized with alginate and chitosan by self-assembly. Food Bioprocess Technol. 2011;4:1119–26. [CrossRef]

[34] Sinha P, Srivastava S, Mishra N, Singh DK, Luqman S, Chanda D, et al. Development, optimization, and characterization of a novel tea tree oil nanogel using response surface methodology. Drug Dev Ind Pharm. 2016;42(9):1434–45. [CrossRef]

[35] Gurpreet K, Singh SK. Review of nanoemulsion formulation and characterization techniques. Indian J Pharm Sci. 2018;80(5):781–9. [CrossRef]

[36] Honary S, Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems - A review (Part 1). Trop J Pharm Res. 2013;12(2):255–64. [CrossRef]

[37] Dias IP, Barbieri SF, Fetzer DEL, Corazza ML, Silveira JLM. Effects of pressurized hot water extraction on the yield and chemical characterization of pectins from Campomanesia xanthocarpa Berg fruits. Vol. 146, International journal of biological macromolecules. 2020. p. 431–43. [CrossRef]

[38] Nurman S, Yulia R, Irmayanti, Noor E, Sunarti TC. The optimization of gel preparations using the active compounds of arabica coffee ground nanoparticles. Sci Pharm. 2019;87(4). [CrossRef]

[39] Kurniawan MF, Sugihartini N, Yuwono T. Iritation properties and evaluation of physical properties emulgel of clove oil using simplex lattice design methods. Farmasains. 2018;5(1):1–8. [CrossRef]

[40] Sari DK, Sugihartini N, Yuwono T. Irritation test and physical properties evaluation of essential oils clove (Syzigium aromaticum) in emulgel. Pharmaciana. 2015;5(2):115–20. [CrossRef]

[41] Kumari M, Gupta SK. Response surface methodological (RSM) approach for optimizing the removal of trihalomethanes (THMs) and its precursor's by surfactant modified magnetic nanoadsorbents (sMNP) - An endeavor to diminish probable cancer risk. Sci Rep. 2019;9(1):1–11. [CrossRef]

[42] Yuliani SH, Hartini M, Pudyastuti B, Istyastono EP. Comparison of physical stability properties of pomegranate seed oil nanoemulsion dosage forms with long-chain triglyceride and medium-chain triglyceride as the oil phase. Tradit Med J. 2016;21(2):93–8. [CrossRef]

[43] Sinko PJ, Singh Y, editors. Martin's physical pharmacy and pharmaceutical sciences: Physical chemical and biopharmaceutical principles in the pharmaceutical sciences. Seventh edit. Philadelphia: Wolter Kluwer Health; 2017.

[44] Elsheikh MA, Elnaggar YSR, Hamdy DA, Abdallah OY. Novel cremochylomicrons for improved oral bioavailability of the antineoplastic phytomedicine berberine chloride: Optimization and pharmacokinetics. Int J Pharm. 2018;535(1–2):316–24. [CrossRef]

[45] Nursal FK, Nining, Rahmah AS. Formulation and development of grape seed oil (Vitis Vinifera L) emulgel peel-off mask using gelling agent hydroxy propyl methyl cellulose (HPMC). IOP Conf Ser Earth Environ Sci. 2021;755(1).

[CrossRef]

[46] Irianto IDK, Purwanto P, Mardan MT. Antibacterial activity and physical evaluation of Piper betle L. decoction gel as an alternative treatment for mastitis. Maj Farm. 2020;16(2):202–10. [CrossRef]

[47] Elfiyani R, Amalia A, Pratama SY. Effect of using the combination of tween 80 and ethanol on the forming and physical stability of microemulsion of eucalyptus oil as antibacterial. J Young Pharm. 2017;9(2):230–3. [CrossRef]

[48] Yati K, Jufri M, Gozan M, Dwita LP. The effect of hidroxy propyl methyl cellulose (HPMC) concentration variation on physical stability of tobacco (Nicotiana tabaccum L.) extract gel and ıts activity against Streptococcus mutans. Pharm Sci Res. 2018;5(3):133–41. [CrossRef]

Tabel 1. Evaluation results of independent variables and dependent variables with the CCD design.

Std	X_1 (%)	X_2 (%)	Y_1 (nm)	Y_2	Y_3 (mV)	Y_4	Y_5 (cm)	Y_6 (sec)
1	5.50	1.02	206.9±0.7	0.571±0.000	35.39±1.50	5.45	5.55±0.07	5.65
2	2.32	1.15	457.0±0.9	0.000±0.000	13.90±1.92	4.60	5.00±0.00	6.99
3	8.68	0.90	166.7±0.4	0.378±0.074	31.64±0.53	6.35	6.20±0.14	4.23
4	2.32	0.90	411.0±5.8	0.000±0.000	30.67±0.89	6.29	5.00±0.00	4.30
5	5.50	1.02	302.1±3.6	0.571±0.000	36.45±0.45	5.46	5.60±0.28	5.63
6	5.50	1.02	263.0±3.7	0.571±0.000	18.49±1.77	5.47	5.55±0.21	5.64
7	8.68	1.15	198.1±1.2	0.242±0.003	57.23±2.91	4.65	6.15±0.07	6.95
8	5.50	1.02	246.9±7.2	0.571±0.000	26.60±0.17	5.46	5.60±0.14	5.67
9	5.50	1.02	206.0±2.3	0.571±0.000	31.77±1.01	5.46	5.60±0.00	5.65
10	10.00	1.02	160.8±1.3	0.285±0.024	46.72±1.97	5.31	6.25±0.21	5.84
11	1.00	1.02	244.2±4.3	0.571±0.000	31.26±0.57	5.78	4.90±0.14	6.88
12	5.50	1.02	238.1±3.0	0.571±0.000	30.78±0.53	5.45	5.55±0.07	5.64
13	5.50	1.20	206.2±1.6	0.571±0.000	30.56±0.78	4.57	5.50±0.14	7.08
14	5.50	0.85	240.5±2.2	0.571±0.000	20.12±0.16	6.39	5.65±0.21	4.22

Y1: Particle size (PS), Y2: Polydispersity index (PDI), Y3: Zeta potensial (ZP), Y4: pH, Y5: Spreadability, Y6: Adhesion

Table 2. Statistical analysis of PS (Y1), PDI (Y2), ZP (Y3), pH (Y4), spreadability (Y5), and adhesion (Y6) TEO-loaded Negs on CCD.

Factors		Y1	Y2	Y3	Y4	Y5	Y6
A	Coefficient	-77.64	0.027	8.27	-0.069	0.53	-0.20
	p-value	0.0063**	0.7325	0.0080**	0.1430	<0.0001**	0.1406
B	Coefficient	3.61	-0.017	2.95	-0.75	-0.033	1.18
	p-value	0.8783	0.8289	0.2668	<0.0001**	0.1157	<0.0001**
AB	Coefficient		-0.034	10.59			
	p-value		0.7602	0.0136*			
A^2	Coefficient		-0.16				
	p-value		0.0818				
B^2	Coefficient		-0.086				
	p-value		0.3087				
Intercept	Coefficient	253.39	0.57	31.54	5.48	5.58	5.74
Degree of freedom		2	5	3	2	2	2
Sum of squares		48332.05	0.24	1065.33	4.48	2.28	11.49
Mean of squares		24166.02	0.047	355.11	2.24	1.14	5.74
F-value		5.69	1.02	7.06	145.08	386.43	46.35
p-value		0.0201	0.4645	0.0078	<0.0001	<0.0001	<0.0001
R-Squared		0.5084	0.3898	0.6794	0.9635	0.9860	0.8939

A: X1 (Span 80-Tween 80); B: X2 (Carbopol 980)
* p-value < 0.05
** p-value < 0.01

388 **Table 3.** Optimized TEO-loaded Negs actual and predicted values for each response.

Responses	Predicted values	Actual value[a]	Error[b] (%)
Y1 (nm)	180.2	182.3 ± 5.5	1.165
Y2	0.250	0.242 ± 0.003	-3.200
Y3 (mV)	56.30	57.23 ± 2.91	1.652
Y4	4.50	4.51 ± 0.02	0.222
Y5 (cm)	6.07	6.0 ± 0.2	-1.153
Y6 (sec)	6.98	6.45 ± 0.19	-7.593

389 a Data listed is the mean ± standart deviation, n = 3
390 b Error (%) = [(Actual value – Predicted value)/Predicted value] * 100%
391
392

393 **Table 4.** Additional evaluation on TEO-loaded Negs with an optimal formula.

Evaluation	Result
Organoleptic	Color: White; Odor: typical turmeric
Homogeneity	Homogeneous
Freeze-Thaw	Cycles 0-6, no separation occurs
Viscosity	32240 cP

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395

396 **Table 5.** The independent and dependent variables with levels and limits in CCD for TEO-loaded
397 Negs development

Variables	Code	Low level	High level	Units
Independent variables				
Span 80-Tween 80 concentration	X1	2.32	5.50	% w/w
Carbopol 980 concentration	X2	0.90	1.02	% w/w
Dependent variables		Limits		
Particle size	Y1	Minimum		nm
Polydispersity index	Y2	Minimum		
Zeta potensial	Y3	Maximum		mV
pH	Y4	is in range		
Spreadability	Y5	is in range		cm
Adhesion	Y6	is in range		

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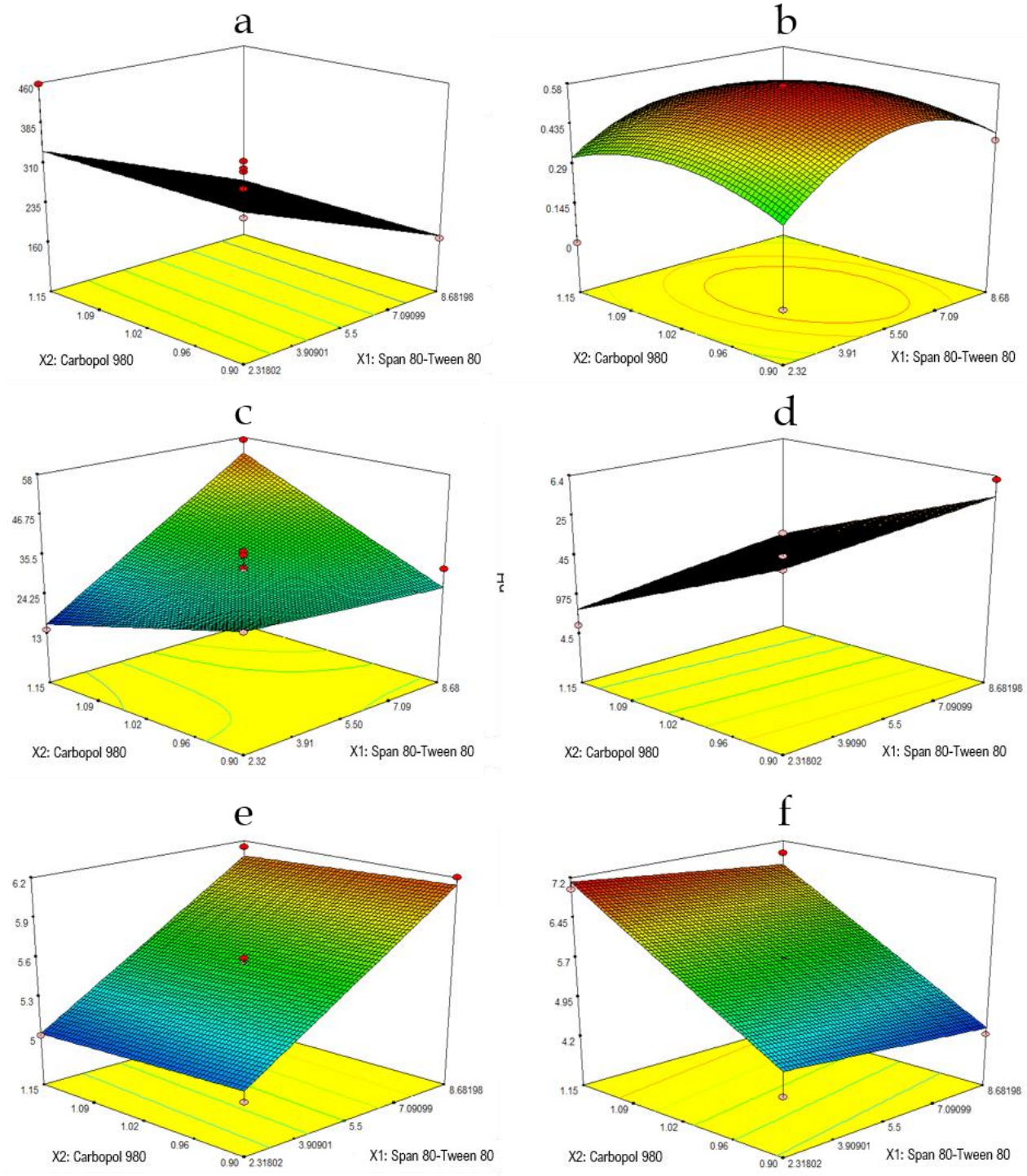

Figure 1. Effect of Span 80-Tween 80 and Carbopol 980 concentration on PS (a) PDI (b) ZP (c) pH (d) spreadability (e) adhesion (f).

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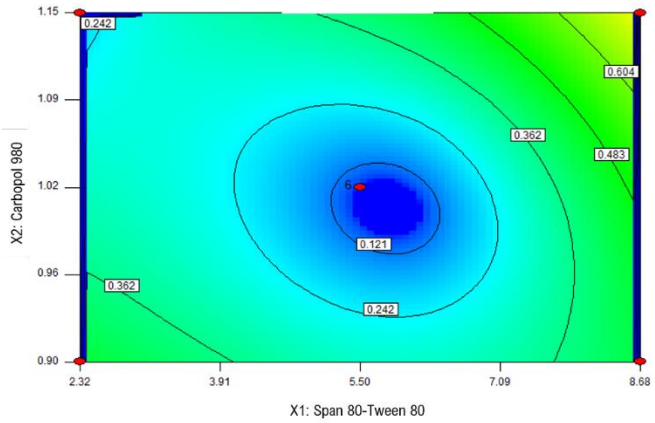

Figure 2. Contour plot desirability value of optimal formula.

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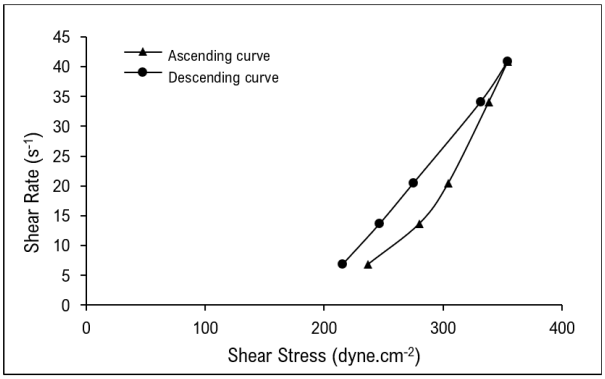

Figure 3. Optimal formula flow properties.

2. Bukti Konfirmasi Review dan Hasil Review Pertama (5 November 2022)

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Suggestions

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Comments to the Author
A very nice and well-designed study about the the turmeric essential oil-loaded nanoemulgel. Authors have fully characterized the prepared formulations while their conclusions are supported by their results. However, the some comments must be taken into consideration for the improvement of the study. I'm sending my comments and recommendations.

1. Please carefully check the text for minor grammar and syntax errors.
2. Abbreviations should be used at the first occurrence of the word.
For example: Nanoemulgels (Negs)
3. Attention should be paid to punctuation marks and spaces In vitro and in vivo should be written in italics.
4. Why was the quantification of turmeric essential oiland drug content study of nanoemulgel not done?
5. Why did the authors choose turmeric essential oil? How the authors analyzed the effectiveness of the oil.
6. Result and discussion part should be supported by the literatures.
7. In methods: Explain what TEA means.
8. Adhesiveness is the work required to overcome the attractive forces between the surface of the sample and the probe surface. The unit of adhesiveness cannot be seconds. The authors should explain the adhesion study in more detail. The results of adhesion should be supported by the literature.

2. Reviewer Comments
The content of the manuscript and the topic seems in line with journals target audience but there are some serious drawbacks need to be addressed:
- The English of the manuscript needs to be revised by a native speaker or a professional editing service,
- The application of CCD method is not clear nor understandable in the manuscript. In particular, how many center points were used, which ±α was preferred in the design etc?
- The most significant missing factor is validation of the CCD. After application of CCD-RSM, suggested optimal points are better presented in table3. Once the optimal points are clarified, three Negs formulation at this suggested optimal point are needed to be prepared experimentally along with all the characterization in order to show the correlation between predicted and experimentally found values.
- The purpose of the Negs formulation is not clear in the manuscript which is another bottleneck of the manuscript. The purpose of this formulation needs to be addressed and further characterized along with the purpose.

Manuscript Information

Manuscript ID: **MPJ-10622**

Title in English: Response surface methodology for optimization of turmeric essential oil-loaded nanoemulgel

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Authors: Nining Nining[1], Anisa Amalia[1], Fatimatuz Zahrok[2]

Institutions: [1]Universitas Muhammadiyah Prof. DR. HAMKA, Pharmaceutical Technology, East Jakarta, Indonesia
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Keywords in English: Central composite design; nanoemulgels; response surface methodology; turmeric essential oil; topical delivery.

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Abstract in English

Turmeric essential oil has antioxidant and anti-inflammatory activities in a topical dosage form. Nanoemulgels are one of them. Using emulsifiers and gelling agents in the formula affects the nanoemulgels (Negs) characteristics and stability. This study focuses on the systematic development, optimization, and characterization of Turmeric Essential Oil-loaded Nanoemulgels (TEO-loaded Negs), having promising topical applications. The TEO-loaded Negs were prepared by the high-speed homogenization method. The formula design used Central Composite Design from Response Surface Methodology (CCD-RSM) to obtain the optimal TEO-loaded Negs formula. The optimization variables of the TEO-loaded Negs formula include the concentrations of Span 80-Tween 80 (X1) and Carbopol 980 (X2) with the response of particle size (PS) (Y1), polydispersity index (PDI) (Y2), zeta potential (ZP) (Y3), pH (Y4), spreadability (Y5) and adhesion (Y6). The actual TEO-loaded Negs responses were compared with the CCD-RSM predictions. In addition, other physical evaluations such as homogeneity observations, phase separation tests, viscosity, and flow properties were carried out. The optimal TEO-loaded Negs developed were made with 8.68% Span 80-Tween 80 and 1.18% Carbopol 980. The evaluation results showed the optimal TEO-loaded Negs nano-metric size (182.3 ± 5.5 nm) with low PDI (0.242 ± 0.003), good ZP (-57.23 ± 2.91 mV), pH (4.51 ± 0.02), spreadability (6.0 ± 0.2 cm), and adhesion (6.45 ± 0.19). TEO-loaded Negs have a good appearance and do not undergo phase separation at extreme temperature storage. Thus, the developed TEO-loaded Negs can be a potential delivery system and a promising suitable approach for topical preparations.

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Score Sheet

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Are the references complete and recent?	Yes

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Are the figures tables and graphics clear ?	No
Is the introduction part	NOT sufficiently developed
Are the experimental procedures sound?	No
Is the results and discussion part	sufficiently developed
Is conclusion sufficient and correlated with the results ?	Yes
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3. Bukti Konfirmasi Review Submit Revisi Pertama, Respon kepada Reviewer, dan Artikel yang Diresubmit (23 November 2022)

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Response surface methodology for optimization of turmeric essential oil-loaded nanoemulgel

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Received: 0 Month 201X / Revised: 0 Month 201X / Accepted: 0 Month 201X

ABSTRACT: Turmeric Essential Oil (TEO) has an antioxidant and anti-inflammatory activity to be formulated in a topical dosage form. Nanoemulgels (Negs) development, based on varying concentrations of emulsifiers and gel formers, affects their characteristics and stability. This study focuses on optimizing TEO-loaded Negs based on physical and mechanical characterization, which have promising topical applications. Negs were created using the high-energy approach and optimized using Response Surface Methodology (RSM) and the Central-Composite Design (CCD) for the optimization of span-80/tween-80 (X_1) and Carbopol® 980. (X_2). Observed variable responses were particle size (PS) (Y_1), polydispersity index (PDI) (Y_2), zeta potential (ZP) (Y_3), pH (Y4), spreadability (Y_5), and adhesion time (AT) (Y_6). Actual responses of Negs were compared with the CCD-RSM predictions to validate the model. In addition, other physical evaluations were observed, such as organoleptic observations, homogeneity, freeze-thaw tests, viscosity, and flow properties. Optimized TEO-loaded Negs were made with 8.68% span-80/tween-80 and 1.18% Carbopol® 980. The evaluation results showed the optimal TEO-loaded Negs on nano-metric size (182.3 ± 5.5 nm) with low PDI (0.242 ± 0.003), good ZP (-57.23 ± 2.91 mV), pH (4.51 ± 0.02), spreadability (6.0 ± 0.2 cm), and AT (6.45 ± 0.19 s). TEO-loaded Negs have an excellent appearance and did not run phase separation at extreme temperature storage with pseudoplastic thixotropy flow. Thus, the developed TEO-loaded Negs can be a potential delivery system and a promising suitable approach for topical preparations.

KEYWORDS: Central composite design; nanoemulgels; response surface methodology; turmeric essential oil; topical delivery.

1. INTRODUCTION

Turmeric is the dried rhizome of *Curcuma longa* L. (Zingiberaceae), which derives from Southeast Asia and is cultivated mainly in India, followed by Bangladesh, China, Thailand, Cambodia, Indonesia, Malaysia, and the Philippines (1). Steam distillation extracts turmeric essential oil (TEO) from the turmeric rhizome (2). Chemical constituents with the most significant proportion were oxygenated monoterpenes and sesquiterpenes, which include β-turmerone, α-turmerone, and ar-turmerone (3–5). The pharmacological activities of TEO have been reported in the form of antioxidants, anti-inflammatory, antinociceptive, antidermatophytic, antifungal, and antibacterial activities (2,6–9). These reducing power and radical scavenging abilities are associated with the high antioxidant potential of TEO (8,10). This pharmacological activity justifies its use in various applications, including cosmetics and phytomedicines (7,11,12). Like other essential oil, TEO has limited use due to its volatility, instability under certain conditions, lipophilicity, and low aqueous solubility (13,14). Many recent studies are oriented toward solving these limitations, so that efficacy of the essential oil lasts longer and increases.

Previously, TEO has been developed for cream as a conventional drug delivery system, patch, and nanoemulsions (7,11,15,16). Most water-based liquid or semisolid systems have limitations in delivering lipophilic drugs (17). Nanoemulsions are an established alternative for delivering lipophilic drugs by increasing topical absorption (18). The main advantage of topically administered nanoemulsions is the ability to increase penetration and permeation of drugs through the skin without adding non-physical enhancers and non-friendly solvents to the formulation, which can cause skin irritation, especially with long-term usage (18,19). However, this system has problems with low viscosity due to poor spreadability and skin

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retention (20). Nanoemulgels (Negs), a combination of nanoemulsion and hydrogel, were made to improve the characteristics (18,19,21).

Negs consist of a hydrogel system and an emulsion with nano-sized globules. An emulsifier in the form of surfactants and co-surfactants stabilizes the emulsion, which serves as a drug delivery platform (18). Surfactants reduce the interfacial surface tension of immiscible liquids and change the entropy of the dispersion, thereby stabilizing a thermodynamically unstable system; co-surfactants are combined with surfactants in the emulsification process by disrupting the surface layer (22). The emulsifier plays a role in the emulsification process to increase stability when the product is kept for an extended time. On the other side, gels are made from polymers, a gel former, that expand after absorbing a liquid (23). Gel former increase the viscosity of the formulation and can react with emulsifiers to change the thickness (24). In addition, topical Negs can increase patient compliance because of their non-irritating, non-greasy characteristics and improved drug release (25). However, the available methods for manufacturing Negs exhibit various limitations, which directly or indirectly affect the quality of the Negs formulations.

Currently, the principle of quality by design is adopted to ensure the quality of drugs, their safety, and efficacy (26). The quality by design (QbD) trend is used to develop, optimize, and investigate the interaction between particular variables and their related responses to achieve the optimal formulation (27). Our study used Central Composite Design (CCD) on Response Surface Methodology (RSM) to select optimal formulations and predict models that rely on statistical analysis (ANOVA) and exact equations (28,29). In this aspect, we intended to develop a TEO-loaded Negs that would be optimized using a complete 2^2-factorial design and determine the independent factors' precise influence on the investigated dependent variables. The choice and procedure factors were span-80/tween-80 (X_1) and Carbopol® 980 (X_2). Response variables investigated were particle size (PS) (Y1), polydispersity index (PDI) (Y2), zeta potential (ZP) (Y3), pH (Y4), spreadability (Y5), and adhesion time AT) (Y6). This work is the first step in developing an optimized TEO preparation suitable for transdermal drug delivery for topical application.

2. RESULTS AND DISCUSSION

2.1. Identification of TEO

TEO identification is carried out to ensure the quality of the active ingredients. The tests included organoleptic, phytochemical identity in the form of phenolics and terpenoids, and antioxidant activity by the DPPH method. The results of this study can be seen in **Figure 1**. Organoleptically, TEO is an orange liquid with a distinctive turmeric aroma. Identification results in the phenolic test showed a black-green color (**Figure 1A**) due to the formation of a phenolic-Fe^{3+} complex (30). In the terpenoid test, reaction results show a purple color (**Figure 1B**) as a positive sign. Based on these results, it can be concluded that TEO contains phenolic and terpenoid compounds. Several studies reported that TEO has powerful antioxidant potential both *in-vitro* and *in-vivo* (2,8,31). Oxygenated monoterpenes and sesquiterpenes, such as ar-turmerone, α-turmerone, and β-turmerone, were included in the terpenoid compounds and reported to play a role in this activity. In addition, contained phenol was also reported to perform as an excellent free radical scavenger because its reduction potential was lower than oxygen's (8). In our study, the antioxidant activity of TEO was presented as an IC_{50} of 9.88 µm/ml, which was included in the powerful antioxidant category because of below 50 µm/ml (32). It differed from other studies' results, which showed an IC_{50} of 2274.02 µm/ml and above 1000 µm/ml (2,10). Meanwhile, similar findings stated IC_{50} values of 10.03 µm/ml, 3.227 µm/ml, and 14.5 µm/ml (33–35). The difference value was due to the use of TEO from different origin sources, regional conditions, and the extraction method used.

2.2. Preparation of TEO-loaded Negs

TEO-loaded Negs were produced using the high-energy method efficiently. A high-pressure homogenizer provides sufficient energy to increase the interfacial area and generate nano-size globules (36). An emulsion system with a nanodroplet size must have flow properties that allow it to pass through the homogenizer (37). Flow properties are inversely correlated with the amount of emulsifier and gel former added to the Negs formulation. In this study, the selected optimized formulation was the one that produces Negs with the smallest size, as shown by the limitations in **Table 3**. Therefore, the range of regulated emulsifiers and gel formers concentrations still provides flow properties that allow the flow to remain good using a high-energy homogenizer. In general, the preparation of Negs was carried out in 3-stages, namely,

nanoemulsions preparation, gel preparation, and Negs preparation. Negs were successfully produced in 14-formulation with variations of emulsifiers and gel former with compositions that can be seen in **Table 1**.

2.3. Experimental Design by CCD-RSM

2.3.1. Fitting the Model

TEO-loaded Negs were optimized based on CCD in the RSM. CCD was used to establish the optimal concentrations of emulsifier (X_1) and gel former (X_2) as the key parameters influencing the dependent response. Prediction of the factorial axial design and the possible curvature in the response could be obtained from the optimization process with an effective second-level design (38). Predicting the significant influence of the independent variable on the dependent variable is essential for generating TEO-loaded Negs. Based on the literature survey, two factors were chosen as independent variables, and six responses were chosen as the dependent variable with the most significant influence on Negs. The independent variables with their levels and the observed response variables are presented in **Table 1**.

2.3.2. Analysis of Design

Statistical data analysis must be carried out to predict and recognize the model. **Table 2** shows the statistical analysis of a quadratic model for PDI, a 2FI model for ZP, and linear models for PS, pH, AT, and spreadability. This table identifies factors with p-values less than a predefined threshold (0.01 and 0.05, with a 99% and 95% confidence level, respectively) as influential factors. Besides the significant p-value, a large F-value minimizes error in the model and lack of fit, preferably non-significant to fit the model data (27,39). Depending on the most significant R-squared value and the least residual predictive sum of squares value, the six responses demonstrated distinct models in their application. The chosen model had a non-statistically significant lack of fit, and model validation was confirmed by the residual plot test of the regression model, which was supported by supplemental information for all responses. Compared to the 2FI and simple linear models, the quadratic model represented the impacts of numerous variables, including individual factors, interactions, and the quadratic influence on the response.

2.3.3. Effect of Independent Variables on Dependent Variables

PS parameters are often used to characterize nanoparticles. Negs globule diameter means (Y_1) was adjusted from 160.8 nm (STD#10) to 457.0 nm (STD#2). Based on **Table 2**, the PS response indicates a significantly linear model with an F-value of 5.69 (p-value 0.0201 < 0.05). The suggested linear model equation can be seen as follows:

$$Y_1 = 253.39 - 77.64X_1 + 3.61X_2$$

The equation shows that the Negs PS is significantly (p-value < 0.01) affected by emulsifier concentration (X_1). The positive coefficient has a synergistic effect on the response. In contrast, the negative coefficient has an antagonistic effect which concludes the inverse relationship of the independent variable with a response (38,40). In addition, this factor has a more significant coefficient. It directly affects the PS, which means that increasing emulsifier concentration causes a decrease in the globule diameter PS in the TEO-loaded Negs. High emulsifier (above 5.5%) resulted in globules measuring below 200 nm, and low emulsifier (below 5.5%) produced globules above 200 nm. This fact is in line with other studies that increasing emulsifiers could reduce droplet Negs PS (41,42). The emulsifier reduced the interfacial surface tension between the water and oil phases, which decreased the free energy required to disrupt or break the globules and resulted in a smaller droplet diameter. It can also produce a protective cover over the globules, preventing them from coalescence. However, the emulsifier must absorb quickly enough around the droplet to form this protective layer (42,43).

Meanwhile, Carbopol® 980 had no significant effect on PS. The same findings were obtained from the globule size Negs from Carbopol® 934 and 940 as gel former (19). A response surface plot (**Figure 2A**) may therefore be used to represent the combined influence of variables X_1 and X_2, which shows that Y_1 changes linearly with the sum of the two variables. Nevertheless, the higher gradient in the response surface with span-80/tween-80 (X_1) – not Carbopol® 980 (X_2) – was evidenced from the comparative plot of the response surface. From this explanation, it can be concluded that the PS can be changed by selecting the right X_1 level.

In **Table 1**, Negs PDI (Y_2) varied from 0.000 (STD#2 and #4) to 0.571 (STD#1, #5, #6, #8, #9, #11 to #14). PDI measures the distribution of molecular mass in a sample. The smaller PDI (close to 0), the more stable the Negs formulation caused; the large PDI indicates particles formed are not uniform, and the formulation will flocculate quickly. An index value less than 0.05 is included in monodisperse, while an index greater than 0.7 indicates that the sample has a broad PS distribution. A-0.2 and below are considered acceptable for nanoparticle preparations (44). Based on **Table 2**, the PDI response shows a non-significant quadratic model with an F-value of 1.02 (p-value 0.4645 > 0.05). Measurements between variables and responses are not a precise cause of that. The suggested quadratic model equation can be seen as follows:

$$Y_2 = 0.57 + 0.027X_1 - 0.017X_2 - 0.034X_1X_2 - 0.16X_1^2 - 0.086X_2^2$$

The equation and p-value of each factor did not significantly affect PDI. The response surface plot (**Figure 2B**) depicts the combined influence of variables X_1 and X_2, which shows that Y_2 changes with the sum of the two variables by model.

The Negs ZP (Y_3) was in the range of 13.90 mV (STD#2) to 46.72 mV (STD#10) (**Table 1**). ZP represents the electric charge between the shear plane of a final outer layer and bulk solution, which significantly affects dispersion stability (45). This factor is strongly influenced by the composition of the Negs and its electrical phenomena. TEO-loaded Negs, which have positive ZP, show good interaction with negatively charged skin (46). ZP is the scientific term for the electrokinetic potential in colloidal systems. The high electric charge on the nanoparticle surface will prevent nanoparticle aggregation because of the strong repulsion between particles. The ZP requirement for stability is above ± 30 mV (19). The higher ZP and the slower aggregation are formed to prevent separation (47). Based on **Table 2**, the ZP response indicates a significant 2FI model with an F-value model of 7.06 (p-value 0.0078 < 0.05). The linear model equation suggested by the software can be seen as follows:

$$Y_3 = 31.54 + 8.27X_1 + 2.95X_2 + 10.59X_1X_2$$

The equation shows that the Negs ZP was significantly affected by the emulsifier (X_1) and gel former-emulsifier interaction (X_1X_2). ZP was usually influenced by the physicochemical properties of the drug, polymer, carrier, electrolyte presence, and their adsorption (48). One study stated that adding Carbopol® only slightly increased the ZP Negs (19). The response surface plot (**Figure 2C**) depicts the combined influence of variables X_1 and X_2, showing that Y_3 changes with the sum of the two variables. Nevertheless, the higher gradient in the response surface with span-80/tween-80 (X_1) – not Carbopol® 980 (X_2) – is the evidence from the comparative plot of the response surface. This description concludes that the ZP can be changed by selecting the proper X_1 level.

The pH test was carried out to measure Negs's acidity or alkalinity level. The pH values (Y_4) were in the range of 4.57 (STD#13) to 6.39 (STD#14) (**Table 1**). The pH requirement of Negs is the same as the skin pH. Too-acidic preparations can irritate the skin and cause a stinging sensation, while too-alkaline preparations can cause dry and itchy skin. The pH result test on 14 formulations was eligible and compatible with the skin (4.5-6.5) (49). Based on **Table 2**, a linear model was found to be significant in pH response with a model F-value of 145.08 (p-value < 0.0001). The linear model equation suggested by the software can be seen as follows:

$$Y_4 = 5.48 - 0.069X_1 - 0.75X_2$$

The equation shows that the pH was significantly (p-value < 0.0001) affected by gel former (X_2). Carbopol is a high molecular weight homopolymer and acrylic acid copolymer crosslinked with polyalkenyl polyethers (50). They are anionic and acidic (2.5–4.0 in 2% dispersion) when not neutralized with bases to achieve a specific viscosity (50,51). Therefore, adding Carbopol to a formulation with a fixed amount of base (triethanolamine) will significantly lower the pH of Negs. The response surface plot (**Figure 2D**) may then be used to depict the combined influence of variables X_1 and X_2, which shows that Y_4 changes linearly with the sum of the two variables. Nevertheless, the higher gradient in the response surface with Carbopol® 980 (X_2) – not span-80/tween-80 (X_1) – is the evidence from the comparative plot of the response surface. This description concludes that the choice of the X_2 level affects the pH Negs.

Spreadability was measured to ensure comfortable use on the skin because it spreads quickly (24). Terms of good dispersion are 5-7 cm. If the dispersion is too small, it is relatively difficult to spread when applied to the skin, while the dispersion tends to spread too quickly when applied, so it will cause an uncomfortable feeling when used (52). Based on the results, only one Neg did not meet the requirements, namely F11. **Table 1** shows the range of spreadability (Y_5) from 4.90 cm (STD#11) to 6.25 cm (STD#10). Based on **Table 2**, the spreadability response indicates a significant linear model with an F-value of 386.43 (p-value <0.0001). The linear model equation suggested by the software can be seen as follows:

$$Y_5 = 5.58 + 0.53X_1 - 0.033X_2$$

The equation shows that the spreadability of Negs was significantly (p-value < 0.0001) positively affected by the emulsifier (X_1). That was also found in other literature studies (24). The higher the Carbopol-contained Negs, the more viscous Negs. AT and spreadability have the opposite results. The higher the Negs' viscosity, the higher the adhesive strength produced, while the smaller the dispersion power (53). The response surface plot (**Figure 2E**) depicts the combined influence of variables X_1 and X_2, which shows that Y_5 changes linearly with the sum of the two variables. Nevertheless, the higher gradient in the response surface with span-80/tween-80 (X_1) – not Carbopol® 980 (X_2) – is the evidence from the comparative plot of the response surface. This description concludes that the scatter can be changed by choosing the right X_1 level.

Topical dosage forms, such as Negs, adhere to the skin in two ways: they adhere directly to the rough surface to form a "mechanical interlock" and to the surface via interaction (54). Good adhesion to Negs supports a higher concentration gradient towards the skin and provides more drug penetration (18). Adhesive strength is directly related to the AT on the Negs as measured using the single-lap shear test method with slight modifications (55,56). The test was carried out by applying a shear load to the plates that flank the sample, which had been pre-loaded, and given a measured force; the time taken for the plates to separate was recorded as AT. In our study, the AT was from 4.22 s (STD#14) to 7.08 s (STD#13) in **Table 1**. They met the requirements based on the AT test results on a 14 formulation. An AT was carried out to see how long a Negs could be attached to the skin. The AT requirement is more than 4 seconds. The longer a Negs could be attached to the skin, it showed the better result, where it is expected that more active substances can be absorbed due to the time the Negs was in contact with the skin (57). Based on **Table 2**, the linear model was found to be significant in the AT response with an F-value model of 46.35 (p-value < 0.0001). The linear model equation suggested by the software can be seen as follows:

$$Y_6 = 5.74 - 0.20X_1 + 1.18X_2$$

The equation reveals that the AT was significantly (p-value < 0.0001) affected by the gel former (X_2) or, indirectly, the same adhesion strength. This finding is in agreement with the literature (58,59). A response surface plot (**Figure 2F**) may therefore be used to depict the combined influence of variables X_1 and X_2, which shows that Y_6 changes linearly with the sum of the two variables. Nevertheless, the higher gradient in the response surface with Carbopol® 980 (X_2) – not span-80/tween-80 (X_1) – is the evidence from the comparative plot of the response surface. From this explanation, it can be concluded that the AT can be changed by selecting the right X_2 level. Details of the ANOVA results for measured responses are also presented in **Table 2**. In the end, the emulsifier factor significantly affected the response of PS, ZP, and spreadability of TEO-loaded Negs. At the same time, gel former affects the AT and pH.

2.4. Optimized TEO-loaded Negs

The formulation was optimized with Design-Expert®, version 13 software. The optimized Negs were selected based on the minimum PS and PDI; maximum ZP; value in pH range, spreadability, and AT (**Table 3**). Variables composition for optimized Negs is span-80/tween-80 of 8.68% and Carbopol® 980 of 1.18% with a desirability value of 0.801. The formulation with the maximum desirability value is the optimal formulation generated from the optimization phase of the program (60) —the optimization value formed as indicated by the desirability value close to one.

The desirability value range is 0-1. **Figure 2** describes the optimization results in the form of a 2D contour. *Contour* is a two-dimensional response image that was presented using a predictive model for PS, PDI, ZP, pH, spreadability, and AT response values. The contour graph shows the desirability value of 0.801, which is the closest value to 1 compared to the other points. **Figure 3** shows the projection in the form of a 3D surface; the low area shows low desirability, while the high area shows high desirability and is getting closer to 1. At this stage, the software predicts the response values shown in **Table 4**. Three confirmation runs need to be performed to validate optimization (61). The optimization model and estimates are validated by the observed optimized Negs, which show an acceptable variation from the predicted values (**Table 4**). We tested the optimized Negs' physical properties for further investigation, such as organoleptic, homogeneity, freeze-thaw, viscosity, and flow properties.

2.5. Evaluation of TEO-loaded Negs

Nanoemulsion systems can cover oily drugs' bitter or unpleasant taste (62). The organoleptic results of TEO-loaded Negs have a less distinctive turmeric odor, which is white and semisolid (**Table 5**). That is due

to the drug entrainment of oil with the oil phase effectively preventing evaporation and masking its specific odor (63). The homogeneity test results of the optimum TEO-loaded Negs formulation showed a homogeneous preparation, as evidenced by the absence of coarse grains. This homogeneity was correlated with the optimal formulation of PS and PDI of 182.3 ± 5.5 and 0.242 ± 0.003, respectively. The low PDI indicates uniformity or homogeneous dispersion of globules Negs (64). In addition, the small size of the globule (±200 nm) is not included in the coarse dispersion (41).

The thermodynamic stability test of the system was carried out using a freeze-thaw cycle to identify the presence of metastable Negs in the optimal formulation. It aims to see the separation of the water and oil phases due to the influence of extreme temperatures (65). The thermodynamic stability of any system is determined by the change in free energy between the system and its surroundings (66). The test results on the optimum formula for six cycles showed promising results; namely, there was no separation. This stability was correlated with the ZP of the optimal formulation of 57.23 ± 2.91 mV. The surface charge's magnitude was directly related to the stability of any Negs. It is evidenced by the high repulsive force between the Negs globules preventing coalescence, which was characterized by the absence of phase separation (67). Similar results were found in the Negs study containing thymoquinone, which had ZPs between −26.7 and −30.6 mV (66).

The pH conditions indirectly affect the viscosity indicated by Negs because they influence the swelling ability of Carbopol® 980. This excipient is a gel former and a thickener (52). It plays an essential role in the viscosity of Negs. Carbopol is dispersed in water to form an acidic colloidal solution with a low viscosity. Neutralizing with triethanolamine increases Negs' viscosity because a stable water-soluble gel was formed (50). Viscosity was carried out with #7 spindle (Brookfield digital RV DV-E) at 50 rpm of 32240 ± 2257.7 cP, indicating significantly high viscosity on Negs with pH 4.5. The magnitude of the viscosity is correlated with AT and spreadability. Viscosity is inversely proportional to spreadability (52). In contrast, the AT is directly proportional to the viscosity. A high-viscosity system will form stronger interfacial interactions and increase intermolecular interactions in the polymer network, increasing cohesion, adhesion strength, and AT (54).

Determining the rheology of a semisolid preparation is essential for controlling the consistency required to ensure the performance and formulation durability and to describe the mechanical (flow properties) system (68). The rheological study was conducted in the shear rate range of 6.81-40.86 s^{-1} at 25°C. The consistency index equals the apparent viscosity at a shear rate of 1 s^{-1}. The consistency index measured on TEO-loaded Negs was 155.67 cP and n = 0.22. The flow index measures the system's deviation from Newtonian behavior (n = 1). A value of n > 1 indicates dilatation or shear thickening flow, and n < 1 indicates pseudoplastic or shear thinning. The flow index typically lowers the thicker the base. Negs produce a 0.22 flow index, which implies pseudoplastic flow behavior. A colloidal network structure aligned with the shear direction and decreases viscosity as the shear rate increases have led to this pseudo-plasticity. The developed system will require a specific force to discharge (69). The results of the flow properties test showed that the optimal formula made was a pseudoplastic thixotropic flow type (**Figure 4**). *Thixotropic* is a flow property expected in pharmaceutical preparations because it has high consistency in the container but can be poured and dispersed easily (70).

4. CONCLUSION

Based on the results of the CCD-RSM analysis, the optimum span-80/tween-80 as an emulsifier is 8.68%, and Carbopol® 980 as a gel former was 1.18%. The resulting response is a PS of 182.3 nm, PDI 0.242, ZP 57.23 mV, pH 4.51, AT 6.45 seconds, and spreadability of 6 cm. Optimized formulation viscosity is 32240 cP with pseudoplastic thixotropic flow properties. Thus, the developed TEO-Negs can be a potential delivery system and a promising suitable approach for topical preparations.

5. MATERIALS AND METHODS

5.1. Materials

TEO (Curcuma longa) was purchased from Darjeeling Sembrani Aroma (Indonesia), sorbilene O E/P from Lamberti (Italy), span-80 from Croda (Singapura), propylene glycol from Dow Chemical Pacific (Singapura), Carbopol® 980 NF from Lubrizol AM (Cleveland), nipagin M from Clariant Produkte (Deutschland), propylparaben from Alpha Chemika (India), triethanolamine from Dow Chemical Pacific (Switzerland), and 1.1-diphenyl-2-picrylhydrazyl (DPPH) from Smart-Lab (Indonesia).

5.2. Methods

5.2.1. Identification of TEO

Organoleptic tests include observing form, color, and odor. Phenolic identification was carried out by adding one drop of 5% $FeCl_3$ to a 1 ml sample. Dark green to black colors indicate phenolic compounds' presence (71). Terpenoid testing was performed by adding Lieberman-Burchard reagent containing anhydrous acetic acid and concentrated sulfuric acid (3:1) into a 1 ml sample. Brownish or violet ring form indicates the presence of terpenoids (72). Spectrophotometry was used to determine antioxidant activity with the DPPH method (5). The calibration curve for the DPPH concentration against absorbance was made at a maximum wavelength of 516 nm. The absorbance was measured in a mixture of sample solution and DPPH with a particular concentration after 30-min of incubation in a dark room. IC_{50} was calculated from the inhibition percentage and absorbance.

5.2.2. Preparation of TEO-loaded Negs

TEO-loaded Negs were produced using a high-energy method, which used a mechanical device to produce a highly disruptive force to break up the water and oil phases to obtain nano-sized globules (18). The oil phase (M1) was prepared by mixing span-80 with 5% turmeric oil using a magnetic stirrer (WiseStir Wisd) at 1,500 rpm for 20 min. A total of 0.18% methylparaben and 0.02% propylparaben dissolved in 15% propylene glycol (M2). Then, the distilled water was stirred with tween-80, and M2 was added gradually until homogeneous at 1,500 rpm for 20 min (M3). M1 was stirred with M3 until homogeneous at 1,500 rpm for 40 min to form a clear and transparent nanoemulsion, then let left for 24 hours. The gel base was prepared by mixing Carbopol® 980 NF with distilled water at 70°C and left for 24 hours. Then, gradually add 1% triethanolamine to form a gel mass. Nanoemulsion was added slowly into the gel base while homogenized using a homogenizer (AEG) at 2,000 rpm for 10 min.

5.2.3. Experimental Design

This study selects the CCD-RSM method to develop the TEO-loaded Negs formulation. For preliminary screening on PS, PDI, ZP, pH, AT, and spreadability, a 2-factor CCD-RSM at two levels (high and low) was used. Based on previous experiments and study literature, high and low variables were determined. The CCD of the statistical package Design-Expert® version 13 software (Stat-Ease Inc., Minneapolis, MN) was used to examine the influence of the specified independent variable on the response variable to obtain the optimal formulation for TEO-loaded Negs. CCD planned 14 experiments were done under controlled circumstances (**Table 3**). The independent variables were span-80/tween-80 (X_1) and Carbopol® 980 concentration (X_2). The observed response of the dependent variables was PS (Y_1), PDI (Y_2), ZP (Y_3), pH (Y_4), spreadability (Y_5), and AT (Y_6).

5.2.4. Determination of PS, PDI, and ZP of Negs

PS and PDI of Negs were assessed using the Delsa Max Pro Particle Size Analyzer LS 100Q (Beckman Coulter, USA) at 25°C utilizing the dynamic light scattering (DLS) method or photo correlation spectroscopy. For analysis, 1 ml of samples was dispersed in 9 ml aqua pro injection. Into the cuvette, 1 ml of suspension and 5 ml of aqua pro injection were added as a diluent, and the results were read on the instrument. All measurements were made at a scattering angle of 90°. ZP was determined by particle size analyzer through mobility and conductivity measurements. The temperature was set to 25°C, and the mean electric field was set to 16 V/cm (73). The final result is the mean of each sample's three repeated measurements.

5.2.5. Determination of pH, Spreadability, and AT

pH was measured at a temperature of 25°C using a pH meter that had been previously calibrated with buffer solutions of pH 4 and 7. The calibration step was completed when the pH value indicated on the screen matched the correct pH value and was stable. Afterward, the electrode was dipped in Negs and recorded the value shown on the screen (74). Spreadability was measured by adding 0.5 g of Negs in the center of a glass covered with another glass. The preparation diameter was measured longitudinally and transversely; for

every minute added, 50 g was to a total weight of 150 g (75). Adhesion time determine by the single-lap shear test method (54). A-0.5 g of Negs was placed on a slide, then covered with another slide, and given a load of 1 kg for 3 min. The glass object was mounted on the test apparatus, and 80 g of the load was released until both glass objects were released, and the time was recorded (75).

5.2.6. Organoleptic Observation and Homogeneity Test

Negs were placed in a glass object and directly observed for color, smell, and shape (76). A-0.1 g Negs were spread over the slide, and homogeneity was observed. Test preparation is declared homogeneous if no coarse grains exist (77).

5.2.7. Viscosity and Rheological Flow

Viscosity and rheology were determined with a Brookfield RV DV-E Viscometer with appropriate spindle and speed. A-500 ml of Negs were put into a beaker glass; the spindle was installed, and the measured value was recorded as viscosity Negs. In this study, the spindle used spindle no. 7. Flow properties were determined by measuring the viscosity using a right spindle from low to high rotational speed and vice versa (74). Flow index and consistency index are determined from the power law equation:

$$\tau = K r^n$$

where τ is the shear stress, *K* is the consistency index, *r* is the shear rate, and *n* is the flow index.
Taking logs on both sides,

$$\log \tau = \log K + n \log r$$

So, from the log, shear stress Vs. Log shear rate plot, the plot slope was used as the flow index and the antilog of the Y-intercept as the consistency index (69).

5.2.8. Freeze-thaw Test

Negs were stored at 4 ± 2 °C, then transferred to 40 ± 2 °C for 48 hours (1-cycle), then repeated for 6-cycles. Phase separation was observed in each cycle (74).

5.2.9. Statistical Analysis

The Design-Expert® version 13 software was used to conduct the statistical study (Stat-Ease Inc., Minneapolis, MN). Analysis was done at a sig—$p < 0.05$ and $p < 0.01$ after three times of each measurement.

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REFERENCES

[1] Rohman A, Widodo H, Lukitaningsih E, Rafi M, Nurrulhidayah AF, Windarsih A. Review on in vitro antioxidant activities of Curcuma species commonly used as. Food Res. 2020;4(2):286–93. [CrossRef]

[2] Liju VB, Jeena K, Kuttan R. An evaluation of antioxidant, anti-inflammatory, and antinociceptive activities of essential oil from Curcuma longa. L. Indian J Pharmacol. 2011;43(5):526–31. [CrossRef]

[3] Devkota L, Rajbhandari M. Composition of essential oils in turmeric rhizome. Nepal J Sci Technol. 2016;16(1):87–94. [CrossRef]

[4] Guimarãesa AF, Vinhas ACA, Gomes, Angélica Ferraz Souza LH, Krepsky PB. Essential oil of Curcuma longa L. rhizomes chemical composition, yield variation and stability. Quim Nov. 2020;43(7):909–13. [CrossRef]

[5] Islamadina R, Can A, Rohman A. Chemometrics application for grouping and determinating volatile compound which related to antioxidant activity of turmeric essential oil (Curcuma longa L). J Food Pharm Sci. 2020;8(2):1.

[CrossRef]

[6] Dosoky NS, Setzer WN. Chemical composition and biological activities of essential oils of curcuma species. Nutrients. 2018;10(9):10–7. [CrossRef]

[7] Jankasem M, Wuthi-udomlert M, Gritsanapan W. Antidermatophytic properties of ar -turmerone, turmeric oil, and curcuma longa preparations. ISRN Dermatol. 2013;2013(April 2009):1–3. [CrossRef]

[8] Mishra R, Gupta AK, Kumar A, Lal RK, Saikia D, Chanotiya CS. Genetic diversity, essential oil composition, and in vitro antioxidant and antimicrobial activity of Curcuma longa L. germplasm collections. J Appl Res Med Aromat Plants. 2018;10(December 2017):75–84. [CrossRef]

[9] Sharifi-Rad J, Rayess Y El, Rizk AA, Sadaka C, Zgheib R, Zam W, et al. Turmeric and ıts major compound curcumin on health: bioactive effects and safety profiles for food, pharmaceutical, biotechnological and medicinal applications. Front Pharmacol. 2020;11(September):1–23. [CrossRef]

[10] Vera JMV, Dueñas-Rivadeneira AA, Díaz JMR, Radice M. Phytochemical characterization of the ethanolic extract, antioxidant activity, phenolic content and toxicity of the essential oil of Curcuma longa L. Rev la Fac Agron. 2022;39(1). [CrossRef]

[11] Ali MS, Alam MS, Imam F, Siddiqui MR. Topical nanoemulsion of turmeric oil for psoriasis: Characterization, ex vivo and in vivo assessment. Int J Drug Deliv. 2012;4(2):184–97. [CrossRef]

[12] Meng F-C, Zhou Y-Q, Ren D, Wang R, Wang C, Lin L-G, et al. Turmeric: A review of ıts chemical composition, quality control, bioactivity, and pharmaceutical application. Natural and Artificial Flavoring Agents and Food Dyes. Elsevier Inc.; 2018. 299–350. [CrossRef]

[13] Ibáñez MD, Blázquez MA. Curcuma longa L. rhizome essential oil from extraction to its agri-food applications. a review. Plants. 2021;10(1):1–31. [CrossRef]

[14] Yan W, Bowen WD, Hopson R, Mathew AE, Jacob JN. Biological studies of turmeric oil, part 2: Isolation and characterization of turmeric oil components; cytotoxic activity against pancreatic cancer cells. Nat Prod Commun. 2013;8(6):811–4. [CrossRef]

[15] Sarojini S, Sundar K, Anjali CH, Ravindran A. Formation and stability of oil-in-water nano emulsion containing turmeric oil. Biotechnol An Indian J. 2014;9(4):171–5.

[16] Vishwakarma AK, Maurya OP, Nimisha, Srivastava D. Formulation and evaluation of transdermal patch containing turmeric oil. Int J Pharm Pharm Sci. 2012;4(SUPPL. 5):358–61.

[17] Kirtane AR, Karavasili C, Wahane A, Freitas D, Collins J, Tamang S, et al. Development of oil-based gels as versatile drug delivery systems for pediatric applications. Sci Adv. 2022;8(21):1–19. [CrossRef]

[18] Sengupta P, Chatterjee B. Potential and future scope of nanoemulgel formulation for topical delivery of lipophilic drugs. Int J Pharm. 2017;526(1–2):353–65. [CrossRef]

[19] Eid AM, El-Enshasy HA, Aziz R, Elmarzugi NA. Preparation, characterization and anti-inflammatory activity of Swietenia macrophylla nanoemulgel. J Nanomedicine Nanotechnol. 2014;5(2). [CrossRef]

[20] Khurana S, Jain NK, Bedi PMS. Nanoemulsion based gel for transdermal delivery of meloxicam: Physico-chemical, mechanistic investigation. Life Sci. 2013;92(6–7):383–92. [CrossRef]

[21] Baibhav J, Gurpreet S, Rana A, Seema S, Vikas S. Emulgel: A comprehensive review on the recent advances in topical drug delivery. Int Res J Pharm. 2011;2(11):66–70.

[22] Sultana N, Akhtar J, Badruddeen, Khan MI, Ahmad U, Arif M, et al. Drug Development Life Cycle. In: Intech. IntechOpen; 2022.

[23] Eswaraiah S, Swetha K, Lohita M, Preethi PJ, Priyanka B, Reddy KK. Emulgel : Review on novel approach to topical drug delivery. Asian J Pharm Res. 2014;4(1):4–11.

[24] Dhawan B, Aggarwal G, Harikumar S. Enhanced transdermal permeability of piroxicam through novel nanoemulgel formulation. Int J Pharm Investig. 2014;4(2):65–76. [CrossRef]

[25] Dev A, Chodankar R, Shelke O. Emulgels : A novel topical drug delivery system. Pharm Biol Eval. 2015;2(4):64–75.

[26] ICH. ICH Guideline Q9 on Quality Risk Management. Vol. 44, Regulatory ICH. 2005. 1–20 p.

[27] Abdallah MH, Elsewedy HS, Abulila AS, Almansour K, Unissa R, Elghamry HA, et al. Quality by design for optimizing a novel liposomal jojoba oil-based emulgel to ameliorate the anti-inflammatory effect of brucine. Gels. 2021;7(219):1–18. [CrossRef]

[28] Sindi AM, Hosny KM, Alharbi WS. Lyophilized composite loaded with meloxicam-peppermint oil nanoemulsion for periodontal pain. Polymers (Basel). 2021;13(14). [CrossRef]

[29] Yalçinkaya SE, Yildiz N, Ak MS, Çalimli A. Preparation of polystyrene/montmorillonite nanocomposites: Optimization by response surface methodology (RSM). Turkish J Chem. 2010;34(4):581–92. [CrossRef]

[30] Klangmanee K, Athipornchai A. A rapid phytochemical screening of the effective phenolic antioxidant agents using $FeCl_3$ reagent. J Pharm Sci Res. 2019;11(10):3475–80.

[31] Orellana-Paucar AM, Machado-Orellana MG. Pharmacological profile, bioactivities, and safety of turmeric oil. Molecules. 2022;27(16):1–16. [CrossRef]

[32] Kusmardiyani S, Novita G, Fidrianny I. Antioxidant activities from various extracts of different parts of kelakai (Stenochlaena palustris) grown in central Kalimantan - Indonesia. Asian J Pharm Clin Res. 2016;9:215–9. [CrossRef]

[33] Avanco GB, Ferreira FD, Bonfim NS, Souza Rodrigues dos Santos PA, Peralta R, Brugnari T, et al. Curcuma longa L. essential oil composition, antioxidant effect, and effect on Fusarium verticillioides and fumonisin production. Food Control. 2017;73:806–13. [CrossRef]

[34] Priya R, Prathapan A, Raghu KG, Menon AN. Chemical composition and in vitro antioxidative potential of essential oil isolated from Curcuma longa L. leaves. Asian Pac J Trop Biomed. 2012;2(2 SUPPL.):S695–9. [CrossRef]

[35] Pino JA, Fon-Fay FM, Pérez JC, Falco AS, Hernández I, Rodeiro I, et al. Chemical composition and biological activities of essential oil from turmeric (Curcuma longa L.) rhizomes grown in Amazonian Ecuador. CENIC Ciene Quím. 2018;49:1–8.

[36] Setya S, Madaan T, Tariq M, Razdan BK, Talegaonkar S. Appraisal of transdermal water-in-oil nanoemulgel of selegiline HCI for the effective management of parkinson's disease: Pharmacodynamic, pharmacokinetic, and biochemical ınvestigations. AAPS PharmSciTech. 2018;19(2):573–89. [CrossRef]

[37] Chang WC, Hu YT, Huang Q, Hsieh SC, Ting Y. Development of a topical applied functional food formulation: Adlay bran oil nanoemulgel. Lwt. 2020;117(February 2019):108619. [CrossRef]

[38] Ye Q, Li J, Li T, Ruan J, Wang H, Wang F, et al. Development and evaluation of puerarin-loaded controlled release nanostructured lipid carries by central composite design. Drug Dev Ind Pharm. 2021;47(1):113–25. [CrossRef]

[39] Shehata TM, Elnahas HM, Elsewedy HS. Development, characterization and optimization of the anti-ınflammatory ınfluence of meloxicam loaded into a eucalyptus oil-based nanoemulgel. Gels. 2022;8(5). [CrossRef]

[40] How CW, Rasedee A, Abbasalipourkabir R. Characterization and cytotoxicity of nanostructured lipid carriers formulated with olive oil, hydrogenated palm oil, and polysorbate 80. IEEE Trans Nanobioscience. 2013;12(2):72–8. [CrossRef]

[41] Chuacharoen T, Prasongsuk S, Sabliov CM. Effect of surfactant concentrations on physicochemical properties and functionality of curcumin nanoemulsions under conditions relevant to commercial utilization. Molecules. 2019;24(15):1–12. [CrossRef]

[42] Sarheed O, Dibi M, Ramesh KVRNS. Studies on the effect of oil and surfactant on the formation of alginate-based O/W lidocaine nanocarriers using nanoemulsion template. Pharmaceutics. 2020;12(12):1–21. [CrossRef]

[43] McClements DJ. Food Emulsions: Principles, Practices, and Techniques. Third Edit. New York: CRC Press; 2016. 719.

[44] Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, et al. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics. 2018;10(2):1–17. [CrossRef]

[45] Choi AJ, Kim CJ, Cho YJ, Hwang JK, Kim CT. Characterization of capsaicin-loaded nanoemulsions stabilized with alginate and chitosan by self-assembly. Food Bioprocess Technol. 2011;4:1119–26. [CrossRef]

[46] Sinha P, Srivastava S, Mishra N, Singh DK, Luqman S, Chanda D, et al. Development, optimization, and characterization of a novel tea tree oil nanogel using response surface methodology. Drug Dev Ind Pharm. 2016;42(9):1434–45. [CrossRef]

[47] Honary S, Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems - a review (part 1). Trop J Pharm Res. 2013;12(2):255–64. [CrossRef]

[48] Gurpreet K, Singh SK. Review of nanoemulsion formulation and characterization techniques. Indian J Pharm Sci. 2018;80(5):781–9. [CrossRef]

[49] Dias IP, Barbieri SF, Fetzer DEL, Corazza ML, Silveira JLM. Effects of pressurized hot water extraction on the yield

and chemical characterization of pectins from Campomanesia xanthocarpa Berg fruits. International journal of biological macromolecules. 2020;146:431–43. [CrossRef]

[50] Kulkarni VS, Shaw C. Use of polymers and thickeners in semisolid and liquid formulations. Essent Chem Formul Semisolid Liq Dosages. 2016;43–69. [CrossRef]

[51] Corporation TL. Product Specification Carbopol 980 NF Polymer. Cleveland; 2017.

[52] Nurman S, Yulia R, Irmayanti, Noor E, Sunarti TC. The optimization of gel preparations using the active compounds of arabica coffee ground nanoparticles. Sci Pharm. 2019;87(4). [CrossRef]

[53] Kurniawan MF, Sugihartini N, Yuwono T. Iritation properties and evaluation of physical properties emulgel of clove oil using simplex lattice design methods. Farmasains J Ilm Ilmu Kefarmasian. 2018;5(1):1–8. [CrossRef]

[54] Sun Z, Li Z, Qu K, Zhang Z, Niu Y, Xu W, et al. A review on recent advances in gel adhesion and their potential applications. J Mol Liq. 2021;325:115254. [CrossRef]

[55] Gupta NV, Natasha S, Getyala A, Bhat RS. Bioadhesive vaginal tablets containing spray dried microspheres loaded with clotrimazole for treatment of vaginal Candidiasis. Acta Pharm. 2013;63(3):359–72. [CrossRef]

[56] Abramowitch S, Easley D. Introduction to Classical Mechanics. Biomechanics of the Female Pelvic Floor. Elsevier Inc.; 2016. 89–107 p. [CrossRef]

[57] Sari DK, Sugihartini N, Yuwono T. Irritation test and physical properties evaluation of essential oils clove (Syzigium aromaticum) in emulgel. Pharmaciana. 2015;5(2):115–20. [CrossRef]

[58] Cevher E, Sensoy D, Taha MAM, Araman A. Effect of thiolated polymers to textural and mucoadhesive properties of vaginal gel formulations prepared with polycarbophil and chitosan. AAPS PharmSciTech. 2008;9(3):953–65. [CrossRef]

[59] Pande V, Patel S, Patil V, Sonawane R. Design expert assisted formulation of topical bioadhesive gel of sertaconazole nitrate. Adv Pharm Bull. 2014;4(2):121–30. [CrossRef]

[60] Badwaik LS, Prasad K, Deka SC. Optimization of extraction conditions by response surface methodology for preparing partially defatted peanut. Int Food Res J. 2012;19(1):341–6.

[61] Mohtashamian S, Boddohi S, Hosseinkhani S. Preparation and optimization of self-assembled chondroitin sulfate-nisin nanogel based on quality by design concept. Int J Biol Macromol. 2018;107:2730–9. [CrossRef]

[62] Jaiswal M, Dudhe R, Sharma PK. Nanoemulsion: An advanced mode of drug delivery system. 3 Biotech. 2015;5(2):123–7. [CrossRef]

[63] Wang X, Gu Y, He Y, Sang L, Dai Y, Wang D. Preparation and optimization formulation of zedoary turmeric oil nanoemulsion based thermo-sensitive gel for improved application in ophthalmology. J Drug Deliv Sci Technol. 2021;65(June):102682. [CrossRef]

[64] Begur M, Kumar Pai V, Gowda D V, Srivastava A, Raghundan H V, Shinde CG, et al. Enhanced permeability of Cyclosporine from a transdermally applied nanoemulgel. Der Pharm Sin. 2015;6(2):69–79.

[65] Yuliani SH, Hartini M, Pudyastuti B, Istyastono EP. Comparison of physical stability properties of pomegranate seed oil nanoemulsion dosage forms with long-chain triglyceride and medium-chain triglyceride as the oil phase. Tradit Med J. 2016;21(2):93–8. [CrossRef]

[66] Algahtani MS, Ahmad MZ, Shaikh IA, Abdel-Wahab BA, Nourein IH, Ahmad J. Thymoquinone loaded topical nanoemulgel for wound healing: Formulation design and in-vivo evaluation. Molecules. 2021;26(13):1–16. [CrossRef]

[67] Ahmad J, Mir SR, Kohli K, Amin S. Effect of oil and co-surfactant on the formation of Solutol HS 15 based colloidal drug carrier by Box-Behnken statistical design. Colloids Surfaces A Physicochem Eng Asp. 2014;453(1):68–77. [CrossRef]

[68] Eid AM, Hawash M. Biological evaluation of Safrole oil and Safrole oil Nanoemulgel as antioxidant, antidiabetic, antibacterial, antifungal and anticancer. BMC Complement Med Ther. 2021;21(1):1–12. [CrossRef]

[69] Arora R, Aggarwal G, Harikumar SL, Kaur K. Nanoemulsion based hydrogel for enhanced transdermal delivery of ketoprofen. Adv Pharm. 2014;2014:1–12. [CrossRef]

[70] Sinko PJ, Singh Y, editors. Martin's physical pharmacy and pharmaceutical sciences 6th edition. Sixth edit. Philadelphia: Wolter Kluwer Health; 2011.

[71] Rahimah SB, Djunaedi DD, Soeroto AY, Bisri T. The phytochemical screening, total phenolic contents and antioxidant activities in vitro of white oyster mushroom (Pleurotus ostreatus) preparations. Open Access Maced J

Med Sci. 2019;7(15):2404–12. [CrossRef]

[72] Farabi K, Harneti D, Darwati, Mayanti T, Nurlelasari, Maharani R, et al. Dammarane-type triterpenoid from the stem bark of aglaia elliptica (meliaceae) and ıts cytotoxic activities. Molecules. 2022;27(19):1–11. [CrossRef]

[73] Elsheikh MA, Elnaggar YSR, Hamdy DA, Abdallah OY. Novel cremochylomicrons for improved oral bioavailability of the antineoplastic phytomedicine berberine chloride: Optimization and pharmacokinetics. Int J Pharm. 2018;535(1–2):316–24. [CrossRef]

[74] Nursal FK, Nining, Rahmah AS. Formulation and development of grape seed oil (Vitis vinifera L) emulgel peel-off mask using gelling agent hydroxy propyl methyl cellulose (HPMC). IOP Conf Ser Earth Environ Sci. 2021;755(1). [CrossRef]

[75] Irianto IDK, Purwanto P, Mardan MT. Antibacterial activity and physical evaluation of Piper betle L. Decoction gel as an alternative treatment for mastitis. Maj Farm. 2020;16(2):202–10. [CrossRef]

[76] Elfiyani R, Amalia A, Pratama SY. Effect of using the combination of tween 80 and ethanol on the forming and physical stability of microemulsion of eucalyptus oil as antibacterial. J Young Pharm. 2017;9(2):230–3. [CrossRef]

[77] Yati K, Jufri M, Gozan M, Dwita LP. The effect of hidroxy propyl methyl cellulose (HPMC) concentration variation on physical stability of tobacco (Nicotiana tabaccum l.) extract gel and ıts activity against streptococcus mutans. Pharm Sci Res. 2018;5(3):133–41. [CrossRef]

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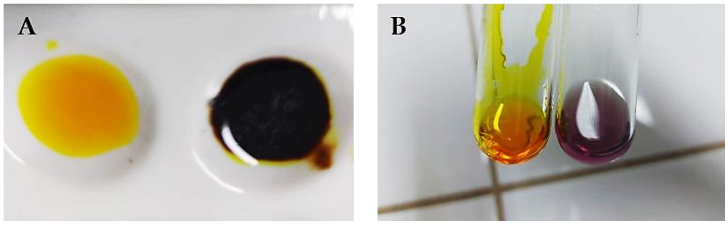

Figure 1. The result of qualitative test observation is a phenolic test with a positive result marked in a blackish green color (a) and a terpenoid test with a positive result marked with a purple color (b).

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Tabel 1. Evaluation results of independent variables and dependent variables with the CCD design for optimizing TEO-loaded Negs.

Std	X_1 (%)	X_2 (%)	Y_1 (nm)	Y_2	Y_3 (mV)	Y_4	Y_5 (cm)	Y_6 (s)
1	5.50	1.02	206.9 ± 0.7	0.571 ± 0.000	35.39 ± 1.50	5.45	5.55	5.65
2	2.32	1.15	457.0 ± 0.9	0.000 ± 0.000	13.90 ± 1.92	4.60	5.00	6.99
3	8.68	0.90	166.7 ± 0.4	0.378 ± 0.074	31.64 ± 0.53	6.35	6.20	4.23
4	2.32	0.90	411.0 ± 5.8	0.000 ± 0.000	30.67 ± 0.89	6.29	5.00	4.30
5	5.50	1.02	302.1 ± 3.6	0.571 ± 0.000	36.45 ± 0.45	5.46	5.60	5.63
6	5.50	1.02	263.0 ± 3.7	0.571 ± 0.000	18.49 ± 1.77	5.47	5.55	5.64
7	8.68	1.15	198.1 ± 1.2	0.242 ± 0.003	57.23 ± 2.91	4.65	6.15	6.95
8	5.50	1.02	246.9 ± 7.2	0.571 ± 0.000	26.60 ± 0.17	5.46	5.60	5.67
9	5.50	1.02	206.0 ± 2.3	0.571 ± 0.000	31.77 ± 1.01	5.46	5.60	5.65
10	10.00	1.02	160.8 ± 1.3	0.285 ± 0.024	46.72 ± 1.97	5.31	6.25	5.84
11	1.00	1.02	244.2 ± 4.3	0.571 ± 0.000	31.26 ± 0.57	5.78	4.90	6.88
12	5.50	1.02	238.1 ± 3.0	0.571 ± 0.000	30.78 ± 0.53	5.45	5.55	5.64
13	5.50	1.20	206.2 ± 1.6	0.571 ± 0.000	30.56 ± 0.78	4.57	5.50	7.08
14	5.50	0.85	240.5 ± 2.2	0.571 ± 0.000	20.12 ± 0.16	6.39	5.65	4.22

Y_1: Particle size (PS), Y_2: Polydispersity index (PDI), Y_3: Zeta potensial (ZP), Y_4: pH, Y_5: Spreadability, Y_6: Adhesion time (AT)

551 **Table 2.** Statistical analysis of PS (Y_1), PDI (Y_2), ZP (Y_3), pH (Y_4), spreadability (Y_5), and AT (Y_6) TEO-loaded Negs on
552 CCD.

Factors		**Y_1**	**Y_2**	**Y_3**	**Y_4**	**Y_5**	**Y_6**
X_1	Coefficient	-77.64	0.027	8.27	-0.069	0.53	-0.20
	p-value	0.0063**	0.7325	0.0080**	0.1430	<0.0001**	0.1406
X_2	Coefficient	3.61	-0.017	2.95	-0.75	-0.033	1.18
	p-value	0.8783	0.8289	0.2668	<0.0001**	0.1157	<0.0001**
X_1X_2	Coefficient		-0.034	10.59			
	p-value		0.7602	0.0136*			
X_1^2	Coefficient		-0.16				
	p-value		0.0818				
X_2^2	Coefficient		-0.086				
	p-value		0.3087				
Intercept	Coefficient	253.39	0.57	31.54	5.48	5.58	5.74
Degree of freedom		2	5	3	2	2	2
Sum of squares		48332.05	0.24	1065.33	4.48	2.28	11.49
Mean of squares		24166.02	0.047	355.11	2.24	1.14	5.74
F-value		5.69	1.02	7.06	145.08	386.43	46.35
p-value		0.0201	0.4645	0.0078	<0.0001	<0.0001	<0.0001
R-Squared		0.5084	0.3898	0.6794	0.9635	0.9860	0.8939

553 X_1: span-80/tween-80; X_2: Carbopol® 980
554 * p-value < 0.05
555 ** p-value < 0.01

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558 **Table 3.** The independent and dependent variables with levels and limits in CCD for TEO-loaded Negs development.

Variables	Code	Start point (-α)	Low level (-1)	Central level	High level (+1)	Start point (+α)	Units
Independent variables							
Span-80/tween-80	X_1	0.85	2.32	1.02	5.50	1.20	% w/w
Carbopol® 980	X_2	1	0.90	5.50	1.02	10	% w/w
Dependent variables		**Limits**					
Particle size (PZ)	Y_1	Minimum					nm
Polydispersity index (PDI)	Y_2	Minimum					
Zeta potensial (ZP)	Y_3	Maximum					mV
pH	Y_4	is in range					
Spreadability	Y_5	is in range					cm
Adhesion time (AT)	Y_6	is in range					s

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561 **Table 4.** Optimized TEO-loaded Negs' actual and predicted values for each response.

Responses	Predicted values	Actual value[a]	Error[b] (%)
Y1 (nm)	180.2	182.3 ± 5.5	1.165
Y2	0.250	0.242 ± 0.003	-3.200
Y3 (mV)	56.30	57.23 ± 2.91	1.652
Y4	4.50	4.51 ± 0.02	0.222
Y5 (cm)	6.07	6.0 ± 0.2	-1.153
Y6 (s)	6.98	6.45 ± 0.19	-7.593

562 a Data listed is the mean ± standart deviation, n = 3
563 b Error (%) = [(Actual value – Predicted value)/Predicted value] * 100%
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565
566

567 **Table 5.** Additional evaluation on TEO-loaded Negs with an optimal formulation.

Evaluation	Result
Organoleptic	Color: White; Odor: typical turmeric
Homogeneity	Homogeneous
Freeze-Thaw	Cycles 0-6, no separation occurs
Viscosity[a]	32240 ± 2257,7 cP

568 a Data listed is the mean ± standart deviation, n = 3

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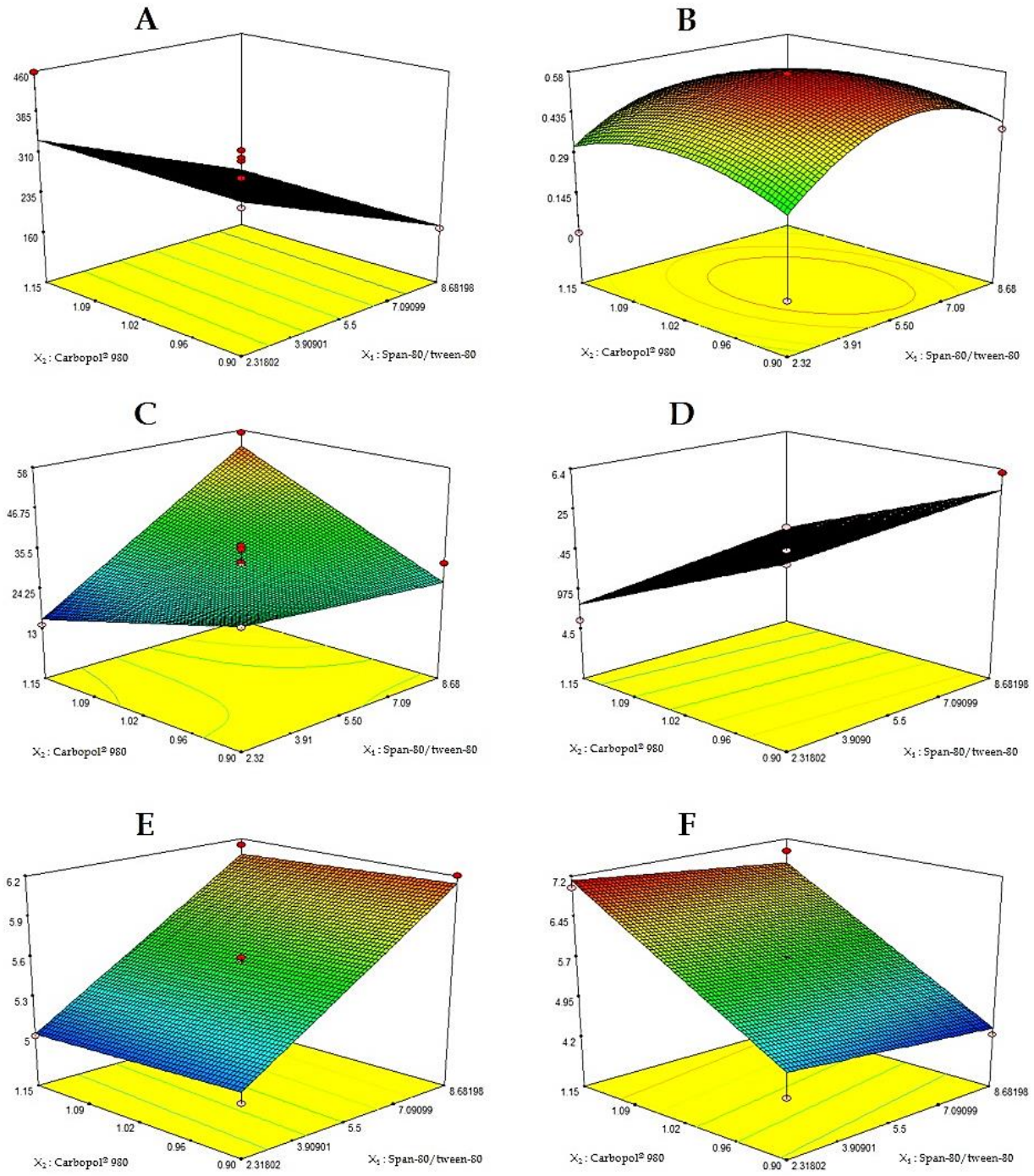

Figure 2. Effect of span-80/tween-80 and Carbopol® 980 concentration on PS (a) PDI (b) ZP (c) pH (d) spreadability (e) AT (f).

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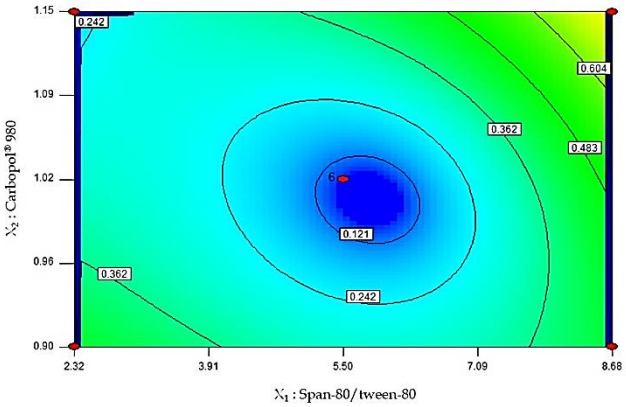

Figure 3. Contour plot desirability value of optimal formulation.

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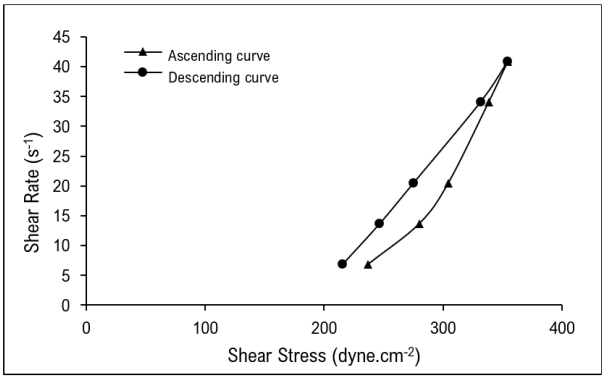

Figure 4. Optimal formulation flow properties.

RESPONSE TO REVIEWERS

N. Nining
Universitas Muhammadiyah Prof. DR. HAMKA
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November 23, 2022

Dear Reviewers:

We appreciate the invaluable review and suggestions from the reviewers to improve our research results. We will respond to several points related to that.

1. We apologize for our poor writing. Improvements have been made with the help of the Language editing service.
2. We have regenerated the abbreviations section, italics, and usage of appropriate terms and units.
3. This study was conducted based on the adequate TEO availability in our country, and several research reports have promising results on its pharmacology activity.
4. This report demonstrates the physical and mechanical characterization of the Negs, while quantitative analysis is a limitation due to limited research funding. We hope to continue this in further research.
5. Several details provide to clarify the CCD design.
6. Results and discussion enriched with sufficient literature.

We hope the revised and updated articles can be published in the Journal of Research in Pharmacy.

We appreciate your consideration.

Sincerely,
Nining apt., M.Si
Lecturer, Faculty of Pharmacy and Science
Universitas Muhammadiyah Prof. DR. HAMKA

4. Bukti Konfirmasi Review dan Hasil Review Kedua (27 Januari 2023)

Uhamka
UNIVERSITAS MUHAMMADIYAH PROF. DR. HAMKA

Nining <nining@uhamka.ac.id>

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1 message

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Suggestions

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The authors' response to my previous comments was presented as a separate file without referring to the comments and İt is impossible to follow the updated manuscript file since the changes made are not traceable. Under these circumstances the manuscript is not acceptable.

2. Reviewer Comments

This revize paper is possibly suitable to be published in "Journal of Research in Pharmacy".

Manuscript Information

Manuscript ID:	**MPJ-10622.REV-1**
Title in English:	Response surface methodology for optimization of turmeric essential oil-loaded nanoemulgel
Small Title in English:	No information entered
Authors:	Nining Nining[1], Anisa Amalia[1], Fatimatuz Zahrok[2]
Institutions:	[1]Universitas Muhammadiyah Prof. DR. HAMKA, Pharmaceutical Technology, East Jakarta, Indonesia [2]Universitas Muhammadiyah Prof. DR. HAMKA, Pharmacy, East Jakarta, Indonesia
Keywords in English:	Central composite design; nanoemulgels; response surface methodology; turmeric essential oil; topical delivery.
Manuscript Type:	Research article
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Abstract in English

Turmeric Essential Oil (TEO) has an antioxidant and anti-inflammatory activity to be formulated in a topical dosage form. Nanoemulgels (Negs) development, based on varying concentrations of emulsifiers and gel formers, affects their characteristics and stability. This study focuses on optimizing TEO-loaded Negs based on physical and mechanical characterization, which have promising topical applications. Negs were created using the high-energy approach and optimized using Response Surface Methodology (RSM) and the Central-Composite Design (CCD) for the optimization of span-80/tween-80 (X1) and Carbopol® 980. (X2). Observed variable responses were particle size (PS) (Y1), polydispersity index (PDI) (Y2), zeta potential (ZP) (Y3), pH (Y4), spreadability (Y5), and adhesion time (AT) (Y6). Actual responses of Negs were compared with the CCD-RSM predictions to validate the model. In addition, other physical evaluations were observed, such as organoleptic observations, homogeneity, freeze-thaw tests, viscosity, and flow properties. Optimized TEO-loaded Negs were made with 8.68% span-80/tween-80 and 1.18% Carbopol® 980. The evaluation results showed the optimal TEO-loaded Negs on nano-metric size (182.3 ± 5.5 nm) with low PDI (0.242 ± 0.003), good ZP (-57.23 ± 2.91 mV), pH (4.51 ± 0.02), spreadability (6.0 ± 0.2 cm), and AT (6.45 ± 0.19 s). TEO-loaded Negs have an excellent appearance and did not run phase separation at extreme temperature storage with pseudoplastic thixotropy flow. Thus, the developed TEO-loaded Negs can be a potential delivery system and a promising suitable approach for topical preparations.

Manuscript Files

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MPJ-10622-9-isi-jrp-checklist.pdf (../pdf-files/in/11427-MPJ-10622-9-isi-jrp-checklist.pdf)	3388 KB	Sep 03, 2022	Author Checklist Form	None
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MPJ-11427-3-response-to-reviewers.rev-1.pdf (../pdf-files/out/11427-MPJ-11427-3-response-to-reviewers.rev-1.pdf)	32 KB	Nov 23, 2022	Response to Reviewers	None

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Is the language adequate?	Yes
Are the nomenclature and scientific terminology correct?	Yes
Are the references complete and recent?	Yes
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Are the experimental procedures sound?	Yes
Is the results and discussion part	sufficiently developed
Is conclusion sufficient and correlated with the results ?	Yes
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Is the language adequate?	Yes
Are the nomenclature and scientific terminology correct?	Yes
Are the references complete and recent?	Yes
Are the figures tables and graphics necessary ?	Yes
Are the figures tables and graphics clear ?	Yes
Is the introduction part	sufficiently developed
Are the experimental procedures sound?	Yes
Is the results and discussion part	sufficiently developed
Is conclusion sufficient and correlated with the results ?	Yes
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Nining <nining@uhamka.ac.id>

Journal of Research in Pharmacy : Confirmation for revised manuscript

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Response surface methodology for optimization of turmeric essential oil-loaded nanoemulgel

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ABSTRACT: Turmeric Essential Oil (TEO) has an antioxidant and anti-inflammatory activity to be formulated in a topical dosage form. Nanoemulgels (Negs) development, based on varying concentrations of emulsifiers and gel formers, affects their characteristics and stability. This study focuses on optimizing TEO-loaded Negs based on physical and mechanical characterization, which have promising topical applications. Negs were created using the high-energy approach and optimized using Response Surface Methodology (RSM) and the Central-Composite Design (CCD) for the optimization of span-80/tween-80 (X_1) and Carbopol® 980. (X_2). Observed variable responses were particle size (PS) (Y_1), polydispersity index (PDI) (Y_2), zeta potential (ZP) (Y_3), pH (Y4), spreadability (Y_5), and adhesion time (AT) (Y_6). Actual responses of Negs were compared with the CCD-RSM predictions to validate the model. In addition, other physical evaluations were observed, such as organoleptic observations, homogeneity, freeze-thaw tests, viscosity, and flow properties. Optimized TEO-loaded Negs were made with 8.68% span-80/tween-80 and 1.18% Carbopol® 980. The evaluation results showed the optimal TEO-loaded Negs on nano-metric size (182.3 ± 5.5 nm) with low PDI (0.242 ± 0.003), good ZP (-57.23 ± 2.91 mV), pH (4.51 ± 0.02), spreadability (6.0 ± 0.2 cm), and AT (6.45 ± 0.19 s). TEO-loaded Negs have an excellent appearance and did not run phase separation at extreme temperature storage with pseudoplastic thixotropy flow. Thus, the developed TEO-loaded Negs can be a potential delivery system and a promising suitable approach for topical preparations.

KEYWORDS: Central composite design; nanoemulgels; response surface methodology; turmeric essential oil; topical delivery.

1. INTRODUCTION

Turmeric is the dried rhizome of *Curcuma longa* L. (Zingiberaceae), which derives from Southeast Asia and is cultivated mainly in India, followed by Bangladesh, China, Thailand, Cambodia, Indonesia, Malaysia, and the Philippines (1). Steam distillation extracts turmeric essential oil (TEO) from the turmeric rhizome (2). Chemical constituents with the most significant proportion were oxygenated monoterpenes and sesquiterpenes, which include β-turmerone, α-turmerone, and ar-turmerone (3–5). The pharmacological activities of TEO have been reported in the form of antioxidants, anti-inflammatory, antinociceptive, antidermatophytic, antifungal, and antibacterial activities (2,6–9). These reducing power and radical scavenging abilities are associated with the high antioxidant potential of TEO (8,10). This pharmacological activity justifies its use in various applications, including cosmetics and phytomedicines (7,11,12). Like other essential oil, TEO has limited use due to its volatility, instability under certain conditions, lipophilicity, and low aqueous solubility (13,14). Many recent studies are oriented toward solving these limitations, so that efficacy of the essential oil lasts longer and increases.

Previously, TEO has been developed for cream as a conventional drug delivery system, patch, and nanoemulsions (7,11,15,16). Most water-based liquid or semisolid systems have limitations in delivering lipophilic drugs (17). Nanoemulsions are an established alternative for delivering lipophilic drugs by increasing topical absorption (18). The main advantage of topically administered nanoemulsions is the ability to increase penetration and permeation of drugs through the skin without adding non-physical enhancers and non-friendly solvents to the formulation, which can cause skin irritation, especially with long-term usage (18,19). However, this system has problems with low viscosity due to poor spreadability and skin

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retention (20). Nanoemulgels (Negs), a combination of nanoemulsion and hydrogel, were made to improve the characteristics (18,19,21).

Negs consist of a hydrogel system and an emulsion with nano-sized globules. An emulsifier in the form of surfactants and co-surfactants stabilizes the emulsion, which serves as a drug delivery platform (18). Surfactants reduce the interfacial surface tension of immiscible liquids and change the entropy of the dispersion, thereby stabilizing a thermodynamically unstable system; co-surfactants are combined with surfactants in the emulsification process by disrupting the surface layer (22). The emulsifier plays a role in the emulsification process to increase stability when the product is kept for an extended time. On the other side, gels are made from polymers, a gel former, that expand after absorbing a liquid (23). Gel former increase the viscosity of the formulation and can react with emulsifiers to change the thickness (24). In addition, topical Negs can increase patient compliance because of their non-irritating, non-greasy characteristics and improved drug release (25). However, the available methods for manufacturing Negs exhibit various limitations, which directly or indirectly affect the quality of the Negs formulations.

Currently, the principle of quality by design is adopted to ensure the quality of drugs, their safety, and efficacy (26). The quality by design (QbD) trend is used to develop, optimize, and investigate the interaction between particular variables and their related responses to achieve the optimal formulation (27). Our study used Central Composite Design (CCD) on Response Surface Methodology (RSM) to select optimal formulations and predict models that rely on statistical analysis (ANOVA) and exact equations (28,29). In this aspect, we intended to develop a TEO-loaded Negs that would be optimized using a complete 2^2-factorial design and determine the independent factors' precise influence on the investigated dependent variables. The choice and procedure factors were span-80/tween-80 (X_1) and Carbopol® 980 (X_2). Response variables investigated were particle size (PS) (Y1), polydispersity index (PDI) (Y2), zeta potential (ZP) (Y3), pH (Y4), spreadability (Y5), and adhesion time AT) (Y6). This work is the first step in developing an optimized TEO preparation suitable for transdermal drug delivery for topical application.

2. RESULTS AND DISCUSSION

2.1. Identification of TEO

TEO identification is carried out to ensure the quality of the active ingredients. The tests included organoleptic, phytochemical identity in the form of phenolics and terpenoids, and antioxidant activity by the DPPH method. The results of this study can be seen in **Figure 1**. Organoleptically, TEO is an orange liquid with a distinctive turmeric aroma. Identification results in the phenolic test showed a black-green color (**Figure 1A**) due to the formation of a phenolic-Fe^{3+} complex (30). In the terpenoid test, reaction results show a purple color (**Figure 1B**) as a positive sign. Based on these results, it can be concluded that TEO contains phenolic and terpenoid compounds. Several studies reported that TEO has powerful antioxidant potential both *in-vitro* and *in-vivo* (2,8,31). Oxygenated monoterpenes and sesquiterpenes, such as ar-turmerone, α-turmerone, and β-turmerone, were included in the terpenoid compounds and reported to play a role in this activity. In addition, contained phenol was also reported to perform as an excellent free radical scavenger because its reduction potential was lower than oxygen's (8). In our study, the antioxidant activity of TEO was presented as an IC_{50} of 9.88 µm/ml, which was included in the powerful antioxidant category because of below 50 µm/ml (32). It differed from other studies' results, which showed an IC_{50} of 2274.02 µm/ml and above 1000 µm/ml (2,10). Meanwhile, similar findings stated IC_{50} values of 10.03 µm/ml, 3.227 µm/ml, and 14.5 µm/ml (33–35). The difference value was due to the use of TEO from different origin sources, regional conditions, and the extraction method used.

2.2. Preparation of TEO-loaded Negs

TEO-loaded Negs were produced using the high-energy method efficiently. A high-pressure homogenizer provides sufficient energy to increase the interfacial area and generate nano-size globules (36). An emulsion system with a nanodroplet size must have flow properties that allow it to pass through the homogenizer (37). Flow properties are inversely correlated with the amount of emulsifier and gel former added to the Negs formulation. In this study, the selected optimized formulation was the one that produces Negs with the smallest size, as shown by the limitations in **Table 3**. Therefore, the range of regulated emulsifiers and gel formers concentrations still provides flow properties that allow the flow to remain good using a high-energy homogenizer. In general, the preparation of Negs was carried out in 3-stages, namely,

nanoemulsions preparation, gel preparation, and Negs preparation. Negs were successfully produced in 14-formulation with variations of emulsifiers and gel former with compositions that can be seen in **Table 1**.

2.3. Experimental Design by CCD-RSM

2.3.1. Fitting the Model

TEO-loaded Negs were optimized based on CCD in the RSM. CCD was used to establish the optimal concentrations of emulsifier (X_1) and gel former (X_2) as the key parameters influencing the dependent response. Prediction of the factorial axial design and the possible curvature in the response could be obtained from the optimization process with an effective second-level design (38). Predicting the significant influence of the independent variable on the dependent variable is essential for generating TEO-loaded Negs. Based on the literature survey, two factors were chosen as independent variables, and six responses were chosen as the dependent variable with the most significant influence on Negs. The independent variables with their levels and the observed response variables are presented in **Table 1**.

2.3.2. Analysis of Design

Statistical data analysis must be carried out to predict and recognize the model. **Table 2** shows the statistical analysis of a quadratic model for PDI, a 2FI model for ZP, and linear models for PS, pH, AT, and spreadability. This table identifies factors with p-values less than a predefined threshold (0.01 and 0.05, with a 99% and 95% confidence level, respectively) as influential factors. Besides the significant p-value, a large F-value minimizes error in the model and lack of fit, preferably non-significant to fit the model data (27,39). Depending on the most significant R-squared value and the least residual predictive sum of squares value, the six responses demonstrated distinct models in their application. The chosen model had a non-statistically significant lack of fit, and model validation was confirmed by the residual plot test of the regression model, which was supported by supplemental information for all responses. Compared to the 2FI and simple linear models, the quadratic model represented the impacts of numerous variables, including individual factors, interactions, and the quadratic influence on the response.

2.3.3. Effect of Independent Variables on Dependent Variables

PS parameters are often used to characterize nanoparticles. Negs globule diameter means (Y_1) was adjusted from 160.8 nm (STD#10) to 457.0 nm (STD#2). Based on **Table 2**, the PS response indicates a significantly linear model with an F-value of 5.69 (p-value 0.0201 < 0.05). The suggested linear model equation can be seen as follows:

$$Y_1 = 253.39 - 77.64X_1 + 3.61X_2$$

The equation shows that the Negs PS is significantly (p-value < 0.01) affected by emulsifier concentration (X_1). The positive coefficient has a synergistic effect on the response. In contrast, the negative coefficient has an antagonistic effect which concludes the inverse relationship of the independent variable with a response (38,40). In addition, this factor has a more significant coefficient. It directly affects the PS, which means that increasing emulsifier concentration causes a decrease in the globule diameter PS in the TEO-loaded Negs. High emulsifier (above 5.5%) resulted in globules measuring below 200 nm, and low emulsifier (below 5.5%) produced globules above 200 nm. This fact is in line with other studies that increasing emulsifiers could reduce droplet Negs PS (41,42). The emulsifier reduced the interfacial surface tension between the water and oil phases, which decreased the free energy required to disrupt or break the globules and resulted in a smaller droplet diameter. It can also produce a protective cover over the globules, preventing them from coalescence. However, the emulsifier must absorb quickly enough around the droplet to form this protective layer (42,43).

Meanwhile, Carbopol® 980 had no significant effect on PS. The same findings were obtained from the globule size Negs from Carbopol® 934 and 940 as gel former (19). A response surface plot (**Figure 2A**) may therefore be used to represent the combined influence of variables X_1 and X_2, which shows that Y_1 changes linearly with the sum of the two variables. Nevertheless, the higher gradient in the response surface with span-80/tween-80 (X_1) – not Carbopol® 980 (X_2) – was evidenced from the comparative plot of the response surface. From this explanation, it can be concluded that the PS can be changed by selecting the right X_1 level.

In **Table 1**, Negs PDI (Y_2) varied from 0.000 (STD#2 and #4) to 0.571 (STD#1, #5, #6, #8, #9, #11 to #14). PDI measures the distribution of molecular mass in a sample. The smaller PDI (close to 0), the more stable the Negs formulation caused; the large PDI indicates particles formed are not uniform, and the formulation will flocculate quickly. An index value less than 0.05 is included in monodisperse, while an index greater than 0.7 indicates that the sample has a broad PS distribution. A-0.2 and below are considered acceptable for nanoparticle preparations (44). Based on **Table 2**, the PDI response shows a non-significant quadratic model with an F-value of 1.02 (p-value 0.4645 > 0.05). Measurements between variables and responses are not a precise cause of that. The suggested quadratic model equation can be seen as follows:

$$Y_2 = 0.57 + 0.027X_1 - 0.017X_2 - 0.034X_1X_2 - 0.16X_1^2 - 0.086X_2^2$$

The equation and p-value of each factor did not significantly affect PDI. The response surface plot (**Figure 2B**) depicts the combined influence of variables X_1 and X_2, which shows that Y_2 changes with the sum of the two variables by model.

The Negs ZP (Y_3) was in the range of 13.90 mV (STD#2) to 46.72 mV (STD#10) (**Table 1**). ZP represents the electric charge between the shear plane of a final outer layer and bulk solution, which significantly affects dispersion stability (45). This factor is strongly influenced by the composition of the Negs and its electrical phenomena. TEO-loaded Negs, which have positive ZP, show good interaction with negatively charged skin (46). ZP is the scientific term for the electrokinetic potential in colloidal systems. The high electric charge on the nanoparticle surface will prevent nanoparticle aggregation because of the strong repulsion between particles. The ZP requirement for stability is above ± 30 mV (19). The higher ZP and the slower aggregation are formed to prevent separation (47). Based on **Table 2**, the ZP response indicates a significant 2FI model with an F-value model of 7.06 (p-value 0.0078 < 0.05). The linear model equation suggested by the software can be seen as follows:

$$Y_3 = 31.54 + 8.27X_1 + 2.95X_2 + 10.59X_1X_2$$

The equation shows that the Negs ZP was significantly affected by the emulsifier (X_1) and gel former-emulsifier interaction (X_1X_2). ZP was usually influenced by the physicochemical properties of the drug, polymer, carrier, electrolyte presence, and their adsorption (48). One study stated that adding Carbopol® only slightly increased the ZP Negs (19). The response surface plot (**Figure 2C**) depicts the combined influence of variables X_1 and X_2, showing that Y_3 changes with the sum of the two variables. Nevertheless, the higher gradient in the response surface with span-80/tween-80 (X_1) – not Carbopol® 980 (X_2) – is the evidence from the comparative plot of the response surface. This description concludes that the ZP can be changed by selecting the proper X_1 level.

The pH test was carried out to measure Negs's acidity or alkalinity level. The pH values (Y_4) were in the range of 4.57 (STD#13) to 6.39 (STD#14) (**Table 1**). The pH requirement of Negs is the same as the skin pH. Too-acidic preparations can irritate the skin and cause a stinging sensation, while too-alkaline preparations can cause dry and itchy skin. The pH result test on 14 formulations was eligible and compatible with the skin (4.5-6.5) (49). Based on **Table 2**, a linear model was found to be significant in pH response with a model F-value of 145.08 (p-value < 0.0001). The linear model equation suggested by the software can be seen as follows:

$$Y_4 = 5.48 - 0.069X_1 - 0.75X_2$$

The equation shows that the pH was significantly (p-value < 0.0001) affected by gel former (X_2). Carbopol is a high molecular weight homopolymer and acrylic acid copolymer crosslinked with polyalkenyl polyethers (50). They are anionic and acidic (2.5–4.0 in 2% dispersion) when not neutralized with bases to achieve a specific viscosity (50,51). Therefore, adding Carbopol to a formulation with a fixed amount of base (triethanolamine) will significantly lower the pH of Negs. The response surface plot (**Figure 2D**) may then be used to depict the combined influence of variables X_1 and X_2, which shows that Y_4 changes linearly with the sum of the two variables. Nevertheless, the higher gradient in the response surface with Carbopol® 980 (X_2) – not span-80/tween-80 (X_1) – is the evidence from the comparative plot of the response surface. This description concludes that the choice of the X_2 level affects the pH Negs.

Spreadability was measured to ensure comfortable use on the skin because it spreads quickly (24). Terms of good dispersion are 5-7 cm. If the dispersion is too small, it is relatively difficult to spread when applied to the skin, while the dispersion tends to spread too quickly when applied, so it will cause an uncomfortable feeling when used (52). Based on the results, only one Neg did not meet the requirements, namely F11. **Table 1** shows the range of spreadability (Y_5) from 4.90 cm (STD#11) to 6.25 cm (STD#10). Based on **Table 2**, the spreadability response indicates a significant linear model with an F-value of 386.43 (p-value <0.0001). The linear model equation suggested by the software can be seen as follows:

$$Y_5 = 5.58 + 0.53X_1 - 0.033X_2$$

The equation shows that the spreadability of Negs was significantly (p-value < 0.0001) positively affected by the emulsifier (X_1). That was also found in other literature studies (24). The higher the Carbopol-contained Negs, the more viscous Negs. AT and spreadability have the opposite results. The higher the Negs' viscosity, the higher the adhesive strength produced, while the smaller the dispersion power (53). The response surface plot (**Figure 2E**) depicts the combined influence of variables X_1 and X_2, which shows that Y_5 changes linearly with the sum of the two variables. Nevertheless, the higher gradient in the response surface with span-80/tween-80 (X_1) – not Carbopol® 980 (X_2) – is the evidence from the comparative plot of the response surface. This description concludes that the scatter can be changed by choosing the right X_1 level.

Topical dosage forms, such as Negs, adhere to the skin in two ways: they adhere directly to the rough surface to form a "mechanical interlock" and to the surface via interaction (54). Good adhesion to Negs supports a higher concentration gradient towards the skin and provides more drug penetration (18). Adhesive strength is directly related to the AT on the Negs as measured using the single-lap shear test method with slight modifications (55,56). The test was carried out by applying a shear load to the plates that flank the sample, which had been pre-loaded, and given a measured force; the time taken for the plates to separate was recorded as AT. In our study, the AT was from 4.22 s (STD#14) to 7.08 s (STD#13) in **Table 1**. They met the requirements based on the AT test results on a 14 formulation. An AT was carried out to see how long a Negs could be attached to the skin. The AT requirement is more than 4 seconds. The longer a Negs could be attached to the skin, it showed the better result, where it is expected that more active substances can be absorbed due to the time the Negs was in contact with the skin (57). Based on **Table 2**, the linear model was found to be significant in the AT response with an F-value model of 46.35 (p-value < 0.0001). The linear model equation suggested by the software can be seen as follows:

$$Y_6 = 5.74 - 0.20X_1 + 1.18X_2$$

The equation reveals that the AT was significantly (p-value < 0.0001) affected by the gel former (X_2) or, indirectly, the same adhesion strength. This finding is in agreement with the literature (58,59). A response surface plot (**Figure 2F**) may therefore be used to depict the combined influence of variables X_1 and X_2, which shows that Y_6 changes linearly with the sum of the two variables. Nevertheless, the higher gradient in the response surface with Carbopol® 980 (X_2) – not span-80/tween-80 (X_1) – is the evidence from the comparative plot of the response surface. From this explanation, it can be concluded that the AT can be changed by selecting the right X_2 level. Details of the ANOVA results for measured responses are also presented in **Table 2**. In the end, the emulsifier factor significantly affected the response of PS, ZP, and spreadability of TEO-loaded Negs. At the same time, gel former affects the AT and pH.

2.4. Optimized TEO-loaded Negs

The formulation was optimized with Design-Expert®, version 13 software. The optimized Negs were selected based on the minimum PS and PDI; maximum ZP; value in pH range, spreadability, and AT (**Table 3**). Variables composition for optimized Negs is span-80/tween-80 of 8.68% and Carbopol® 980 of 1.18% with a desirability value of 0.801. The formulation with the maximum desirability value is the optimal formulation generated from the optimization phase of the program (60) —the optimization value formed as indicated by the desirability value close to one.

The desirability value range is 0-1. **Figure 2** describes the optimization results in the form of a 2D contour. *Contour* is a two-dimensional response image that was presented using a predictive model for PS, PDI, ZP, pH, spreadability, and AT response values. The contour graph shows the desirability value of 0.801, which is the closest value to 1 compared to the other points. **Figure 3** shows the projection in the form of a 3D surface; the low area shows low desirability, while the high area shows high desirability and is getting closer to 1. At this stage, the software predicts the response values shown in **Table 4**. Three confirmation runs need to be performed to validate optimization (61). The optimization model and estimates are validated by the observed optimized Negs, which show an acceptable variation from the predicted values (**Table 4**). We tested the optimized Negs' physical properties for further investigation, such as organoleptic, homogeneity, freeze-thaw, viscosity, and flow properties.

2.5. Evaluation of TEO-loaded Negs

Nanoemulsion systems can cover oily drugs' bitter or unpleasant taste (62). The organoleptic results of TEO-loaded Negs have a less distinctive turmeric odor, which is white and semisolid (**Table 5**). That is due

to the drug entrainment of oil with the oil phase effectively preventing evaporation and masking its specific odor (63). The homogeneity test results of the optimum TEO-loaded Negs formulation showed a homogeneous preparation, as evidenced by the absence of coarse grains. This homogeneity was correlated with the optimal formulation of PS and PDI of 182.3 ± 5.5 and 0.242 ± 0.003, respectively. The low PDI indicates uniformity or homogeneous dispersion of globules Negs (64). In addition, the small size of the globule (±200 nm) is not included in the coarse dispersion (41).

The thermodynamic stability test of the system was carried out using a freeze-thaw cycle to identify the presence of metastable Negs in the optimal formulation. It aims to see the separation of the water and oil phases due to the influence of extreme temperatures (65). The thermodynamic stability of any system is determined by the change in free energy between the system and its surroundings (66). The test results on the optimum formula for six cycles showed promising results; namely, there was no separation. This stability was correlated with the ZP of the optimal formulation of 57.23 ± 2.91 mV. The surface charge's magnitude was directly related to the stability of any Negs. It is evidenced by the high repulsive force between the Negs globules preventing coalescence, which was characterized by the absence of phase separation (67). Similar results were found in the Negs study containing thymoquinone, which had ZPs between −26.7 and −30.6 mV (66).

The pH conditions indirectly affect the viscosity indicated by Negs because they influence the swelling ability of Carbopol® 980. This excipient is a gel former and a thickener (52). It plays an essential role in the viscosity of Negs. Carbopol is dispersed in water to form an acidic colloidal solution with a low viscosity. Neutralizing with triethanolamine increases Negs' viscosity because a stable water-soluble gel was formed (50). Viscosity was carried out with #7 spindle (Brookfield digital RV DV-E) at 50 rpm of 32240 ± 2257.7 cP, indicating significantly high viscosity on Negs with pH 4.5. The magnitude of the viscosity is correlated with AT and spreadability. Viscosity is inversely proportional to spreadability (52). In contrast, the AT is directly proportional to the viscosity. A high-viscosity system will form stronger interfacial interactions and increase intermolecular interactions in the polymer network, increasing cohesion, adhesion strength, and AT (54).

Determining the rheology of a semisolid preparation is essential for controlling the consistency required to ensure the performance and formulation durability and to describe the mechanical (flow properties) system (68). The rheological study was conducted in the shear rate range of 6.81-40.86 s^{-1} at 25°C. The consistency index equals the apparent viscosity at a shear rate of 1 s^{-1}. The consistency index measured on TEO-loaded Negs was 155.67 cP and n = 0.22. The flow index measures the system's deviation from Newtonian behavior (n = 1). A value of n > 1 indicates dilatation or shear thickening flow, and n < 1 indicates pseudoplastic or shear thinning. The flow index typically lowers the thicker the base. Negs produce a 0.22 flow index, which implies pseudoplastic flow behavior. A colloidal network structure aligned with the shear direction and decreases viscosity as the shear rate increases have led to this pseudo-plasticity. The developed system will require a specific force to discharge (69). The results of the flow properties test showed that the optimal formula made was a pseudoplastic thixotropic flow type (**Figure 4**). *Thixotropic* is a flow property expected in pharmaceutical preparations because it has high consistency in the container but can be poured and dispersed easily (70).

4. CONCLUSION

Based on the results of the CCD-RSM analysis, the optimum span-80/tween-80 as an emulsifier is 8.68%, and Carbopol® 980 as a gel former was 1.18%. The resulting response is a PS of 182.3 nm, PDI 0.242, ZP 57.23 mV, pH 4.51, AT 6.45 seconds, and spreadability of 6 cm. Optimized formulation viscosity is 32240 cP with pseudoplastic thixotropic flow properties. Thus, the developed TEO-Negs can be a potential delivery system and a promising suitable approach for topical preparations.

5. MATERIALS AND METHODS

5.1. Materials

TEO (Curcuma longa) was purchased from Darjeeling Sembrani Aroma (Indonesia), sorbilene O E/P from Lamberti (Italy), span-80 from Croda (Singapura), propylene glycol from Dow Chemical Pacific (Singapura), Carbopol® 980 NF from Lubrizol AM (Cleveland), nipagin M from Clariant Produkte (Deutschland), propylparaben from Alpha Chemika (India), triethanolamine from Dow Chemical Pacific (Switzerland), and 1.1-diphenyl-2-picrylhydrazyl (DPPH) from Smart-Lab (Indonesia).

5.2. Methods

5.2.1. Identification of TEO

Organoleptic tests include observing form, color, and odor. Phenolic identification was carried out by adding one drop of 5% $FeCl_3$ to a 1 ml sample. Dark green to black colors indicate phenolic compounds' presence (71). Terpenoid testing was performed by adding Lieberman-Burchard reagent containing anhydrous acetic acid and concentrated sulfuric acid (3:1) into a 1 ml sample. Brownish or violet ring form indicates the presence of terpenoids (72). Spectrophotometry was used to determine antioxidant activity with the DPPH method (5). The calibration curve for the DPPH concentration against absorbance was made at a maximum wavelength of 516 nm. The absorbance was measured in a mixture of sample solution and DPPH with a particular concentration after 30-min of incubation in a dark room. IC_{50} was calculated from the inhibition percentage and absorbance.

5.2.2. Preparation of TEO-loaded Negs

TEO-loaded Negs were produced using a high-energy method, which used a mechanical device to produce a highly disruptive force to break up the water and oil phases to obtain nano-sized globules (18). The oil phase (M1) was prepared by mixing span-80 with 5% turmeric oil using a magnetic stirrer (WiseStir Wisd) at 1,500 rpm for 20 min. A total of 0.18% methylparaben and 0.02% propylparaben dissolved in 15% propylene glycol (M2). Then, the distilled water was stirred with tween-80, and M2 was added gradually until homogeneous at 1,500 rpm for 20 min (M3). M1 was stirred with M3 until homogeneous at 1,500 rpm for 40 min to form a clear and transparent nanoemulsion, then let left for 24 hours. The gel base was prepared by mixing Carbopol® 980 NF with distilled water at 70°C and left for 24 hours. Then, gradually add 1% triethanolamine to form a gel mass. Nanoemulsion was added slowly into the gel base while homogenized using a homogenizer (AEG) at 2,000 rpm for 10 min.

5.2.3. Experimental Design

This study selects the CCD-RSM method to develop the TEO-loaded Negs formulation. For preliminary screening on PS, PDI, ZP, pH, AT, and spreadability, a 2-factor CCD-RSM at two levels (high and low) was used. Based on previous experiments and study literature, high and low variables were determined. The CCD of the statistical package Design-Expert® version 13 software (Stat-Ease Inc., Minneapolis, MN) was used to examine the influence of the specified independent variable on the response variable to obtain the optimal formulation for TEO-loaded Negs. CCD planned 14 experiments were done under controlled circumstances (**Table 3**). The independent variables were span-80/tween-80 (X_1) and Carbopol® 980 concentration (X_2). The observed response of the dependent variables was PS (Y_1), PDI (Y_2), ZP (Y_3), pH (Y_4), spreadability (Y_5), and AT (Y_6).

5.2.4. Determination of PS, PDI, and ZP of Negs

PS and PDI of Negs were assessed using the Delsa Max Pro Particle Size Analyzer LS 100Q (Beckman Coulter, USA) at 25°C utilizing the dynamic light scattering (DLS) method or photo correlation spectroscopy. For analysis, 1 ml of samples was dispersed in 9 ml aqua pro injection. Into the cuvette, 1 ml of suspension and 5 ml of aqua pro injection were added as a diluent, and the results were read on the instrument. All measurements were made at a scattering angle of 90°. ZP was determined by particle size analyzer through mobility and conductivity measurements. The temperature was set to 25°C, and the mean electric field was set to 16 V/cm (73). The final result is the mean of each sample's three repeated measurements.

5.2.5. Determination of pH, Spreadability, and AT

pH was measured at a temperature of 25°C using a pH meter that had been previously calibrated with buffer solutions of pH 4 and 7. The calibration step was completed when the pH value indicated on the screen matched the correct pH value and was stable. Afterward, the electrode was dipped in Negs and recorded the value shown on the screen (74). Spreadability was measured by adding 0.5 g of Negs in the center of a glass covered with another glass. The preparation diameter was measured longitudinally and transversely; for

every minute added, 50 g was to a total weight of 150 g (75). Adhesion time determine by the single-lap shear test method (54). A-0.5 g of Negs was placed on a slide, then covered with another slide, and given a load of 1 kg for 3 min. The glass object was mounted on the test apparatus, and 80 g of the load was released until both glass objects were released, and the time was recorded (75).

5.2.6. Organoleptic Observation and Homogeneity Test

Negs were placed in a glass object and directly observed for color, smell, and shape (76). A-0.1 g Negs were spread over the slide, and homogeneity was observed. Test preparation is declared homogeneous if no coarse grains exist (77).

5.2.7. Viscosity and Rheological Flow

Viscosity and rheology were determined with a Brookfield RV DV-E Viscometer with appropriate spindle and speed. A-500 ml of Negs were put into a beaker glass; the spindle was installed, and the measured value was recorded as viscosity Negs. In this study, the spindle used spindle no. 7. Flow properties were determined by measuring the viscosity using a right spindle from low to high rotational speed and vice versa (74). Flow index and consistency index are determined from the power law equation:

$$\tau = K r^n$$

where τ is the shear stress, *K* is the consistency index, *r* is the shear rate, and *n* is the flow index.
Taking logs on both sides,

$$\log \tau = \log K + n \log r$$

So, from the log, shear stress Vs. Log shear rate plot, the plot slope was used as the flow index and the antilog of the Y-intercept as the consistency index (69).

5.2.8. Freeze-thaw Test

Negs were stored at 4 ± 2 °C, then transferred to 40 ± 2 °C for 48 hours (1-cycle), then repeated for 6-cycles. Phase separation was observed in each cycle (74).

5.2.9. Statistical Analysis

The Design-Expert® version 13 software was used to conduct the statistical study (Stat-Ease Inc., Minneapolis, MN). Analysis was done at a sig—$p < 0.05$ and $p < 0.01$ after three times of each measurement.

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REFERENCES

[1] Rohman A, Widodo H, Lukitaningsih E, Rafi M, Nurrulhidayah AF, Windarsih A. Review on *in-vitro* antioxidant activities of Curcuma species commonly used as. Food Res. 2020;4(2):286–93. [CrossRef]

[2] Liju VB, Jeena K, Kuttan R. An evaluation of antioxidant, anti-inflammatory, and antinociceptive activities of essential oil from Curcuma longa. L. Indian J Pharmacol. 2011;43(5):526–31. [CrossRef]

[3] Devkota L, Rajbhandari M. Composition of essential oils in turmeric rhizome. Nepal J Sci Technol. 2016;16(1):87–94. [CrossRef]

[4] Guimarãesa AF, Vinhas ACA, Gomes, Angélica Ferraz Souza LH, Krepsky PB. Essential oil of Curcuma longa L. rhizomes chemical composition, yield variation and stability. Quim Nov. 2020;43(7):909–13. [CrossRef]

[5] Islamadina R, Can A, Rohman A. Chemometrics application for grouping and determinating volatile compound which related to antioxidant activity of turmeric essential oil (Curcuma longa L). J Food Pharm Sci. 2020;8(2):1.

[CrossRef]

[6] Dosoky NS, Setzer WN. Chemical composition and biological activities of essential oils of curcuma species. Nutrients. 2018;10(9):10–7. [CrossRef]

[7] Jankasem M, Wuthi-udomlert M, Gritsanapan W. Antidermatophytic properties of ar -turmerone, turmeric oil, and curcuma longa preparations. ISRN Dermatol. 2013;2013(April 2009):1–3. [CrossRef]

[8] Mishra R, Gupta AK, Kumar A, Lal RK, Saikia D, Chanotiya CS. Genetic diversity, essential oil composition, and *in-vitro* antioxidant and antimicrobial activity of Curcuma longa L. germplasm collections. J Appl Res Med Aromat Plants. 2018;10(December 2017):75–84. [CrossRef]

[9] Sharifi-Rad J, Rayess Y El, Rizk AA, Sadaka C, Zgheib R, Zam W, et al. Turmeric and ıts major compound curcumin on health: bioactive effects and safety profiles for food, pharmaceutical, biotechnological and medicinal applications. Front Pharmacol. 2020;11(September):1–23. [CrossRef]

[10] Vera JMV, Dueñas-Rivadeneira AA, Díaz JMR, Radice M. Phytochemical characterization of the ethanolic extract, antioxidant activity, phenolic content and toxicity of the essential oil of Curcuma longa L. Rev la Fac Agron. 2022;39(1). [CrossRef]

[11] Ali MS, Alam MS, Imam F, Siddiqui MR. Topical nanoemulsion of turmeric oil for psoriasis: Characterization, *ex-vivo* and *in-vivo* assessment. Int J Drug Deliv. 2012;4(2):184–97. [CrossRef]

[12] Meng F-C, Zhou Y-Q, Ren D, Wang R, Wang C, Lin L-G, et al. Turmeric: A review of ıts chemical composition, quality control, bioactivity, and pharmaceutical application. Natural and Artificial Flavoring Agents and Food Dyes. Elsevier Inc.; 2018. 299–350. [CrossRef]

[13] Ibáñez MD, Blázquez MA. Curcuma longa L. rhizome essential oil from extraction to its agri-food applications. a review. Plants. 2021;10(1):1–31. [CrossRef]

[14] Yan W, Bowen WD, Hopson R, Mathew AE, Jacob JN. Biological studies of turmeric oil, part 2: Isolation and characterization of turmeric oil components; cytotoxic activity against pancreatic cancer cells. Nat Prod Commun. 2013;8(6):811–4. [CrossRef]

[15] Sarojini S, Sundar K, Anjali CH, Ravindran A. Formation and stability of oil-in-water nano emulsion containing turmeric oil. Biotechnol An Indian J. 2014;9(4):171–5.

[16] Vishwakarma AK, Maurya OP, Nimisha, Srivastava D. Formulation and evaluation of transdermal patch containing turmeric oil. Int J Pharm Pharm Sci. 2012;4(SUPPL. 5):358–61.

[17] Kirtane AR, Karavasili C, Wahane A, Freitas D, Collins J, Tamang S, et al. Development of oil-based gels as versatile drug delivery systems for pediatric applications. Sci Adv. 2022;8(21):1–19. [CrossRef]

[18] Sengupta P, Chatterjee B. Potential and future scope of nanoemulgel formulation for topical delivery of lipophilic drugs. Int J Pharm. 2017;526(1–2):353–65. [CrossRef]

[19] Eid AM, El-Enshasy HA, Aziz R, Elmarzugi NA. Preparation, characterization and anti-inflammatory activity of Swietenia macrophylla nanoemulgel. J Nanomedicine Nanotechnol. 2014;5(2). [CrossRef]

[20] Khurana S, Jain NK, Bedi PMS. Nanoemulsion based gel for transdermal delivery of meloxicam: Physico-chemical, mechanistic investigation. Life Sci. 2013;92(6–7):383–92. [CrossRef]

[21] Baibhav J, Gurpreet S, Rana A, Seema S, Vikas S. Emulgel: A comprehensive review on the recent advances in topical drug delivery. Int Res J Pharm. 2011;2(11):66–70.

[22] Sultana N, Akhtar J, Badruddeen, Khan MI, Ahmad U, Arif M, et al. Drug Development Life Cycle. In: Intech. IntechOpen; 2022.

[23] Eswaraiah S, Swetha K, Lohita M, Preethi PJ, Priyanka B, Reddy KK. Emulgel : Review on novel approach to topical drug delivery. Asian J Pharm Res. 2014;4(1):4–11.

[24] Dhawan B, Aggarwal G, Harikumar S. Enhanced transdermal permeability of piroxicam through novel nanoemulgel formulation. Int J Pharm Investig. 2014;4(2):65–76. [CrossRef]

[25] Dev A, Chodankar R, Shelke O. Emulgels : A novel topical drug delivery system. Pharm Biol Eval. 2015;2(4):64–75.

[26] ICH. ICH Guideline Q9 on Quality Risk Management. Vol. 44, Regulatory ICH. 2005. 1–20 p.

[27] Abdallah MH, Elsewedy HS, Abulila AS, Almansour K, Unissa R, Elghamry HA, et al. Quality by design for optimizing a novel liposomal jojoba oil-based emulgel to ameliorate the anti-inflammatory effect of brucine. Gels. 2021;7(219):1–18. [CrossRef]

[28] Sindi AM, Hosny KM, Alharbi WS. Lyophilized composite loaded with meloxicam-peppermint oil nanoemulsion for periodontal pain. Polymers (Basel). 2021;13(14). [CrossRef]

[29] Yalçinkaya SE, Yildiz N, Ak MS, Çalimli A. Preparation of polystyrene/montmorillonite nanocomposites: Optimization by response surface methodology (RSM). Turkish J Chem. 2010;34(4):581–92. [CrossRef]

[30] Klangmanee K, Athipornchai A. A rapid phytochemical screening of the effective phenolic antioxidant agents using $FeCl_3$ reagent. J Pharm Sci Res. 2019;11(10):3475–80.

[31] Orellana-Paucar AM, Machado-Orellana MG. Pharmacological profile, bioactivities, and safety of turmeric oil. Molecules. 2022;27(16):1–16. [CrossRef]

[32] Kusmardiyani S, Novita G, Fidrianny I. Antioxidant activities from various extracts of different parts of kelakai (Stenochlaena palustris) grown in central Kalimantan - Indonesia. Asian J Pharm Clin Res. 2016;9:215–9. [CrossRef]

[33] Avanco GB, Ferreira FD, Bonfim NS, Souza Rodrigues dos Santos PA, Peralta R, Brugnari T, et al. Curcuma longa L. essential oil composition, antioxidant effect, and effect on Fusarium verticillioides and fumonisin production. Food Control. 2017;73:806–13. [CrossRef]

[34] Priya R, Prathapan A, Raghu KG, Menon AN. Chemical composition and *in-vitro* antioxidative potential of essential oil isolated from Curcuma longa L. leaves. Asian Pac J Trop Biomed. 2012;2(2 SUPPL.):S695–9. [CrossRef]

[35] Pino JA, Fon-Fay FM, Pérez JC, Falco AS, Hernández I, Rodeiro I, et al. Chemical composition and biological activities of essential oil from turmeric (Curcuma longa L.) rhizomes grown in Amazonian Ecuador. CENIC Ciene Quím. 2018;49:1–8.

[36] Setya S, Madaan T, Tariq M, Razdan BK, Talegaonkar S. Appraisal of transdermal water-in-oil nanoemulgel of selegiline HCI for the effective management of parkinson's disease: Pharmacodynamic, pharmacokinetic, and biochemical ınvestigations. AAPS PharmSciTech. 2018;19(2):573–89. [CrossRef]

[37] Chang WC, Hu YT, Huang Q, Hsieh SC, Ting Y. Development of a topical applied functional food formulation: Adlay bran oil nanoemulgel. Lwt. 2020;117(February 2019):108619. [CrossRef]

[38] Ye Q, Li J, Li T, Ruan J, Wang H, Wang F, et al. Development and evaluation of puerarin-loaded controlled release nanostructured lipid carries by central composite design. Drug Dev Ind Pharm. 2021;47(1):113–25. [CrossRef]

[39] Shehata TM, Elnahas HM, Elsewedy HS. Development, characterization and optimization of the anti-ınflammatory ınfluence of meloxicam loaded into a eucalyptus oil-based nanoemulgel. Gels. 2022;8(5). [CrossRef]

[40] How CW, Rasedee A, Abbasalipourkabir R. Characterization and cytotoxicity of nanostructured lipid carriers formulated with olive oil, hydrogenated palm oil, and polysorbate 80. IEEE Trans Nanobioscience. 2013;12(2):72–8. [CrossRef]

[41] Chuacharoen T, Prasongsuk S, Sabliov CM. Effect of surfactant concentrations on physicochemical properties and functionality of curcumin nanoemulsions under conditions relevant to commercial utilization. Molecules. 2019;24(15):1–12. [CrossRef]

[42] Sarheed O, Dibi M, Ramesh KVRNS. Studies on the effect of oil and surfactant on the formation of alginate-based O/W lidocaine nanocarriers using nanoemulsion template. Pharmaceutics. 2020;12(12):1–21. [CrossRef]

[43] McClements DJ. Food Emulsions: Principles, Practices, and Techniques. Third Edit. New York: CRC Press; 2016. 719.

[44] Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, et al. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics. 2018;10(2):1–17. [CrossRef]

[45] Choi AJ, Kim CJ, Cho YJ, Hwang JK, Kim CT. Characterization of capsaicin-loaded nanoemulsions stabilized with alginate and chitosan by self-assembly. Food Bioprocess Technol. 2011;4:1119–26. [CrossRef]

[46] Sinha P, Srivastava S, Mishra N, Singh DK, Luqman S, Chanda D, et al. Development, optimization, and characterization of a novel tea tree oil nanogel using response surface methodology. Drug Dev Ind Pharm. 2016;42(9):1434–45. [CrossRef]

[47] Honary S, Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems - a review (part 1). Trop J Pharm Res. 2013;12(2):255–64. [CrossRef]

[48] Gurpreet K, Singh SK. Review of nanoemulsion formulation and characterization techniques. Indian J Pharm Sci. 2018;80(5):781–9. [CrossRef]

[49] Dias IP, Barbieri SF, Fetzer DEL, Corazza ML, Silveira JLM. Effects of pressurized hot water extraction on the yield

and chemical characterization of pectins from Campomanesia xanthocarpa Berg fruits. International journal of biological macromolecules. 2020;146:431–43. [CrossRef]

[50] Kulkarni VS, Shaw C. Use of polymers and thickeners in semisolid and liquid formulations. Essent Chem Formul Semisolid Liq Dosages. 2016;43–69. [CrossRef]

[51] Corporation TL. Product Specification Carbopol 980 NF Polymer. Cleveland; 2017.

[52] Nurman S, Yulia R, Irmayanti, Noor E, Sunarti TC. The optimization of gel preparations using the active compounds of arabica coffee ground nanoparticles. Sci Pharm. 2019;87(4). [CrossRef]

[53] Kurniawan MF, Sugihartini N, Yuwono T. Iritation properties and evaluation of physical properties emulgel of clove oil using simplex lattice design methods. Farmasains J Ilm Ilmu Kefarmasian. 2018;5(1):1–8. [CrossRef]

[54] Sun Z, Li Z, Qu K, Zhang Z, Niu Y, Xu W, et al. A review on recent advances in gel adhesion and their potential applications. J Mol Liq. 2021;325:115254. [CrossRef]

[55] Gupta NV, Natasha S, Getyala A, Bhat RS. Bioadhesive vaginal tablets containing spray dried microspheres loaded with clotrimazole for treatment of vaginal Candidiasis. Acta Pharm. 2013;63(3):359–72. [CrossRef]

[56] Abramowitch S, Easley D. Introduction to Classical Mechanics. Biomechanics of the Female Pelvic Floor. Elsevier Inc.; 2016. 89–107 p. [CrossRef]

[57] Sari DK, Sugihartini N, Yuwono T. Irritation test and physical properties evaluation of essential oils clove (Syzigium aromaticum) in emulgel. Pharmaciana. 2015;5(2):115–20. [CrossRef]

[58] Cevher E, Sensoy D, Taha MAM, Araman A. Effect of thiolated polymers to textural and mucoadhesive properties of vaginal gel formulations prepared with polycarbophil and chitosan. AAPS PharmSciTech. 2008;9(3):953–65. [CrossRef]

[59] Pande V, Patel S, Patil V, Sonawane R. Design expert assisted formulation of topical bioadhesive gel of sertaconazole nitrate. Adv Pharm Bull. 2014;4(2):121–30. [CrossRef]

[60] Badwaik LS, Prasad K, Deka SC. Optimization of extraction conditions by response surface methodology for preparing partially defatted peanut. Int Food Res J. 2012;19(1):341–6.

[61] Mohtashamian S, Boddohi S, Hosseinkhani S. Preparation and optimization of self-assembled chondroitin sulfate-nisin nanogel based on quality by design concept. Int J Biol Macromol. 2018;107:2730–9. [CrossRef]

[62] Jaiswal M, Dudhe R, Sharma PK. Nanoemulsion: An advanced mode of drug delivery system. 3 Biotech. 2015;5(2):123–7. [CrossRef]

[63] Wang X, Gu Y, He Y, Sang L, Dai Y, Wang D. Preparation and optimization formulation of zedoary turmeric oil nanoemulsion based thermo-sensitive gel for improved application in ophthalmology. J Drug Deliv Sci Technol. 2021;65(June):102682. [CrossRef]

[64] Begur M, Kumar Pai V, Gowda D V, Srivastava A, Raghundan H V, Shinde CG, et al. Enhanced permeability of Cyclosporine from a transdermally applied nanoemulgel. Der Pharm Sin. 2015;6(2):69–79.

[65] Yuliani SH, Hartini M, Pudyastuti B, Istyastono EP. Comparison of physical stability properties of pomegranate seed oil nanoemulsion dosage forms with long-chain triglyceride and medium-chain triglyceride as the oil phase. Tradit Med J. 2016;21(2):93–8. [CrossRef]

[66] Algahtani MS, Ahmad MZ, Shaikh IA, Abdel-Wahab BA, Nourein IH, Ahmad J. Thymoquinone loaded topical nanoemulgel for wound healing: Formulation design and *in-vivo* evaluation. Molecules. 2021;26(13):1–16. [CrossRef]

[67] Ahmad J, Mir SR, Kohli K, Amin S. Effect of oil and co-surfactant on the formation of Solutol HS 15 based colloidal drug carrier by Box-Behnken statistical design. Colloids Surfaces A Physicochem Eng Asp. 2014;453(1):68–77. [CrossRef]

[68] Eid AM, Hawash M. Biological evaluation of Safrole oil and Safrole oil Nanoemulgel as antioxidant, antidiabetic, antibacterial, antifungal and anticancer. BMC Complement Med Ther. 2021;21(1):1–12. [CrossRef]

[69] Arora R, Aggarwal G, Harikumar SL, Kaur K. Nanoemulsion based hydrogel for enhanced transdermal delivery of ketoprofen. Adv Pharm. 2014;2014:1–12. [CrossRef]

[70] Sinko PJ, Singh Y, editors. Martin's physical pharmacy and pharmaceutical sciences 6th edition. Sixth edit. Philadelphia: Wolter Kluwer Health; 2011.

[71] Rahimah SB, Djunaedi DD, Soeroto AY, Bisri T. The phytochemical screening, total phenolic contents and antioxidant activities *in-vitro* of white oyster mushroom (Pleurotus ostreatus) preparations. Open Access Maced J

Med Sci. 2019;7(15):2404–12. [CrossRef]

[72] Farabi K, Harneti D, Darwati, Mayanti T, Nurlelasari, Maharani R, et al. Dammarane-type triterpenoid from the stem bark of aglaia elliptica (meliaceae) and ıts cytotoxic activities. Molecules. 2022;27(19):1–11. [CrossRef]

[73] Elsheikh MA, Elnaggar YSR, Hamdy DA, Abdallah OY. Novel cremochylomicrons for improved oral bioavailability of the antineoplastic phytomedicine berberine chloride: Optimization and pharmacokinetics. Int J Pharm. 2018;535(1–2):316–24. [CrossRef]

[74] Nursal FK, Nining, Rahmah AS. Formulation and development of grape seed oil (Vitis vinifera L) emulgel peel-off mask using gelling agent hydroxy propyl methyl cellulose (HPMC). IOP Conf Ser Earth Environ Sci. 2021;755(1). [CrossRef]

[75] Irianto IDK, Purwanto P, Mardan MT. Antibacterial activity and physical evaluation of Piper betle L. Decoction gel as an alternative treatment for mastitis. Maj Farm. 2020;16(2):202–10. [CrossRef]

[76] Elfiyani R, Amalia A, Pratama SY. Effect of using the combination of tween 80 and ethanol on the forming and physical stability of microemulsion of eucalyptus oil as antibacterial. J Young Pharm. 2017;9(2):230–3. [CrossRef]

[77] Yati K, Jufri M, Gozan M, Dwita LP. The effect of hidroxy propyl methyl cellulose (HPMC) concentration variation on physical stability of tobacco (Nicotiana tabaccum l.) extract gel and ıts activity against streptococcus mutans. Pharm Sci Res. 2018;5(3):133–41. [CrossRef]

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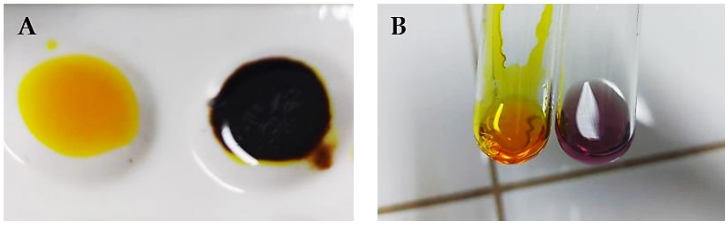

Figure 1. The result of qualitative test observation is a phenolic test with a positive result marked in a blackish green color (a) and a terpenoid test with a positive result marked with a purple color (b).

https://doi.org/10.12991/jrp.2019.00
J Res Pharm 2019; 23(6): 1-XX

Tabel 1. Evaluation results of independent variables and dependent variables with the CCD design for optimizing TEO-loaded Negs.

Std	X_1 (%)	X_2 (%)	Y_1 (nm)	Y_2	Y_3 (mV)	Y_4	Y_5 (cm)	Y_6 (s)
1	5.50	1.02	206.9 ± 0.7	0.571 ± 0.000	35.39 ± 1.50	5.45	5.55	5.65
2	2.32	1.15	457.0 ± 0.9	0.000 ± 0.000	13.90 ± 1.92	4.60	5.00	6.99
3	8.68	0.90	166.7 ± 0.4	0.378 ± 0.074	31.64 ± 0.53	6.35	6.20	4.23
4	2.32	0.90	411.0 ± 5.8	0.000 ± 0.000	30.67 ± 0.89	6.29	5.00	4.30
5	5.50	1.02	302.1 ± 3.6	0.571 ± 0.000	36.45 ± 0.45	5.46	5.60	5.63
6	5.50	1.02	263.0 ± 3.7	0.571 ± 0.000	18.49 ± 1.77	5.47	5.55	5.64
7	8.68	1.15	198.1 ± 1.2	0.242 ± 0.003	57.23 ± 2.91	4.65	6.15	6.95
8	5.50	1.02	246.9 ± 7.2	0.571 ± 0.000	26.60 ± 0.17	5.46	5.60	5.67
9	5.50	1.02	206.0 ± 2.3	0.571 ± 0.000	31.77 ± 1.01	5.46	5.60	5.65
10	10.00	1.02	160.8 ± 1.3	0.285 ± 0.024	46.72 ± 1.97	5.31	6.25	5.84
11	1.00	1.02	244.2 ± 4.3	0.571 ± 0.000	31.26 ± 0.57	5.78	4.90	6.88
12	5.50	1.02	238.1 ± 3.0	0.571 ± 0.000	30.78 ± 0.53	5.45	5.55	5.64
13	5.50	1.20	206.2 ± 1.6	0.571 ± 0.000	30.56 ± 0.78	4.57	5.50	7.08
14	5.50	0.85	240.5 ± 2.2	0.571 ± 0.000	20.12 ± 0.16	6.39	5.65	4.22

Y_1: Particle size (PS), Y_2: Polydispersity index (PDI), Y_3: Zeta potensial (ZP), Y_4: pH, Y_5: Spreadability, Y_6: Adhesion time (AT)

551 **Table 2.** Statistical analysis of PS (Y_1), PDI (Y_2), ZP (Y_3), pH (Y_4), spreadability (Y_5), and AT (Y_6) TEO-loaded Negs on
552 CCD.

Factors		**Y_1**	**Y_2**	**Y_3**	**Y_4**	**Y_5**	**Y_6**
X_1	Coefficient	-77.64	0.027	8.27	-0.069	0.53	-0.20
	p-value	0.0063**	0.7325	0.0080**	0.1430	<0.0001**	0.1406
X_2	Coefficient	3.61	-0.017	2.95	-0.75	-0.033	1.18
	p-value	0.8783	0.8289	0.2668	<0.0001**	0.1157	<0.0001**
X_1X_2	Coefficient		-0.034	10.59			
	p-value		0.7602	0.0136*			
X_1^2	Coefficient		-0.16				
	p-value		0.0818				
X_2^2	Coefficient		-0.086				
	p-value		0.3087				
Intercept	Coefficient	253.39	0.57	31.54	5.48	5.58	5.74
Degree of freedom		2	5	3	2	2	2
Sum of squares		48332.05	0.24	1065.33	4.48	2.28	11.49
Mean of squares		24166.02	0.047	355.11	2.24	1.14	5.74
F-value		5.69	1.02	7.06	145.08	386.43	46.35
p-value		0.0201	0.4645	0.0078	<0.0001	<0.0001	<0.0001
R-Squared		0.5084	0.3898	0.6794	0.9635	0.9860	0.8939

553 X_1: span-80/tween-80; X_2: Carbopol® 980
554 * p-value < 0.05
555 ** p-value < 0.01

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558 **Table 3.** The independent and dependent variables with levels and limits in CCD for TEO-loaded Negs development.

Variables	Code	Start point (-α)	Low level (-1)	Central level	High level (+1)	Start point (+α)	Units
Independent variables							
Span-80/tween-80	X_1	0.85	2.32	1.02	5.50	1.20	% w/w
Carbopol® 980	X_2	1	0.90	5.50	1.02	10	% w/w
Dependent variables		**Limits**					
Particle size (PZ)	Y_1	Minimum					nm
Polydispersity index (PDI)	Y_2	Minimum					
Zeta potensial (ZP)	Y_3	Maximum					mV
pH	Y_4	is in range					
Spreadability	Y_5	is in range					cm
Adhesion time (AT)	Y_6	is in range					s

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561 **Table 4.** Optimized TEO-loaded Negs' actual and predicted values for each response.

Responses	Predicted values	Actual value[a]	Error[b] (%)
Y1 (nm)	180.2	182.3 ± 5.5	1.165
Y2	0.250	0.242 ± 0.003	-3.200
Y3 (mV)	56.30	57.23 ± 2.91	1.652
Y4	4.50	4.51 ± 0.02	0.222
Y5 (cm)	6.07	6.0 ± 0.2	-1.153
Y6 (s)	6.98	6.45 ± 0.19	-7.593

562 a Data listed is the mean ± standart deviation, n = 3
563 b Error (%) = [(Actual value – Predicted value)/Predicted value] * 100%
564
565
566

567 **Table 5.** Additional evaluation on TEO-loaded Negs with an optimal formulation.

Evaluation	Result
Organoleptic	Color: White; Odor: typical turmeric
Homogeneity	Homogeneous
Freeze-Thaw	Cycles 0-6, no separation occurs
Viscosity[a]	32240 ± 2257,7 cP

568 a Data listed is the mean ± standart deviation, n = 3

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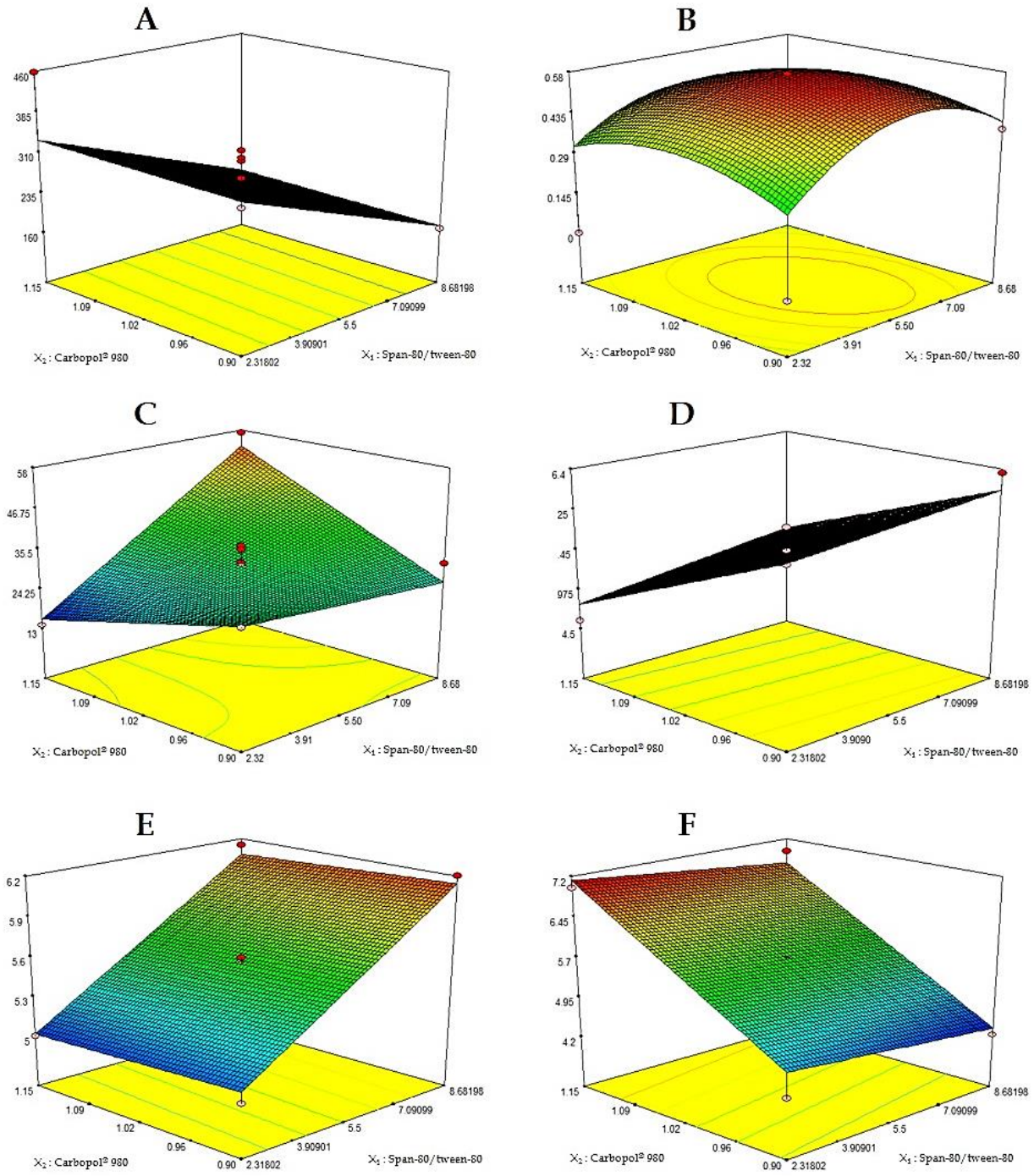

Figure 2. Effect of span-80/tween-80 and Carbopol® 980 concentration on PS (a) PDI (b) ZP (c) pH (d) spreadability (e) AT (f).

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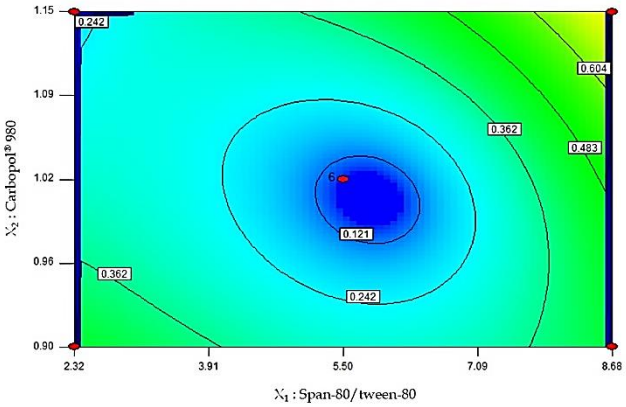

Figure 3. Contour plot desirability value of optimal formulation.

https://doi.org/10.12991/jrp.2019.00
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Research Article

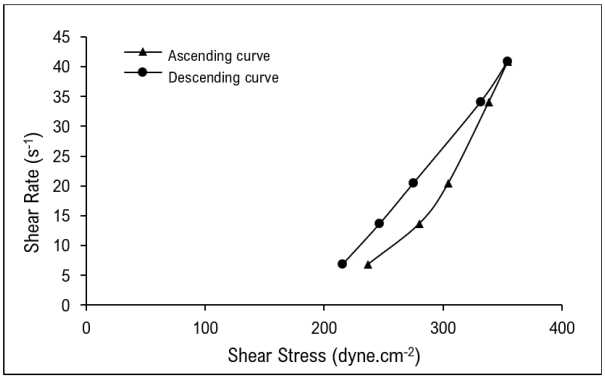

Figure 4. Optimal formulation flow properties.

RESPONSE TO REVIEWERS
OF THE JOURNAL OF RESEARCH IN PHARMACY

MANUSCRIPT ID: MPJ-10622

Reviewer: 1

Comments to the Author
A very nice and well-designed study about the the turmeric essential oil-loaded nanoemulgel. Authors have fully characterized the prepared formulations while their conclusions are supported by their results. However, the some comments must be taken into consideration for the improvement of the study. I'm sending my comments and recommendations.

1) Please carefully check the text for minor grammar and syntax errors.

Response:
We apologize for our poor writing. Improvements have been made with the help of the Language editing service.

2) Abbreviations should be used at the first occurrence of the word.
For example: Nanoemulgels (Negs)

Response:
We accepted your suggestion. We have regenerated the abbreviations section, italics, and usage of appropriate terms and units. The words "Turmeric Essential Oil (TEO)" and "Nanoemulgels (Negs)" are written at the beginning of the abstract and introduction (lines 16 and 33). After that, the following writing will be TEO and Negs only.

3) Attention should be paid to punctuation marks and spaces In vitro and in vivo should be written in italics.

Response:
We accepted your suggestion. The labelling "in-vitro" and "in-vivo" has been updated on lines 66, 364, 380, 436, and 522.

4) Why was the quantification of turmeric essential oil and drug content study of nanoemulgel not done?

Response:
This report demonstrates the physical and mechanical characterization of the Negs, while quantitative analysis is a limitation due to limited research funding. We hope to continue this in further research. Instead, we add qualitative TEO identification data with a discussion of the test results (lines 58-74) accompanied by a photo in Figure 1 (lines 541-543) with the test procedures carried out (lines 292-301).

2.1. Identification of TEO

TEO identification is carried out to ensure the quality of the active ingredients. The tests included organoleptic, phytochemical identity in the form of phenolics and terpenoids, and antioxidant activity by the DPPH method. The results of this study can be seen in **Figure 1**. Organoleptically, TEO is an orange liquid with a distinctive

turmeric aroma. Identification results in the phenolic test showed a black-green color (**Figure 1A**) due to the formation of a phenolic-Fe^{3+} complex (30). In the terpenoid test, reaction results show a purple color (**Figure 1B**) as a positive sign. Based on these results, it can be concluded that TEO contains phenolic and terpenoid compounds. Several studies reported that TEO has powerful antioxidant potential both *in-vitro* and *in-vivo* (2,8,31). Oxygenated monoterpenes and sesquiterpenes, such as ar-turmerone, α-turmerone, and β-turmerone, were included in the terpenoid compounds and reported to play a role in this activity. In addition, contained phenol was also reported to act as an excellent free radical scavenger because its reduction potential was lower than oxygen's (8). In our study, the antioxidant activity of TEO was expressed as an IC_{50} of 9.88 µm/ml, which was included in the powerful antioxidant category because of below 50 µm/ml (32). It differed from other studies' results, which showed an IC_{50} of 2274.02 µm/ml and above 1000 µm/ml (2,10). Meanwhile, similar findings stated IC_{50} values of 10.03 µm/ml, 3.227 µm/ml, and 14.5 µm/ml (33–35). The difference value was due to the use of TEO from different origin sources, regional conditions, and the extraction method used.

5.2.1. Identification of TEO

Organoleptic tests include observing form, color, and odor. Phenolic identification was carried out by adding one drop of 5% $FeCl_3$ to a 1 ml sample. Dark green to black colors indicate phenolic compounds' presence (71). Terpenoid testing was performed by adding Lieberman-Burchard reagent containing anhydrous acetic acid and concentrated sulfuric acid (3:1) into a 1 ml sample. Brownish or violet ring form indicates the presence of terpenoids (72). Spectrophotometry was used to determine antioxidant activity with the DPPH method (5). The calibration curve for the DPPH concentration against absorbance was made at a maximum wavelength of 516 nm. The absorbance was measured in a mixture of sample solution and DPPH with a particular concentration after 30-min of incubation in a dark room. IC_{50} was calculated from the inhibition percentage and absorbance.

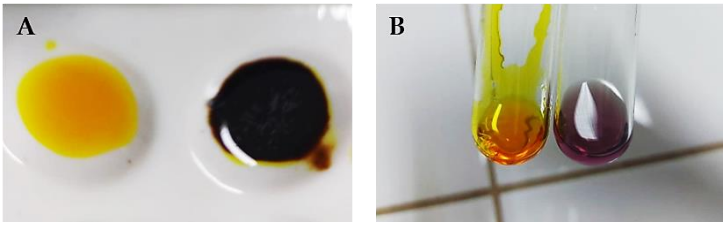

Figure 1. The result of qualitative test observation is a phenolic test with a positive result marked in a blackish green color (a) and a terpenoid test with a positive result marked with a purple color (b).

5) Why did the authors choose turmeric essential oil? How the authors analyzed the effectiveness of the oil.

Response:

This study was conducted based on the adequate TEO availability in our country, and several research reports have promising results on its pharmacology activity. As we said earlier, the qualitative identification of TEO showed that the TEO used was positive for containing phenolic and terpenoid compounds. Both groups of these compounds were reported to provide antioxidant activity. Accordingly, tests of antioxidant activity test on TEO were carried out and noted in the section "Identification of TEO" (lines 69-70 and 298-301).

6) Result and discussion part should be supported by the literatures.

Response:

We accepted your advice. Results and discussion enriched with sufficient literature. We added 32 additional references that support our discussion of the results we found in this study. The distribution of the supplementary literature is as follows:

1. *Identification of TEO: References 2, 8, 10*
2. *Preparation of TEO-loaded Negs: References 36, 37*
3. *Experimental Design by CCD-RSM: References 18, 19, 24, 27, 30, 31, 32, 33, 34, 35, 36, 37, 39, 50, 51, 54, 55, 56, 58, 59*
4. *Optimized TEO-loaded Negs: References 60, 61*
5. *Evaluation of TEO-loaded Negs: References 62, 63, 64, 66, 67, 68, 69*

7) In methods: Explain what TEA means.

Response:

The TEA in question is triethanolamine. We have changed the term "TEA" to "triethanolamine" (line 310).

8) Adhesiveness is the work required to overcome the attractive forces between the surface of the sample and the probe surface. The unit of adhesiveness cannot be seconds. The authors should explain the adhesion study in more detail. The results of adhesion should be supported by the literature.

Response:

We accepted your suggestion. We have regenerated the abbreviations section, italics, and usage of appropriate terms and units. An explanation regarding the adhesive properties of the preparation has been written on lines 196-218. The use of appropriate units has also been updated.

Topical dosage forms, such as Negs, adhere to the skin in two ways: they adhere directly to the rough substrate to form a "mechanical interlock" and to the substrate via interaction (54). Good adhesion to Negs supports a higher concentration gradient towards the skin and provides more drug penetration (18). Adhesive strength is directly related to the AT on the Negs as measured using the single-lap shear test method with slight modifications (55,56). The test was carried out by applying a shear load to the plates that flank the sample, which had been pre-loaded, and given a measured force; the time taken for the plates to separate was recorded as AT. In our study, the AT was from 4.22 s (STD#14) to 7.08 s (STD#13) in **Table 1**. They met the requirements based on the AT test results on a 14 formulation. An AT was carried out to see how long a Negs could be attached to the skin. The AT requirement is more than 4 seconds. The longer a Negs could be attached to the skin, it showed the better result, where it is expected that more active substances can be absorbed due to the time the Negs was in contact with the skin (57). Based on **Table 2**, the linear model was found to be significant in the AT response with an F-value model of 46.35 (p-value < 0.0001). The linear model equation suggested by the software can be seen as follows:

$$Y_6 = 5.74 - 0.20X_1 + 1.18X_2$$

The equation reveals that the AT was significantly (p-value < 0.0001) affected by the gel former (X_2) or, indirectly, the same adhesion strength. This finding is in agreement with the literature (58,59). A response surface plot (**Figure 2F**) may therefore be used to depict the combined influence of factors X_1 and X_2, which shows that Y_6 changes linearly with the sum of the two factors. However, the higher gradient in the response surface with Carbopol® 980 (X_2) – not span-80/tween-80 (X_1) – is the evidence from the comparative plot of the response surface. From this explanation, it can be concluded that the AT can be changed by selecting the right X_2 level. Details of the ANOVA results for measured responses are also presented in **Table 2**. In the end, the emulsifier factor significantly affected the response of PS, ZP, and spreadability of TEO-loaded Negs. At the same time, gel former affects the AT and pH.

Reviewer: 2

Comments to the Author

The content of the manuscript and the topic seems in line with journals target audience but there are some serious drawbacks need to be addressed:

1) The English of the manuscript needs to be revised by a native speaker or a professional editing service,

Response:

We apologize for our poor writing. Improvements have been made with the help of the Language editing service.

2) The application of CCD method is not clear nor understandable in the manuscript. In particular, how many center points were used, which ±α was preferred in the design etc?

Response:

We accepted your suggestion and added this table. Several details provide to clarify the CCD design.

Table 3. The independent and dependent variables with levels and limits in CCD for TEO-loaded Negs development.

Variables	Code	Start point (-α)	Low level (-1)	Central level	High level (+1)	Start point (+α)	Units
Independent variables							
Span-80/tween-80	X_1	0.85	2.32	1.02	5.50	1.20	% w/w
Carbopol® 980	X_2	1	0.90	5.50	1.02	10	% w/w
Dependent variables		**Limits**					
Particle size (PZ)	Y_1	Minimum					nm
Polydispersity index (PDI)	Y_2	Minimum					
Zeta potensial (ZP)	Y_3	Maximum					mV
pH	Y_4	is in range					
Spreadability	Y_5	is in range					cm
Adhesion time (AT)	Y_6	is in range					s

3) The most significant missing factor is validation of the CCD. After application of CCD-RSM, suggested optimal points are better presented in table3. Once the optimal points are clarified, three Negs formulation at this suggested optimal point are needed to be prepared experimentally along with all the characterization in order to show the correlation between predicted and experimentally found values.

Response:

We accepted your advice. We have added a discussion of the CCD validation process (lines 231-233).

4) The purpose of the Negs formulation is not clear in the manuscript which is another bottleneck of the manuscript. The purpose of this formulation needs to be addressed and further characterized along with the purpose.

Response:

We accepted your advice. In the introduction section, we improve the writing to clarify the choice of nanoemulgel in delivering TEO.

RESPONSE TO REVIEWERS
OF THE JOURNAL OF RESEARCH IN PHARMACY

MANUSCRIPT ID: MPJ-10622.REV-1

Reviewer: 1

Comments to the Author
The authors' response to my previous comments was presented as a separate file without referring to the comments and İt is impossible to follow the updated manuscript file since the changes made are not traceable. Under these circumstances the manuscript is not acceptable.

Response:
We apologize for the ignorance of the "response to reviewers" writing. We have improved the response writing. Hopefully, it can be accepted by reviewers. Thank you.

6. Bukti Konfirmasi Review dan Hasil Review Ketiga (6 Maret 2023)

Uhamka
UNIVERSITAS MUHAMMADIYAH PROF. DR. HAMKA

Nining <nining@uhamka.ac.id>

Journal of Research in Pharmacy : Revision request for your manuscript

1 message

Journal of Research in Pharmacy <editor@marmarapharmj.com> Mon, Mar 6, 2023 at 12:42 AM
To: nining@uhamka.ac.id

JRP
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Dear Other Nining Nining,

The Editors have now assessed the reviewer response and have concluded that, in its present form, the manuscript is not yet ready for publication in the Journal. Below you will find the relevant review comments and editorial notes. Acceptance of the paper is contingent upon effectively revising the work by taking these comments into serious consideration, and by responding or rebutting them in detail. We ask you to submit your revision through the online system within 30 days. The web site is located at https://www.jrespharm.com.
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Suggestions

1. Reviewer Comments
Updated manuscript covers my earlier points but there are some minors points:
- The whole manuscript needs to be checked in terms of non-scientific and non-specific statements such as "...non-friendly solvents to the formulation....". In particular what does "non-friendly" statement refer to??? Please be specific.
- The resolution of the Figure 2 and Figure3 should be enhanced. The number and the letters are pixelized.

Manuscript Information

Manuscript ID:	**MPJ-10622.REV-2**
Title in English:	Response surface methodology for optimization of turmeric essential oil-loaded nanoemulgel
Small Title in English:	No information entered
Authors:	Nining Nining[1], Anisa Amalia[1], Fatimatuz Zahrok[2]
Institutions:	[1]Universitas Muhammadiyah Prof. DR. HAMKA, Pharmaceutical Technology, East Jakarta, Indonesia [2]Universitas Muhammadiyah Prof. DR. HAMKA, Pharmacy, East Jakarta, Indonesia
Keywords in English:	Central composite design; nanoemulgels; response surface methodology; turmeric essential oil; topical delivery.
Manuscript Type:	Research article
Processing Status:	Minor Revision

Manuscript Files

File Name	File Size	Date Created	Category	Description
MPJ-10622-1-cover-letter-nining-et-al..pdf (../pdf-files/out/12282-MPJ-10622-1-cover-letter-nining-et-al..pdf)	31 KB	Sep 03, 2022	Cover letter	None
MPJ-10622-9-isi-jrp-checklist.pdf (../pdf-files/in/12282-MPJ-10622-9-isi-jrp-checklist.pdf)	3388 KB	Sep 03, 2022	Author Checklist Form	None
MPJ-10622-8-isi-jrp-copyright-form-integrated.pdf (../pdf-files/in/12282-MPJ-10622-8-isi-jrp-copyright-form-integrated.pdf)	327 KB	Sep 03, 2022	Copyright Transfer Form	None
MPJ-10622-4-manuscript-nining-et-al..pdf (../pdf-files/out/12282-MPJ-10622-4-manuscript-nining-et-al..pdf)	818 KB	Sep 03, 2022	Main Document	None
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MPJ-11427-7-figure-2..rev-1.jpg (../pdf-files/in/12282-MPJ-11427-7-figure-2..rev-1.jpg)	232 KB	Nov 23, 2022	Figure	None
MPJ-11427-9-figure-3..rev-1.jpg (../pdf-files/in/12282-MPJ-11427-9-figure-3..rev-1.jpg)	82 KB	Nov 23, 2022	Figure	None
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Response surface methodology for optimization of turmeric essential oil-loaded nanoemulgel

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ABSTRACT: Turmeric Essential Oil (TEO) has an antioxidant and anti-inflammatory activity to be formulated in a topical dosage form. Nanoemulgels (Negs) development, based on varying concentrations of emulsifiers and gel formers, affects their characteristics and stability. This study focuses on optimizing TEO-loaded Negs based on physical and mechanical characterization, which have promising topical applications. Negs were created using the high-energy approach and optimized using Response Surface Methodology (RSM) and the Central-Composite Design (CCD) for the optimization of span-80/tween-80 (X_1) and Carbopol® 980. (X_2). Observed variable responses were particle size (PS) (Y_1), polydispersity index (PDI) (Y_2), zeta potential (ZP) (Y_3), pH (Y4), spreadability (Y_5), and adhesion time (AT) (Y_6). Actual responses of Negs were compared with the CCD-RSM predictions to validate the model. In addition, other physical evaluations were observed, such as organoleptic observations, homogeneity, freeze-thaw tests, viscosity, and flow properties. Optimized TEO-loaded Negs were made with 8.68% span-80/tween-80 and 1.18% Carbopol® 980. The evaluation results showed the optimal TEO-loaded Negs on nano-metric size (182.3 ± 5.5 nm) with low PDI (0.242 ± 0.003), good ZP (-57.23 ± 2.91 mV), pH (4.51 ± 0.02), spreadability (6.0 ± 0.2 cm), and AT (6.45 ± 0.19 s). TEO-loaded Negs have an excellent appearance and did not run phase separation at extreme temperature storage with pseudoplastic thixotropy flow. Thus, the developed TEO-loaded Negs can be a potential delivery system and a promising suitable approach for topical preparations.

KEYWORDS: Central composite design; nanoemulgels; response surface methodology; turmeric essential oil; topical delivery.

1. INTRODUCTION

Turmeric is the dried rhizome of *Curcuma longa* L. (Zingiberaceae), which derives from Southeast Asia and is cultivated mainly in India, followed by Bangladesh, China, Thailand, Cambodia, Indonesia, Malaysia, and the Philippines (1). Steam distillation extracts turmeric essential oil (TEO) from the turmeric rhizome (2). Chemical constituents with the most significant proportion were oxygenated monoterpenes and sesquiterpenes, which include β-turmerone, α-turmerone, and ar-turmerone (3–5). The pharmacological activities of TEO have been reported in the form of antioxidants, anti-inflammatory, antinociceptive, antidermatophytic, antifungal, and antibacterial activities (2,6–9). These reducing power and radical scavenging abilities are associated with the high antioxidant potential of TEO (8,10). This pharmacological activity justifies its use in various applications, including cosmetics and phytomedicines (7,11,12). Like other essential oil, TEO has limited use due to its volatility, instability under certain conditions, lipophilicity, and low aqueous solubility (13,14). Many recent studies are oriented toward solving these limitations, so that efficacy of the essential oil lasts longer and increases.

Previously, TEO has been developed for cream as a conventional drug delivery system, patch, and nanoemulsions (7,11,15,16). Most water-based liquid or semisolid systems have limitations in delivering lipophilic drugs (17). Nanoemulsions are an established alternative for delivering lipophilic drugs by increasing topical absorption (18). The main advantage of topically administered nanoemulsions is the ability to increase penetration and permeation of drugs through the skin without adding non-physical enhancers and a large number of surfactants to the formulation, which can cause skin irritation, especially with long-term usage (18,19). However, this system has problems with low viscosity due to poor

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spreadability and skin retention (20). Nanoemulgels (Negs), a combination of nanoemulsion and hydrogel, were made to improve the characteristics (18,19,21).

Negs consist of a hydrogel system and an emulsion with nano-sized globules. An emulsifier in the form of surfactants and co-surfactants stabilizes the emulsion, which serves as a drug delivery platform (18). Surfactants reduce the interfacial surface tension of immiscible liquids and change the entropy of the dispersion, thereby stabilizing a thermodynamically unstable system; co-surfactants are combined with surfactants in the emulsification process by disrupting the surface layer (22). The emulsifier plays a role in the emulsification process to increase stability when the product is kept for an extended time. On the other side, gels are made from polymers, a gel former, that expand after absorbing a liquid (23). Gel former increase the viscosity of the formulation and can react with emulsifiers to change the thickness (24). In addition, topical Negs can increase patient compliance because of their non-irritating, non-greasy characteristics and improved drug release (25). However, the available methods for manufacturing Negs exhibit various limitations, which directly or indirectly affect the quality of the Negs formulations.

Currently, the principle of quality by design is adopted to ensure the quality of drugs, their safety, and efficacy (26). The quality by design (QbD) trend is used to develop, optimize, and investigate the interaction between particular variables and their related responses to achieve the optimal formulation (27). Our study used Central Composite Design (CCD) on Response Surface Methodology (RSM) to select optimal formulations and predict models that rely on statistical analysis (ANOVA) and exact equations (28,29). In this aspect, we intended to develop a TEO-loaded Negs that would be optimized using a complete 2^2-factorial design and determine the independent factors' precise influence on the investigated dependent variables. The choice and procedure factors were span-80/tween-80 (X_1) and Carbopol® 980 (X_2). Response variables investigated were particle size (PS) (Y1), polydispersity index (PDI) (Y2), zeta potential (ZP) (Y3), pH (Y4), spreadability (Y5), and adhesion time AT) (Y6). This work is the first step in developing an optimized TEO preparation suitable for transdermal drug delivery for topical application.

2. RESULTS AND DISCUSSION

2.1. Identification of TEO

TEO identification is carried out to ensure the quality of the active ingredients. The tests included organoleptic, phytochemical identity in the form of phenolics and terpenoids, and antioxidant activity by the DPPH method. The results of this study can be seen in **Figure 1**. Organoleptically, TEO is an orange liquid with a distinctive turmeric aroma. Identification results in the phenolic test showed a black-green color (**Figure 1A**) due to the formation of a phenolic-Fe^{3+} complex (30). In the terpenoid test, reaction results show a purple color (**Figure 1B**) as a positive sign. Based on these results, it can be concluded that TEO contains phenolic and terpenoid compounds. Several studies reported that TEO has powerful antioxidant potential both *in-vitro* and *in-vivo* (2,8,31). Oxygenated monoterpenes and sesquiterpenes, such as ar-turmerone, α-turmerone, and β-turmerone, were included in the terpenoid compounds and reported to play a role in this activity. In addition, contained phenol was also reported to perform as an excellent free radical scavenger because its reduction potential was lower than oxygen's (8). In our study, the antioxidant activity of TEO was presented as an IC_{50} of 9.88 µm/ml, which was included in the powerful antioxidant category because of below 50 µm/ml (32). It differed from other studies' results, which showed an IC_{50} of 2274.02 µm/ml and above 1000 µm/ml (2,10). Meanwhile, similar findings stated IC_{50} values of 10.03 µm/ml, 3.227 µm/ml, and 14.5 µm/ml (33–35). The difference value was due to the use of TEO from different origin sources, regional conditions, and the extraction method used.

2.2. Preparation of TEO-loaded Negs

TEO-loaded Negs were produced using the high-energy method efficiently. A high-pressure homogenizer provides sufficient energy to increase the interfacial area and generate nano-size globules (36). An emulsion system with a nanodroplet size must have flow properties that allow it to pass through the homogenizer (37). Flow properties are inversely correlated with the amount of emulsifier and gel former added to the Negs formulation. In this study, the selected optimized formulation was the one that produces Negs with the smallest size, as shown by the limitations in **Table 3**. Therefore, the range of regulated emulsifiers and gel formers concentrations still provides flow properties that allow the flow to remain good using a high-energy homogenizer. In general, the preparation of Negs was carried out in 3-stages, namely,

nanoemulsions preparation, gel preparation, and Negs preparation. Negs were successfully produced in 14-formulation with variations of emulsifiers and gel former with compositions that can be seen in **Table 1**.

2.3. Experimental Design by CCD-RSM

2.3.1. Fitting the Model

TEO-loaded Negs were optimized based on CCD in the RSM. CCD was used to establish the optimal concentrations of emulsifier (X_1) and gel former (X_2) as the key parameters influencing the dependent response. Prediction of the factorial axial design and the possible curvature in the response could be obtained from the optimization process with an effective second-level design (38). Predicting the significant influence of the independent variable on the dependent variable is essential for generating TEO-loaded Negs. Based on the literature survey, two factors were chosen as independent variables, and six responses were chosen as the dependent variable with the most significant influence on Negs. The independent variables with their levels and the observed response variables are presented in **Table 1**.

2.3.2. Analysis of Design

Statistical data analysis must be carried out to predict and recognize the model. **Table 2** shows the statistical analysis of a quadratic model for PDI, a 2FI model for ZP, and linear models for PS, pH, AT, and spreadability. This table identifies factors with p-values less than a predefined threshold (0.01 and 0.05, with a 99% and 95% confidence level, respectively) as influential factors. Besides the significant p-value, a large F-value minimizes error in the model and lack of fit, preferably non-significant to fit the model data (27,39). Depending on the most significant R-squared value and the least residual predictive sum of squares value, the six responses demonstrated distinct models in their application. The chosen model had a non-statistically significant lack of fit, and model validation was confirmed by the residual plot test of the regression model, which was supported by supplemental information for all responses. Compared to the 2FI and simple linear models, the quadratic model represented the impacts of numerous variables, including individual factors, interactions, and the quadratic influence on the response.

2.3.3. Effect of Independent Variables on Dependent Variables

PS parameters are often used to characterize nanoparticles. Negs globule diameter means (Y_1) was adjusted from 160.8 nm (STD#10) to 457.0 nm (STD#2). Based on **Table 2**, the PS response indicates a significantly linear model with an F-value of 5.69 (p-value 0.0201 < 0.05). The suggested linear model equation can be seen as follows:

$$Y_1 = 253.39 - 77.64X_1 + 3.61X_2$$

The equation shows that the Negs PS is significantly (p-value < 0.01) affected by emulsifier concentration (X_1). The positive coefficient has a synergistic effect on the response. In contrast, the negative coefficient has an antagonistic effect which concludes the inverse relationship of the independent variable with a response (38,40). In addition, this factor has a more significant coefficient. It directly affects the PS, which means that increasing emulsifier concentration causes a decrease in the globule diameter PS in the TEO-loaded Negs. High emulsifier (above 5.5%) resulted in globules measuring below 200 nm, and low emulsifier (below 5.5%) produced globules above 200 nm. This fact is in line with other studies that increasing emulsifiers could reduce droplet Negs PS (41,42). The emulsifier reduced the interfacial surface tension between the water and oil phases, which decreased the free energy required to disrupt or break the globules and resulted in a smaller droplet diameter. It can also produce a protective cover over the globules, preventing them from coalescence. However, the emulsifier must absorb quickly enough around the droplet to form this protective layer (42,43).

Meanwhile, Carbopol® 980 had no significant effect on PS. The same findings were obtained from the globule size Negs from Carbopol® 934 and 940 as gel former (19). A response surface plot (**Figure 2A**) may therefore be used to represent the combined influence of variables X_1 and X_2, which shows that Y_1 changes linearly with the sum of the two variables. Nevertheless, the higher gradient in the response surface with span-80/tween-80 (X_1) – not Carbopol® 980 (X_2) – was evidenced from the comparative plot of the response surface. From this explanation, it can be concluded that the PS can be changed by selecting the right X_1 level.

In **Table 1**, Negs PDI (Y_2) varied from 0.000 (STD#2 and #4) to 0.571 (STD#1, #5, #6, #8, #9, #11 to #14). PDI measures the distribution of molecular mass in a sample. The smaller PDI (close to 0), the more stable the Negs formulation caused; the large PDI indicates particles formed are not uniform, and the formulation will flocculate quickly. An index value less than 0.05 is included in monodisperse, while an index greater than 0.7 indicates that the sample has a broad PS distribution. A-0.2 and below are considered acceptable for nanoparticle preparations (44). Based on **Table 2**, the PDI response shows a non-significant quadratic model with an F-value of 1.02 (p-value 0.4645 > 0.05). Measurements between variables and responses are not a precise cause of that. The suggested quadratic model equation can be seen as follows:

$$Y_2 = 0.57 + 0.027X_1 - 0.017X_2 - 0.034X_1X_2 - 0.16X_1^2 - 0.086X_2^2$$

The equation and p-value of each factor did not significantly affect PDI. The response surface plot (**Figure 2B**) depicts the combined influence of variables X_1 and X_2, which shows that Y_2 changes with the sum of the two variables by model.

The Negs ZP (Y_3) was in the range of 13.90 mV (STD#2) to 46.72 mV (STD#10) (**Table 1**). ZP represents the electric charge between the shear plane of a final outer layer and bulk solution, which significantly affects dispersion stability (45). This factor is strongly influenced by the composition of the Negs and its electrical phenomena. TEO-loaded Negs, which have positive ZP, show good interaction with negatively charged skin (46). ZP is the scientific term for the electrokinetic potential in colloidal systems. The high electric charge on the nanoparticle surface will prevent nanoparticle aggregation because of the strong repulsion between particles. The ZP requirement for stability is above ± 30 mV (19). The higher ZP and the slower aggregation are formed to prevent separation (47). Based on **Table 2**, the ZP response indicates a significant 2FI model with an F-value model of 7.06 (p-value 0.0078 < 0.05). The linear model equation suggested by the software can be seen as follows:

$$Y_3 = 31.54 + 8.27X_1 + 2.95X_2 + 10.59X_1X_2$$

The equation shows that the Negs ZP was significantly affected by the emulsifier (X_1) and gel former-emulsifier interaction (X_1X_2). ZP was usually influenced by the physicochemical properties of the drug, polymer, carrier, electrolyte presence, and their adsorption (48). One study stated that adding Carbopol® only slightly increased the ZP Negs (19). The response surface plot (**Figure 2C**) depicts the combined influence of variables X_1 and X_2, showing that Y_3 changes with the sum of the two variables. Nevertheless, the higher gradient in the response surface with span-80/tween-80 (X_1) – not Carbopol® 980 (X_2) – is the evidence from the comparative plot of the response surface. This description concludes that the ZP can be changed by selecting the proper X_1 level.

The pH test was carried out to measure Negs's acidity or alkalinity level. The pH values (Y_4) were in the range of 4.57 (STD#13) to 6.39 (STD#14) (**Table 1**). The pH requirement of Negs is the same as the skin pH. Too-acidic preparations can irritate the skin and cause a stinging sensation, while too-alkaline preparations can cause dry and itchy skin. The pH result test on 14 formulations was eligible and compatible with the skin (4.5-6.5) (49). Based on **Table 2**, a linear model was found to be significant in pH response with a model F-value of 145.08 (p-value < 0.0001). The linear model equation suggested by the software can be seen as follows:

$$Y_4 = 5.48 - 0.069X_1 - 0.75X_2$$

The equation shows that the pH was significantly (p-value < 0.0001) affected by gel former (X_2). Carbopol is a high molecular weight homopolymer and acrylic acid copolymer crosslinked with polyalkenyl polyethers (50). They are anionic and acidic (2.5–4.0 in 2% dispersion) when not neutralized with bases to achieve a specific viscosity (50,51). Therefore, adding Carbopol to a formulation with a fixed amount of base (triethanolamine) will significantly lower the pH of Negs. The response surface plot (**Figure 2D**) may then be used to depict the combined influence of variables X_1 and X_2, which shows that Y_4 changes linearly with the sum of the two variables. Nevertheless, the higher gradient in the response surface with Carbopol® 980 (X_2) – not span-80/tween-80 (X_1) – is the evidence from the comparative plot of the response surface. This description concludes that the choice of the X_2 level affects the pH Negs.

Spreadability was measured to ensure comfortable use on the skin because it spreads quickly (24). Terms of good dispersion are 5-7 cm. If the dispersion is too small, it is relatively difficult to spread when applied to the skin, while the dispersion tends to spread too quickly when applied, so it will cause an uncomfortable feeling when used (52). Based on the results, only one Neg did not meet the requirements, namely F11. **Table 1** shows the range of spreadability (Y_5) from 4.90 cm (STD#11) to 6.25 cm (STD#10). Based on **Table 2**, the spreadability response indicates a significant linear model with an F-value of 386.43 (p-value <0.0001). The linear model equation suggested by the software can be seen as follows:

$$Y_5 = 5.58 + 0.53X_1 - 0.033X_2$$

The equation shows that the spreadability of Negs was significantly (p-value < 0.0001) positively affected by the emulsifier (X_1). That was also found in other literature studies (24). The higher the Carbopol-contained Negs, the more viscous Negs. AT and spreadability have the opposite results. The higher the Negs' viscosity, the higher the adhesive strength produced, while the smaller the dispersion power (53). The response surface plot (**Figure 2E**) depicts the combined influence of variables X_1 and X_2, which shows that Y_5 changes linearly with the sum of the two variables. Nevertheless, the higher gradient in the response surface with span-80/tween-80 (X_1) – not Carbopol® 980 (X_2) – is the evidence from the comparative plot of the response surface. This description concludes that the scatter can be changed by choosing the right X_1 level.

Topical dosage forms, such as Negs, adhere to the skin in two ways: they adhere directly to the rough surface to form a "mechanical interlock" and to the surface via interaction (54). Good adhesion to Negs supports a higher concentration gradient towards the skin and provides more drug penetration (18). Adhesive strength is directly related to the AT on the Negs as measured using the single-lap shear test method with slight modifications (55,56). The test was carried out by applying a shear load to the plates that flank the sample, which had been pre-loaded, and given a measured force; the time taken for the plates to separate was recorded as AT. In our study, the AT was from 4.22 s (STD#14) to 7.08 s (STD#13) in **Table 1**. They met the requirements based on the AT test results on a 14 formulation. An AT was carried out to see how long a Negs could be attached to the skin. The AT requirement is more than 4 seconds. The longer a Negs could be attached to the skin, it showed the better result, where it is expected that more active substances can be absorbed due to the time the Negs was in contact with the skin (57). Based on **Table 2**, the linear model was found to be significant in the AT response with an F-value model of 46.35 (p-value < 0.0001). The linear model equation suggested by the software can be seen as follows:

$$Y_6 = 5.74 - 0.20X_1 + 1.18X_2$$

The equation reveals that the AT was significantly (p-value < 0.0001) affected by the gel former (X_2) or, indirectly, the same adhesion strength. This finding is in agreement with the literature (58,59). A response surface plot (**Figure 2F**) may therefore be used to depict the combined influence of variables X_1 and X_2, which shows that Y_6 changes linearly with the sum of the two variables. Nevertheless, the higher gradient in the response surface with Carbopol® 980 (X_2) – not span-80/tween-80 (X_1) – is the evidence from the comparative plot of the response surface. From this explanation, it can be concluded that the AT can be changed by selecting the right X_2 level. Details of the ANOVA results for measured responses are also presented in **Table 2**. In the end, the emulsifier factor significantly affected the response of PS, ZP, and spreadability of TEO-loaded Negs. At the same time, gel former affects the AT and pH.

2.4. Optimized TEO-loaded Negs

The formulation was optimized with Design-Expert®, version 13 software. The optimized Negs were selected based on the minimum PS and PDI; maximum ZP; value in pH range, spreadability, and AT (**Table 3**). Variables composition for optimized Negs is span-80/tween-80 of 8.68% and Carbopol® 980 of 1.18% with a desirability value of 0.801. The formulation with the maximum desirability value is the optimal formulation generated from the optimization phase of the program (60) —the optimization value formed as indicated by the desirability value close to one.

The desirability value range is 0-1. **Figure 2** describes the optimization results in the form of a 2D contour. *Contour* is a two-dimensional response image that was presented using a predictive model for PS, PDI, ZP, pH, spreadability, and AT response values. The contour graph shows the desirability value of 0.801, which is the closest value to 1 compared to the other points. **Figure 3** shows the projection in the form of a 3D surface; the low area shows low desirability, while the high area shows high desirability and is getting closer to 1. At this stage, the software predicts the response values shown in **Table 4**. Three confirmation runs need to be performed to validate optimization (61). The optimization model and estimates are validated by the observed optimized Negs, which show an acceptable variation from the predicted values (**Table 4**). We tested the optimized Negs' physical properties for further investigation, such as organoleptic, homogeneity, freeze-thaw, viscosity, and flow properties.

2.5. Evaluation of TEO-loaded Negs

Nanoemulsion systems can cover oily drugs' bitter or unpleasant taste (62). The organoleptic results of TEO-loaded Negs have a less distinctive turmeric odor, which is white and semisolid (**Table 5**). That is due

to the drug entrainment of oil with the oil phase effectively preventing evaporation and masking its specific odor (63). The homogeneity test results of the optimum TEO-loaded Negs formulation showed a homogeneous preparation, as evidenced by the absence of coarse grains. This homogeneity was correlated with the optimal formulation of PS and PDI of 182.3 ± 5.5 and 0.242 ± 0.003, respectively. The low PDI indicates uniformity or homogeneous dispersion of globules Negs (64). In addition, the small size of the globule (±200 nm) is not included in the coarse dispersion (41).

The thermodynamic stability test of the system was carried out using a freeze-thaw cycle to identify the presence of metastable Negs in the optimal formulation. It aims to see the separation of the water and oil phases due to the influence of extreme temperatures (65). The thermodynamic stability of any system is determined by the change in free energy between the system and its surroundings (66). The test results on the optimum formula for six cycles showed promising results; namely, there was no separation. This stability was correlated with the ZP of the optimal formulation of 57.23 ± 2.91 mV. The surface charge's magnitude was directly related to the stability of any Negs. It is evidenced by the high repulsive force between the Negs globules preventing coalescence, which was characterized by the absence of phase separation (67). Similar results were found in the Negs study containing thymoquinone, which had ZPs between −26.7 and −30.6 mV (66).

The pH conditions indirectly affect the viscosity indicated by Negs because they influence the swelling ability of Carbopol® 980. This excipient is a gel former and a thickener (52). It plays an essential role in the viscosity of Negs. Carbopol is dispersed in water to form an acidic colloidal solution with a low viscosity. Neutralizing with triethanolamine increases Negs' viscosity because a stable water-soluble gel was formed (50). Viscosity was carried out with #7 spindle (Brookfield digital RV DV-E) at 50 rpm of 32240 ± 2257.7 cP, indicating significantly high viscosity on Negs with pH 4.5. The magnitude of the viscosity is correlated with AT and spreadability. Viscosity is inversely proportional to spreadability (52). In contrast, the AT is directly proportional to the viscosity. A high-viscosity system will form stronger interfacial interactions and increase intermolecular interactions in the polymer network, increasing cohesion, adhesion strength, and AT (54).

Determining the rheology of a semisolid preparation is essential for controlling the consistency required to ensure the performance and formulation durability and to describe the mechanical (flow properties) system (68). The rheological study was conducted in the shear rate range of 6.81-40.86 s^{-1} at 25°C. The consistency index equals the apparent viscosity at a shear rate of 1 s^{-1}. The consistency index measured on TEO-loaded Negs was 155.67 cP and n = 0.22. The flow index measures the system's deviation from Newtonian behavior (n = 1). A value of n > 1 indicates dilatation or shear thickening flow, and n < 1 indicates pseudoplastic or shear thinning. The flow index typically lowers the thicker the base. Negs produce a 0.22 flow index, which implies pseudoplastic flow behavior. A colloidal network structure aligned with the shear direction and decreases viscosity as the shear rate increases have led to this pseudo-plasticity. The developed system will require a specific force to discharge (69). The results of the flow properties test showed that the optimal formula made was a pseudoplastic thixotropic flow type (**Figure 4**). *Thixotropic* is a flow property expected in pharmaceutical preparations because it has high consistency in the container but can be poured and dispersed easily (70).

3. CONCLUSION

Based on the results of the CCD-RSM analysis, the optimum span-80/tween-80 as an emulsifier is 8.68%, and Carbopol® 980 as a gel former was 1.18%. The resulting response is a PS of 182.3 nm, PDI 0.242, ZP 57.23 mV, pH 4.51, AT 6.45 seconds, and spreadability of 6 cm. Optimized formulation viscosity is 32240 cP with pseudoplastic thixotropic flow properties. Thus, the developed TEO-Negs can be a potential delivery system and a promising suitable approach for topical preparations.

4. MATERIALS AND METHODS

4.1. Materials

TEO (Curcuma longa) was purchased from Darjeeling Sembrani Aroma (Indonesia), sorbilene O E/P from Lamberti (Italy), span-80 from Croda (Singapura), propylene glycol from Dow Chemical Pacific (Singapura), Carbopol® 980 NF from Lubrizol AM (Cleveland), nipagin M from Clariant Produkte (Deutschland), propylparaben from Alpha Chemika (India), triethanolamine from Dow Chemical Pacific (Switzerland), and 1.1-diphenyl-2-picrylhydrazyl (DPPH) from Smart-Lab (Indonesia).

4.2. Methods

4.2.1. Identification of TEO

Organoleptic tests include observing form, color, and odor. Phenolic identification was carried out by adding one drop of 5% $FeCl_3$ to a 1 ml sample. Dark green to black colors indicate phenolic compounds' presence (71). Terpenoid testing was performed by adding Lieberman-Burchard reagent containing anhydrous acetic acid and concentrated sulfuric acid (3:1) into a 1 ml sample. Brownish or violet ring form indicates the presence of terpenoids (72). Spectrophotometry was used to determine antioxidant activity with the DPPH method (5). The calibration curve for the DPPH concentration against absorbance was made at a maximum wavelength of 516 nm. The absorbance was measured in a mixture of sample solution and DPPH with a particular concentration after 30-min of incubation in a dark room. IC_{50} was calculated from the inhibition percentage and absorbance.

4.2.2. Preparation of TEO-loaded Negs

TEO-loaded Negs were produced using a high-energy method, which used a mechanical device to produce a highly disruptive force to break up the water and oil phases to obtain nano-sized globules (18). The oil phase (M1) was prepared by mixing span-80 with 5% turmeric oil using a magnetic stirrer (WiseStir Wisd) at 1,500 rpm for 20 min. A total of 0.18% methylparaben and 0.02% propylparaben dissolved in 15% propylene glycol (M2). Then, the distilled water was stirred with tween-80, and M2 was added gradually until homogeneous at 1,500 rpm for 20 min (M3). M1 was stirred with M3 until homogeneous at 1,500 rpm for 40 min to form a clear and transparent nanoemulsion, then let left for 24 hours. The gel base was prepared by mixing Carbopol® 980 NF with distilled water at 70°C and left for 24 hours. Then, gradually add 1% triethanolamine to form a gel mass. Nanoemulsion was added slowly into the gel base while homogenized using a homogenizer (AEG) at 2,000 rpm for 10 min.

4.2.3. Experimental Design

This study selects the CCD-RSM method to develop the TEO-loaded Negs formulation. For preliminary screening on PS, PDI, ZP, pH, AT, and spreadability, a 2-factor CCD-RSM at two levels (high and low) was used. Based on previous experiments and study literature, high and low variables were determined. The CCD of the statistical package Design-Expert® version 13 software (Stat-Ease Inc., Minneapolis, MN) was used to examine the influence of the specified independent variable on the response variable to obtain the optimal formulation for TEO-loaded Negs. CCD planned 14 experiments were done under controlled circumstances (**Table 3**). The independent variables were span-80/tween-80 (X_1) and Carbopol® 980 concentration (X_2). The observed response of the dependent variables was PS (Y_1), PDI (Y_2), ZP (Y_3), pH (Y_4), spreadability (Y_5), and AT (Y_6).

4.2.4. Determination of PS, PDI, and ZP of Negs

PS and PDI of Negs were assessed using the Delsa Max Pro Particle Size Analyzer LS 100Q (Beckman Coulter, USA) at 25°C utilizing the dynamic light scattering (DLS) method or photo correlation spectroscopy. For analysis, 1 ml of samples was dispersed in 9 ml aqua pro injection. Into the cuvette, 1 ml of suspension and 5 ml of aqua pro injection were added as a diluent, and the results were read on the instrument. All measurements were made at a scattering angle of 90°. ZP was determined by particle size analyzer through mobility and conductivity measurements. The temperature was set to 25°C, and the mean electric field was set to 16 V/cm (73). The final result is the mean of each sample's three repeated measurements.

4.2.5. Determination of pH, Spreadability, and AT

pH was measured at a temperature of 25°C using a pH meter that had been previously calibrated with buffer solutions of pH 4 and 7. The calibration step was completed when the pH value indicated on the screen matched the correct pH value and was stable. Afterward, the electrode was dipped in Negs and recorded the value shown on the screen (74). Spreadability was measured by adding 0.5 g of Negs in the center of a glass covered with another glass. The preparation diameter was measured longitudinally and transversely; for

every minute added, 50 g was to a total weight of 150 g (75). Adhesion time determine by the single-lap shear test method (54). A-0.5 g of Negs was placed on a slide, then covered with another slide, and given a load of 1 kg for 3 min. The glass object was mounted on the test apparatus, and 80 g of the load was released until both glass objects were released, and the time was recorded (75).

4.2.6. Organoleptic Observation and Homogeneity Test

Negs were placed in a glass object and directly observed for color, smell, and shape (76). A-0.1 g Negs were spread over the slide, and homogeneity was observed. Test preparation is declared homogeneous if no coarse grains exist (77).

4.2.7. Viscosity and Rheological Flow

Viscosity and rheology were determined with a Brookfield RV DV-E Viscometer with appropriate spindle and speed. A-500 ml of Negs were put into a beaker glass; the spindle was installed, and the measured value was recorded as viscosity Negs. In this study, the spindle used spindle no. 7. Flow properties were determined by measuring the viscosity using a right spindle from low to high rotational speed and vice versa (74). Flow index and consistency index are determined from the power law equation:

$$\tau = K r^n$$

where τ is the shear stress, K is the consistency index, r is the shear rate, and n is the flow index.
Taking logs on both sides,

$$\log \tau = \log K + n \log r$$

So, from the log, shear stress Vs. Log shear rate plot, the plot slope was used as the flow index and the antilog of the Y-intercept as the consistency index (69).

4.2.8. Freeze-thaw Test

Negs were stored at 4 ± 2 °C, then transferred to 40 ± 2 °C for 48 hours (1-cycle), then repeated for 6-cycles. Phase separation was observed in each cycle (74).

4.2.9. Statistical Analysis

The Design-Expert® version 13 software was used to conduct the statistical study (Stat-Ease Inc., Minneapolis, MN). Analysis was done at a sig—$p < 0.05$ and $p < 0.01$ after three times of each measurement.

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REFERENCES

[1] Rohman A, Widodo H, Lukitaningsih E, Rafi M, Nurrulhidayah AF, Windarsih A. Review on *in-vitro* antioxidant activities of Curcuma species commonly used as. Food Res. 2020;4(2):286–93. [CrossRef]

[2] Liju VB, Jeena K, Kuttan R. An evaluation of antioxidant, anti-inflammatory, and antinociceptive activities of essential oil from Curcuma longa. L. Indian J Pharmacol. 2011;43(5):526–31. [CrossRef]

[3] Devkota L, Rajbhandari M. Composition of essential oils in turmeric rhizome. Nepal J Sci Technol. 2016;16(1):87–94. [CrossRef]

[4] Guimarãesa AF, Vinhas ACA, Gomes, Angélica Ferraz Souza LH, Krepsky PB. Essential oil of Curcuma longa L. rhizomes chemical composition, yield variation and stability. Quim Nov. 2020;43(7):909–13. [CrossRef]

[5] Islamadina R, Can A, Rohman A. Chemometrics application for grouping and determinating volatile compound which related to antioxidant activity of turmeric essential oil (Curcuma longa L). J Food Pharm Sci. 2020;8(2):1.

[CrossRef]

[6] Dosoky NS, Setzer WN. Chemical composition and biological activities of essential oils of curcuma species. Nutrients. 2018;10(9):10–7. [CrossRef]

[7] Jankasem M, Wuthi-udomlert M, Gritsanapan W. Antidermatophytic properties of ar -turmerone, turmeric oil, and curcuma longa preparations. ISRN Dermatol. 2013;2013(April 2009):1–3. [CrossRef]

[8] Mishra R, Gupta AK, Kumar A, Lal RK, Saikia D, Chanotiya CS. Genetic diversity, essential oil composition, and *in-vitro* antioxidant and antimicrobial activity of Curcuma longa L. germplasm collections. J Appl Res Med Aromat Plants. 2018;10(December 2017):75–84. [CrossRef]

[9] Sharifi-Rad J, Rayess Y El, Rizk AA, Sadaka C, Zgheib R, Zam W, et al. Turmeric and ıts major compound curcumin on health: bioactive effects and safety profiles for food, pharmaceutical, biotechnological and medicinal applications. Front Pharmacol. 2020;11(September):1–23. [CrossRef]

[10] Vera JMV, Dueñas-Rivadeneira AA, Díaz JMR, Radice M. Phytochemical characterization of the ethanolic extract, antioxidant activity, phenolic content and toxicity of the essential oil of Curcuma longa L. Rev la Fac Agron. 2022;39(1). [CrossRef]

[11] Ali MS, Alam MS, Imam F, Siddiqui MR. Topical nanoemulsion of turmeric oil for psoriasis: Characterization, *ex-vivo* and *in-vivo* assessment. Int J Drug Deliv. 2012;4(2):184–97. [CrossRef]

[12] Meng F-C, Zhou Y-Q, Ren D, Wang R, Wang C, Lin L-G, et al. Turmeric: A review of ıts chemical composition, quality control, bioactivity, and pharmaceutical application. Natural and Artificial Flavoring Agents and Food Dyes. Elsevier Inc.; 2018. 299–350. [CrossRef]

[13] Ibáñez MD, Blázquez MA. Curcuma longa L. rhizome essential oil from extraction to its agri-food applications. a review. Plants. 2021;10(1):1–31. [CrossRef]

[14] Yan W, Bowen WD, Hopson R, Mathew AE, Jacob JN. Biological studies of turmeric oil, part 2: Isolation and characterization of turmeric oil components; cytotoxic activity against pancreatic cancer cells. Nat Prod Commun. 2013;8(6):811–4. [CrossRef]

[15] Sarojini S, Sundar K, Anjali CH, Ravindran A. Formation and stability of oil-in-water nano emulsion containing turmeric oil. Biotechnol An Indian J. 2014;9(4):171–5.

[16] Vishwakarma AK, Maurya OP, Nimisha, Srivastava D. Formulation and evaluation of transdermal patch containing turmeric oil. Int J Pharm Pharm Sci. 2012;4(SUPPL. 5):358–61.

[17] Kirtane AR, Karavasili C, Wahane A, Freitas D, Collins J, Tamang S, et al. Development of oil-based gels as versatile drug delivery systems for pediatric applications. Sci Adv. 2022;8(21):1–19. [CrossRef]

[18] Sengupta P, Chatterjee B. Potential and future scope of nanoemulgel formulation for topical delivery of lipophilic drugs. Int J Pharm. 2017;526(1–2):353–65. [CrossRef]

[19] Eid AM, El-Enshasy HA, Aziz R, Elmarzugi NA. Preparation, characterization and anti-inflammatory activity of Swietenia macrophylla nanoemulgel. J Nanomedicine Nanotechnol. 2014;5(2). [CrossRef]

[20] Khurana S, Jain NK, Bedi PMS. Nanoemulsion based gel for transdermal delivery of meloxicam: Physico-chemical, mechanistic investigation. Life Sci. 2013;92(6–7):383–92. [CrossRef]

[21] Baibhav J, Gurpreet S, Rana A, Seema S, Vikas S. Emulgel: A comprehensive review on the recent advances in topical drug delivery. Int Res J Pharm. 2011;2(11):66–70.

[22] Sultana N, Akhtar J, Badruddeen, Khan MI, Ahmad U, Arif M, et al. Drug Development Life Cycle. In: Intech. IntechOpen; 2022.

[23] Eswaraiah S, Swetha K, Lohita M, Preethi PJ, Priyanka B, Reddy KK. Emulgel : Review on novel approach to topical drug delivery. Asian J Pharm Res. 2014;4(1):4–11.

[24] Dhawan B, Aggarwal G, Harikumar S. Enhanced transdermal permeability of piroxicam through novel nanoemulgel formulation. Int J Pharm Investig. 2014;4(2):65–76. [CrossRef]

[25] Dev A, Chodankar R, Shelke O. Emulgels : A novel topical drug delivery system. Pharm Biol Eval. 2015;2(4):64–75.

[26] ICH. ICH Guideline Q9 on Quality Risk Management. Vol. 44, Regulatory ICH. 2005. 1–20 p.

[27] Abdallah MH, Elsewedy HS, Abulila AS, Almansour K, Unissa R, Elghamry HA, et al. Quality by design for optimizing a novel liposomal jojoba oil-based emulgel to ameliorate the anti-inflammatory effect of brucine. Gels. 2021;7(219):1–18. [CrossRef]

[28] Sindi AM, Hosny KM, Alharbi WS. Lyophilized composite loaded with meloxicam-peppermint oil nanoemulsion for periodontal pain. Polymers (Basel). 2021;13(14). [CrossRef]

[29] Yalçinkaya SE, Yildiz N, Ak MS, Çalimli A. Preparation of polystyrene/montmorillonite nanocomposites: Optimization by response surface methodology (RSM). Turkish J Chem. 2010;34(4):581–92. [CrossRef]

[30] Klangmanee K, Athipornchai A. A rapid phytochemical screening of the effective phenolic antioxidant agents using $FeCl_3$ reagent. J Pharm Sci Res. 2019;11(10):3475–80.

[31] Orellana-Paucar AM, Machado-Orellana MG. Pharmacological profile, bioactivities, and safety of turmeric oil. Molecules. 2022;27(16):1–16. [CrossRef]

[32] Kusmardiyani S, Novita G, Fidrianny I. Antioxidant activities from various extracts of different parts of kelakai (Stenochlaena palustris) grown in central Kalimantan - Indonesia. Asian J Pharm Clin Res. 2016;9:215–9. [CrossRef]

[33] Avanco GB, Ferreira FD, Bonfim NS, Souza Rodrigues dos Santos PA, Peralta R, Brugnari T, et al. Curcuma longa L. essential oil composition, antioxidant effect, and effect on Fusarium verticillioides and fumonisin production. Food Control. 2017;73:806–13. [CrossRef]

[34] Priya R, Prathapan A, Raghu KG, Menon AN. Chemical composition and *in-vitro* antioxidative potential of essential oil isolated from Curcuma longa L. leaves. Asian Pac J Trop Biomed. 2012;2(2 SUPPL.):S695–9. [CrossRef]

[35] Pino JA, Fon-Fay FM, Pérez JC, Falco AS, Hernández I, Rodeiro I, et al. Chemical composition and biological activities of essential oil from turmeric (Curcuma longa L.) rhizomes grown in Amazonian Ecuador. CENIC Ciene Quím. 2018;49:1–8.

[36] Setya S, Madaan T, Tariq M, Razdan BK, Talegaonkar S. Appraisal of transdermal water-in-oil nanoemulgel of selegiline HCI for the effective management of parkinson's disease: Pharmacodynamic, pharmacokinetic, and biochemical ınvestigations. AAPS PharmSciTech. 2018;19(2):573–89. [CrossRef]

[37] Chang WC, Hu YT, Huang Q, Hsieh SC, Ting Y. Development of a topical applied functional food formulation: Adlay bran oil nanoemulgel. Lwt. 2020;117(February 2019):108619. [CrossRef]

[38] Ye Q, Li J, Li T, Ruan J, Wang H, Wang F, et al. Development and evaluation of puerarin-loaded controlled release nanostructured lipid carries by central composite design. Drug Dev Ind Pharm. 2021;47(1):113–25. [CrossRef]

[39] Shehata TM, Elnahas HM, Elsewedy HS. Development, characterization and optimization of the anti-ınflammatory ınfluence of meloxicam loaded into a eucalyptus oil-based nanoemulgel. Gels. 2022;8(5). [CrossRef]

[40] How CW, Rasedee A, Abbasalipourkabir R. Characterization and cytotoxicity of nanostructured lipid carriers formulated with olive oil, hydrogenated palm oil, and polysorbate 80. IEEE Trans Nanobioscience. 2013;12(2):72–8. [CrossRef]

[41] Chuacharoen T, Prasongsuk S, Sabliov CM. Effect of surfactant concentrations on physicochemical properties and functionality of curcumin nanoemulsions under conditions relevant to commercial utilization. Molecules. 2019;24(15):1–12. [CrossRef]

[42] Sarheed O, Dibi M, Ramesh KVRNS. Studies on the effect of oil and surfactant on the formation of alginate-based O/W lidocaine nanocarriers using nanoemulsion template. Pharmaceutics. 2020;12(12):1–21. [CrossRef]

[43] McClements DJ. Food Emulsions: Principles, Practices, and Techniques. Third Edit. New York: CRC Press; 2016. 719.

[44] Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, et al. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics. 2018;10(2):1–17. [CrossRef]

[45] Choi AJ, Kim CJ, Cho YJ, Hwang JK, Kim CT. Characterization of capsaicin-loaded nanoemulsions stabilized with alginate and chitosan by self-assembly. Food Bioprocess Technol. 2011;4:1119–26. [CrossRef]

[46] Sinha P, Srivastava S, Mishra N, Singh DK, Luqman S, Chanda D, et al. Development, optimization, and characterization of a novel tea tree oil nanogel using response surface methodology. Drug Dev Ind Pharm. 2016;42(9):1434–45. [CrossRef]

[47] Honary S, Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems - a review (part 1). Trop J Pharm Res. 2013;12(2):255–64. [CrossRef]

[48] Gurpreet K, Singh SK. Review of nanoemulsion formulation and characterization techniques. Indian J Pharm Sci. 2018;80(5):781–9. [CrossRef]

[49] Dias IP, Barbieri SF, Fetzer DEL, Corazza ML, Silveira JLM. Effects of pressurized hot water extraction on the yield

and chemical characterization of pectins from Campomanesia xanthocarpa Berg fruits. International journal of biological macromolecules. 2020;146:431–43. [CrossRef]

[50] Kulkarni VS, Shaw C. Use of polymers and thickeners in semisolid and liquid formulations. Essent Chem Formul Semisolid Liq Dosages. 2016;43–69. [CrossRef]

[51] Corporation TL. Product Specification Carbopol 980 NF Polymer. Cleveland; 2017.

[52] Nurman S, Yulia R, Irmayanti, Noor E, Sunarti TC. The optimization of gel preparations using the active compounds of arabica coffee ground nanoparticles. Sci Pharm. 2019;87(4). [CrossRef]

[53] Kurniawan MF, Sugihartini N, Yuwono T. Iritation properties and evaluation of physical properties emulgel of clove oil using simplex lattice design methods. Farmasains J Ilm Ilmu Kefarmasian. 2018;5(1):1–8. [CrossRef]

[54] Sun Z, Li Z, Qu K, Zhang Z, Niu Y, Xu W, et al. A review on recent advances in gel adhesion and their potential applications. J Mol Liq. 2021;325:115254. [CrossRef]

[55] Gupta NV, Natasha S, Getyala A, Bhat RS. Bioadhesive vaginal tablets containing spray dried microspheres loaded with clotrimazole for treatment of vaginal Candidiasis. Acta Pharm. 2013;63(3):359–72. [CrossRef]

[56] Abramowitch S, Easley D. Introduction to Classical Mechanics. Biomechanics of the Female Pelvic Floor. Elsevier Inc.; 2016. 89–107 p. [CrossRef]

[57] Sari DK, Sugihartini N, Yuwono T. Irritation test and physical properties evaluation of essential oils clove (Syzigium aromaticum) in emulgel. Pharmaciana. 2015;5(2):115–20. [CrossRef]

[58] Cevher E, Sensoy D, Taha MAM, Araman A. Effect of thiolated polymers to textural and mucoadhesive properties of vaginal gel formulations prepared with polycarbophil and chitosan. AAPS PharmSciTech. 2008;9(3):953–65. [CrossRef]

[59] Pande V, Patel S, Patil V, Sonawane R. Design expert assisted formulation of topical bioadhesive gel of sertaconazole nitrate. Adv Pharm Bull. 2014;4(2):121–30. [CrossRef]

[60] Badwaik LS, Prasad K, Deka SC. Optimization of extraction conditions by response surface methodology for preparing partially defatted peanut. Int Food Res J. 2012;19(1):341–6.

[61] Mohtashamian S, Boddohi S, Hosseinkhani S. Preparation and optimization of self-assembled chondroitin sulfate-nisin nanogel based on quality by design concept. Int J Biol Macromol. 2018;107:2730–9. [CrossRef]

[62] Jaiswal M, Dudhe R, Sharma PK. Nanoemulsion: An advanced mode of drug delivery system. 3 Biotech. 2015;5(2):123–7. [CrossRef]

[63] Wang X, Gu Y, He Y, Sang L, Dai Y, Wang D. Preparation and optimization formulation of zedoary turmeric oil nanoemulsion based thermo-sensitive gel for improved application in ophthalmology. J Drug Deliv Sci Technol. 2021;65(June):102682. [CrossRef]

[64] Begur M, Kumar Pai V, Gowda D V, Srivastava A, Raghundan H V, Shinde CG, et al. Enhanced permeability of Cyclosporine from a transdermally applied nanoemulgel. Der Pharm Sin. 2015;6(2):69–79.

[65] Yuliani SH, Hartini M, Pudyastuti B, Istyastono EP. Comparison of physical stability properties of pomegranate seed oil nanoemulsion dosage forms with long-chain triglyceride and medium-chain triglyceride as the oil phase. Tradit Med J. 2016;21(2):93–8. [CrossRef]

[66] Algahtani MS, Ahmad MZ, Shaikh IA, Abdel-Wahab BA, Nourein IH, Ahmad J. Thymoquinone loaded topical nanoemulgel for wound healing: Formulation design and *in-vivo* evaluation. Molecules. 2021;26(13):1–16. [CrossRef]

[67] Ahmad J, Mir SR, Kohli K, Amin S. Effect of oil and co-surfactant on the formation of Solutol HS 15 based colloidal drug carrier by Box-Behnken statistical design. Colloids Surfaces A Physicochem Eng Asp. 2014;453(1):68–77. [CrossRef]

[68] Eid AM, Hawash M. Biological evaluation of Safrole oil and Safrole oil Nanoemulgel as antioxidant, antidiabetic, antibacterial, antifungal and anticancer. BMC Complement Med Ther. 2021;21(1):1–12. [CrossRef]

[69] Arora R, Aggarwal G, Harikumar SL, Kaur K. Nanoemulsion based hydrogel for enhanced transdermal delivery of ketoprofen. Adv Pharm. 2014;2014:1–12. [CrossRef]

[70] Sinko PJ, Singh Y, editors. Martin's physical pharmacy and pharmaceutical sciences 6th edition. Sixth edit. Philadelphia: Wolter Kluwer Health; 2011.

[71] Rahimah SB, Djunaedi DD, Soeroto AY, Bisri T. The phytochemical screening, total phenolic contents and antioxidant activities *in-vitro* of white oyster mushroom (Pleurotus ostreatus) preparations. Open Access Maced J

Med Sci. 2019;7(15):2404–12. [CrossRef]

[72] Farabi K, Harneti D, Darwati, Mayanti T, Nurlelasari, Maharani R, et al. Dammarane-type triterpenoid from the stem bark of aglaia elliptica (meliaceae) and ıts cytotoxic activities. Molecules. 2022;27(19):1–11. [CrossRef]

[73] Elsheikh MA, Elnaggar YSR, Hamdy DA, Abdallah OY. Novel cremochylomicrons for improved oral bioavailability of the antineoplastic phytomedicine berberine chloride: Optimization and pharmacokinetics. Int J Pharm. 2018;535(1–2):316–24. [CrossRef]

[74] Nursal FK, Nining, Rahmah AS. Formulation and development of grape seed oil (Vitis vinifera L) emulgel peel-off mask using gelling agent hydroxy propyl methyl cellulose (HPMC). IOP Conf Ser Earth Environ Sci. 2021;755(1). [CrossRef]

[75] Irianto IDK, Purwanto P, Mardan MT. Antibacterial activity and physical evaluation of Piper betle L. Decoction gel as an alternative treatment for mastitis. Maj Farm. 2020;16(2):202–10. [CrossRef]

[76] Elfiyani R, Amalia A, Pratama SY. Effect of using the combination of tween 80 and ethanol on the forming and physical stability of microemulsion of eucalyptus oil as antibacterial. J Young Pharm. 2017;9(2):230–3. [CrossRef]

[77] Yati K, Jufri M, Gozan M, Dwita LP. The effect of hidroxy propyl methyl cellulose (HPMC) concentration variation on physical stability of tobacco (Nicotiana tabaccum l.) extract gel and ıts activity against streptococcus mutans. Pharm Sci Res. 2018;5(3):133–41. [CrossRef]

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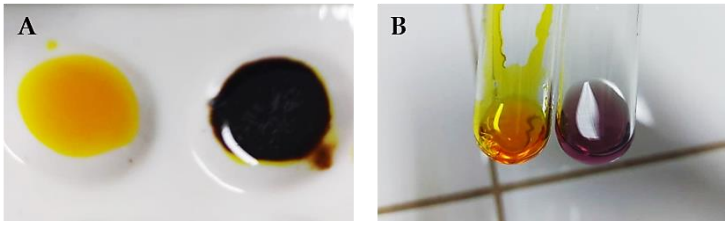

Figure 1. The result of qualitative test observation is a phenolic test with a positive result marked in a blackish green color (a) and a terpenoid test with a positive result marked with a purple color (b).

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Tabel 1. Evaluation results of independent variables and dependent variables with the CCD design for optimizing TEO-loaded Negs.

Std	X_1 (%)	X_2 (%)	Y_1 (nm)	Y_2	Y_3 (mV)	Y_4	Y_5 (cm)	Y_6 (s)
1	5.50	1.02	206.9 ± 0.7	0.571 ± 0.000	35.39 ± 1.50	5.45	5.55	5.65
2	2.32	1.15	457.0 ± 0.9	0.000 ± 0.000	13.90 ± 1.92	4.60	5.00	6.99
3	8.68	0.90	166.7 ± 0.4	0.378 ± 0.074	31.64 ± 0.53	6.35	6.20	4.23
4	2.32	0.90	411.0 ± 5.8	0.000 ± 0.000	30.67 ± 0.89	6.29	5.00	4.30
5	5.50	1.02	302.1 ± 3.6	0.571 ± 0.000	36.45 ± 0.45	5.46	5.60	5.63
6	5.50	1.02	263.0 ± 3.7	0.571 ± 0.000	18.49 ± 1.77	5.47	5.55	5.64
7	8.68	1.15	198.1 ± 1.2	0.242 ± 0.003	57.23 ± 2.91	4.65	6.15	6.95
8	5.50	1.02	246.9 ± 7.2	0.571 ± 0.000	26.60 ± 0.17	5.46	5.60	5.67
9	5.50	1.02	206.0 ± 2.3	0.571 ± 0.000	31.77 ± 1.01	5.46	5.60	5.65
10	10.00	1.02	160.8 ± 1.3	0.285 ± 0.024	46.72 ± 1.97	5.31	6.25	5.84
11	1.00	1.02	244.2 ± 4.3	0.571 ± 0.000	31.26 ± 0.57	5.78	4.90	6.88
12	5.50	1.02	238.1 ± 3.0	0.571 ± 0.000	30.78 ± 0.53	5.45	5.55	5.64
13	5.50	1.20	206.2 ± 1.6	0.571 ± 0.000	30.56 ± 0.78	4.57	5.50	7.08
14	5.50	0.85	240.5 ± 2.2	0.571 ± 0.000	20.12 ± 0.16	6.39	5.65	4.22

Y_1: Particle size (PS), Y_2: Polydispersity index (PDI), Y_3: Zeta potensial (ZP), Y_4: pH, Y_5: Spreadability, Y_6: Adhesion time (AT)

551 **Table 2.** Statistical analysis of PS (Y_1), PDI (Y_2), ZP (Y_3), pH (Y_4), spreadability (Y_5), and AT (Y_6) TEO-loaded Negs on
552 CCD.

Factors		**Y_1**	**Y_2**	**Y_3**	**Y_4**	**Y_5**	**Y_6**
X_1	Coefficient	-77.64	0.027	8.27	-0.069	0.53	-0.20
	p-value	0.0063**	0.7325	0.0080**	0.1430	<0.0001**	0.1406
X_2	Coefficient	3.61	-0.017	2.95	-0.75	-0.033	1.18
	p-value	0.8783	0.8289	0.2668	<0.0001**	0.1157	<0.0001**
X_1X_2	Coefficient		-0.034	10.59			
	p-value		0.7602	0.0136*			
X_1^2	Coefficient		-0.16				
	p-value		0.0818				
X_2^2	Coefficient		-0.086				
	p-value		0.3087				
Intercept	Coefficient	253.39	0.57	31.54	5.48	5.58	5.74
Degree of freedom		2	5	3	2	2	2
Sum of squares		48332.05	0.24	1065.33	4.48	2.28	11.49
Mean of squares		24166.02	0.047	355.11	2.24	1.14	5.74
F-value		5.69	1.02	7.06	145.08	386.43	46.35
p-value		0.0201	0.4645	0.0078	<0.0001	<0.0001	<0.0001
R-Squared		0.5084	0.3898	0.6794	0.9635	0.9860	0.8939

553 X_1: span-80/tween-80; X_2: Carbopol® 980
554 * p-value < 0.05
555 ** p-value < 0.01

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558 **Table 3.** The independent and dependent variables with levels and limits in CCD for TEO-loaded Negs development.

Variables	Code	Start point (-α)	Low level (-1)	Central level	High level (+1)	Start point (+α)	Units
Independent variables							
Span-80/tween-80	X_1	0.85	2.32	1.02	5.50	1.20	% w/w
Carbopol® 980	X_2	1	0.90	5.50	1.02	10	% w/w
Dependent variables		**Limits**					
Particle size (PZ)	Y_1	Minimum					nm
Polydispersity index (PDI)	Y_2	Minimum					
Zeta potensial (ZP)	Y_3	Maximum					mV
pH	Y_4	is in range					
Spreadability	Y_5	is in range					cm
Adhesion time (AT)	Y_6	is in range					s

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561 **Table 4.** Optimized TEO-loaded Negs' actual and predicted values for each response.

Responses	Predicted values	Actual value[a]	Error[b] (%)
Y1 (nm)	180.2	182.3 ± 5.5	1.165
Y2	0.250	0.242 ± 0.003	-3.200
Y3 (mV)	56.30	57.23 ± 2.91	1.652
Y4	4.50	4.51 ± 0.02	0.222
Y5 (cm)	6.07	6.0 ± 0.2	-1.153
Y6 (s)	6.98	6.45 ± 0.19	-7.593

562 a Data listed is the mean ± standart deviation, n = 3
563 b Error (%) = [(Actual value – Predicted value)/Predicted value] * 100%
564
565
566

567 **Table 5.** Additional evaluation on TEO-loaded Negs with an optimal formulation.

Evaluation	Result
Organoleptic	Color: White; Odor: typical turmeric
Homogeneity	Homogeneous
Freeze-Thaw	Cycles 0-6, no separation occurs
Viscosity[a]	32240 ± 2257.7 cP

568 a Data listed is the mean ± standart deviation, n = 3

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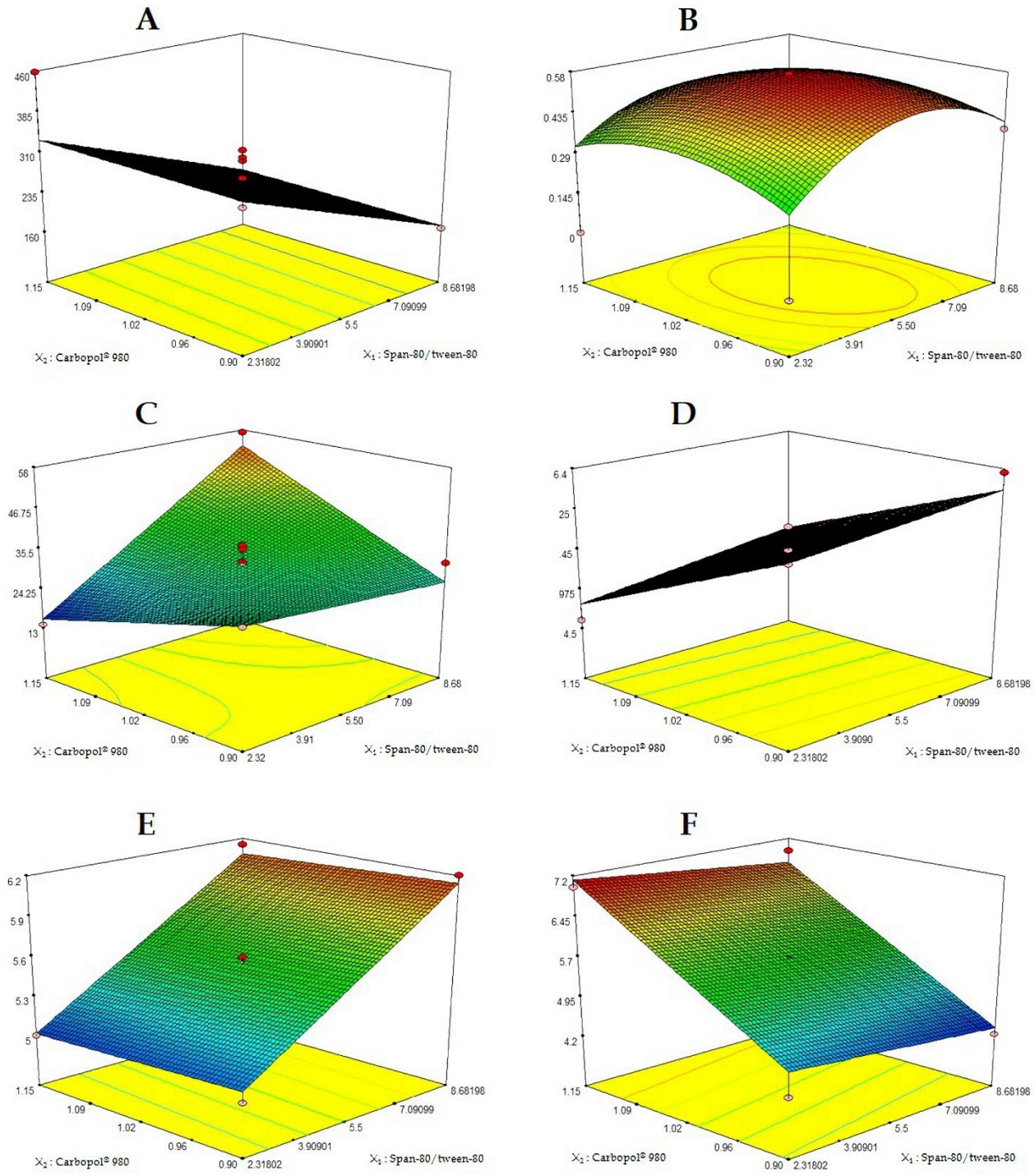

573

574 **Figure 2.** Effect of span-80/tween-80 and Carbopol® 980 concentration on PS (a) PDI (b) ZP (c) pH
575 (d) spreadability (e) AT (f).

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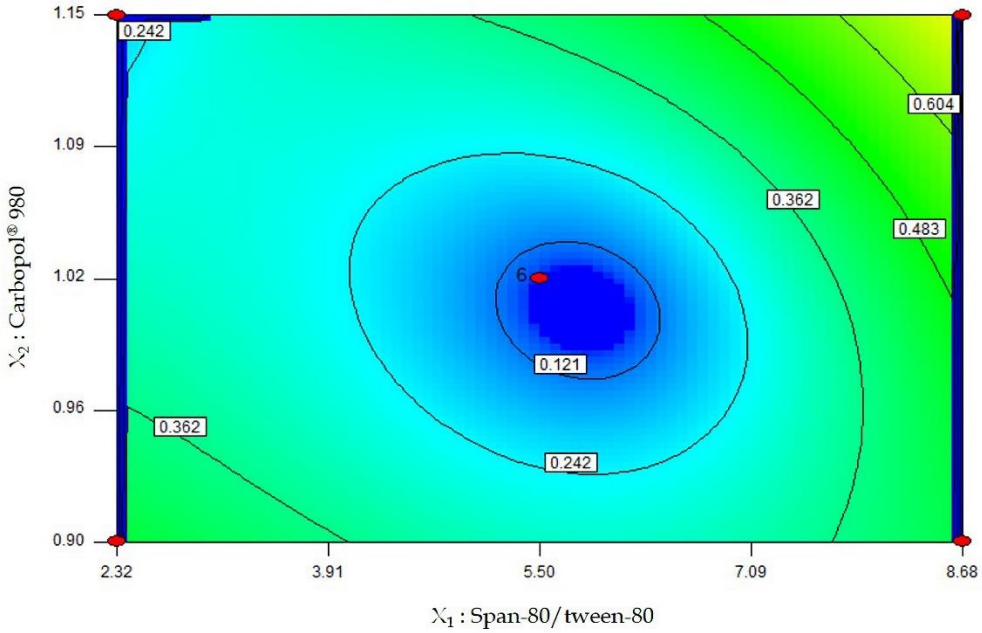

Figure 3. Contour plot desirability value of optimal formulation.

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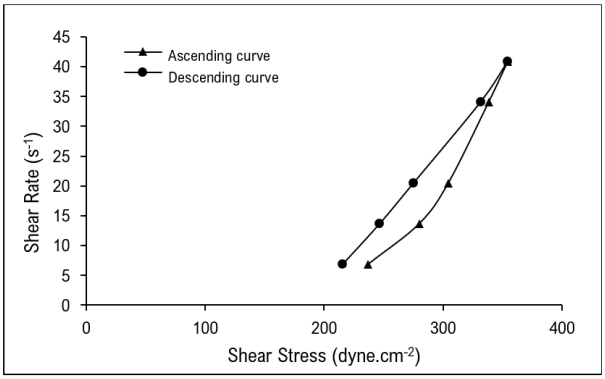

Figure 4. Optimal formulation flow properties.

RESPONSE TO REVIEWERS
OF THE JOURNAL OF RESEARCH IN PHARMACY

MANUSCRIPT ID: MPJ-10622.REV-2

Reviewer: 1

Comments to the Author
Updated manuscript covers my earlier points but there are some minors points:

1) The whole manuscript needs to be checked in terms of non-scientific and non-specific statements such as "...non-friendly solvents to the formulation....". In particular what does "non-friendly" statement refer to??? Please be specific.

Response:
We accepted your suggestion. The term "non-friendly solvents to the formulation" refer to large amounts of surfactant that components may add in nanoemulsion formulations. These components can irritate the skin based on some of the literature we cite, namely Sengupta and Chatterjee (2017) and Eid et al. (2014).
To clarify the information, we changed the sentence to: (line 31)
The main advantage of topically administered nanoemulsions is the ability to increase penetration and permeation of drugs through the skin without adding non-physical enhancers and a large amount of surfactant to the formulation, which can cause skin irritation, especially with long-term usage (18,19).

2) The resolution of the Figure 2 and Figure3 should be enhanced. The number and the letters are pixelized.

Response:
We accepted your suggestion. Figures 2 and 3 have been updated to 400 dpi from 150 dpi to enhance the quality of numbers and letters pixels.

8. Bukti Konfirmasi Artikel *Accepted* (9 Maret 2023)

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Nining <nining@uhamka.ac.id>

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Editor-in-Chief The manuscript could be accepted for publication without any more revision.

Accepted

Manuscript Information

Manuscript ID:	**MPJ-10622.REV-3**
Title in English:	Response surface methodology for optimization of turmeric essential oil-loaded nanoemulgel
Small Title in English:	No information entered
Authors:	Nining Nining[1], Anisa Amalia[1], Fatimatuz Zahrok[2]
Institutions:	[1]Universitas Muhammadiyah Prof. DR. HAMKA, Pharmaceutical Technology, East Jakarta, Indonesia [2]Universitas Muhammadiyah Prof. DR. HAMKA, Pharmacy, East Jakarta, Indonesia
Keywords in English:	Central composite design; nanoemulgels; response surface methodology; turmeric essential oil; topical delivery.
Manuscript Type:	Research article
Processing Status:	Accepted

Abstract in English

Turmeric Essential Oil (TEO) has an antioxidant and anti-inflammatory activity to be formulated in a topical dosage form. Nanoemulgels (Negs) development, based on varying concentrations of emulsifiers and gel formers, affects their characteristics and stability. This study focuses on optimizing TEO-loaded Negs based on physical and mechanical characterization, which have promising topical applications. Negs were created using the high-energy approach and optimized using Response Surface Methodology (RSM) and the Central-Composite Design (CCD) for the optimization of span-80/tween-80 (X1) and Carbopol® 980. (X2). Observed variable responses were particle size (PS) (Y1), polydispersity index (PDI) (Y2), zeta potential (ZP) (Y3), pH (Y4), spreadability (Y5), and adhesion time (AT) (Y6). Actual responses of Negs were compared with the CCD-RSM predictions to validate the model. In addition, other physical evaluations were observed, such as organoleptic observations, homogeneity, freeze-thaw tests, viscosity, and flow properties. Optimized TEO-loaded Negs were made with 8.68% span-80/tween-80 and 1.18% Carbopol® 980. The evaluation results showed the optimal TEO-loaded Negs on nano-metric size (182.3 ± 5.5 nm) with low PDI (0.242 ± 0.003), good ZP (-57.23 ± 2.91 mV), pH (4.51 ± 0.02), spreadability (6.0 ± 0.2 cm), and AT (6.45 ± 0.19 s). TEO-loaded Negs have an excellent appearance and did not run phase separation at extreme temperature storage with pseudoplastic thixotropy flow. Thus, the developed TEO-loaded Negs can be a potential delivery system and a promising suitable approach for topical preparations.

Manuscript Files

File Name	File Size	Date Created	Category	Description
MPJ-10622-1-cover-letter-nining-et-al..pdf (../pdf-files/out/12462-MPJ-10622-1-cover-letter-nining-et-al..pdf)	31 KB	Sep 03, 2022	Cover letter	None
MPJ-10622-9-isi-jrp-checklist.pdf (../pdf-files/in/12462-MPJ-10622-9-isi-jrp-checklist.pdf)	3388 KB	Sep 03, 2022	Author Checklist Form	None
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MPJ-10622-7-figure-1..jpeg (../pdf-files/in/12462-MPJ-10622-7-figure-1..jpeg)	513 KB	Sep 03, 2022	Figure	None
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